Leucocyte CD-markers and receptors expression (TiO_2 concentration influence)	The group of patients: Often consumed TiO_2	Rarely consumed TiO_2
CD19 density on B-lymphocytes (0.0005 mg/mL)	2,5[−2,1; +3,0]*	1,8[−1,7; +2,3]
$Fc\varepsilon RI^+$ eosinophils number (0.005 mg/mL)	193[−135; +251]*	103[−66; +141]
$Fc\varepsilon RI^+$ eosinophils number (0.0005 mg/mL)	181[−118; +245]*	108[−37; +178]
$Fc\varepsilon RI^+$ eosinophils number (without TiO_2)	196[−130; +261]*	114[−55; +173]
Receptor-associated IL10 density on lymphocytes (0.005 mg/mL)	4,4[−4,0; +4,8]*	3,5[−2,7; +4,2]
Receptor-associated IL10 density on lymphocytes (0.0005 mg/mL)	4,4[−4,0; +4,9]*	3,6[−2,8; +4,4]
Receptor-associated IL10 density on lymphocytes (without TiO_2)	4,5[−4,1; +4,9]*	3,7[−2,9; +4,5]
$CD69^+$ T-lymphocytes number (0.005 mg/mL)	212[−5,0; +417]*	589[−90; +1088]
$CD69^+$ T-lymphocytes number (without TiO_2)	189[−133; +512]*	765[−113; +1642]
$CD69^+CD154^-$ density on T-lymphocytes (0.005 mg/mL)	3,5[−2,4; +4,6]*	2,0[−2,0; +2,1]
$CD69^+CD154^-$ density on T-lymphocytes (0.0005 mg/mL)	3,1[−2,5; +3,6]*	2,2[−1,8; +2,6]
CD3CD45 density on T–lymphocytes (0.005 mg/mL)	7,2[−5,8; +8,6]*	4,9[−2,7; +7,1]

1389 | In vitro NLRP3 inflammasome activation assay assists diagnosis of genetically negative CAPS patients and guides anti-IL1 therapy

Mendonca LO[1]; Toledo-Barros MAM[1]; Franco PA[1]; Giavina-Bianchi Junior PF[1]; Kalil JE[1]; Morato Castro FF[1]; Caroli F[2]; Grossi A[2]; Ceccherini I[2]; Robazzi T[3]; Pileggi GS[4]; Rivitti MC[5]; Sanches Junior JA[5]; Grumach AS[6]; Gattorno M[7]; Pontillo A[8]

[1]*Autoinflammatory Unit, Clinical Immunology and Allergy Discipline, School of Medicine, University of São Paulo, São Paulo, Brazil;* [2]*UOSD Genetics and Genomics of Rare Diseases, Istituto Giannina Gaslini, Genoa, Italy;* [3]*Pediatric Rheumatology Department, School of Medicine, Federal University of Bahia, Bahia, Brazil;* [4]*Pediatric Rheumatology Department, School of Medicine, Barretos School of Health Science, Barretos, Brazil;* [5]*Dermatology Department, Hospital das Clinicas, School of Medicine, University of São Paulo, São Paulo, Brazil;* [6]*Clinical Immunology Department, Medical School, University Center Health ABC, Santo André, Brazil;* [7]*Center for Autoinflammatory Diseases and Primary Immunodeficiencies, Istituto Giannina Gaslini, Genoa, Italy;* [8]*Laboratory of Immunogenetics, Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil*

Background: Cryopyrin/NLRP3-associated periodic syndromes (CAPS) is a group of rare spectral autoinflammatory diseases characterized by periodic fever, early onset urticarial rash, positive acute reactants markers (CRP, SAA), arthritis and neurologic involvement, including ocular disorders and progressive deafness. The constitutive activation of NLRP3 inflammasome and the consequent elevate production of inflammatory cytokine IL-1ß are the pathogenic mechanisms involved in CAPS. Gain-of-function mutations in NLRP3 were detected in about 60% of patients. However due to the heterogeneity of clinical presentation and to the missing of genetic proving in 40% of patients, both diagnosis and therapeutic choice are often tricky.

Method: Eight patients (P1-P8) with a suspect of CAPS were included in a pilot study for the development of in vitro test for NLRP3 inflammasome activation (NLRP3-IA) between 2016 and 2019. All patients were examined according to standard protocols for autoinflammatory syndromes, including genomic DNA screening for mutations in candidate genes already associated with CAPS spectrum (NLRP3, NLPR12 and NLRC4). All patients were also sequenced for genes associated to other monogenic autoinflammatory syndromes. In parallel, patient' peripheral blood was used for serum collection and monocytes isolation. Monocytes were stimulated with LPS and ATP. IL-1ß concentration was measured in serum and culture supernatants by ELISA.

Results: *Clinical*: All patients presented clinical features compatible with a CAPS (Table 1). Genetic: Candidate genes revealed pathogenic heterozygous mutations in NLRP3 of P1 (exon 3 - c.913G>C, p.Asp305His) and P2 (exon 3 - c.926G>T, p.Gly309Val). One Schnitzler Syndrome found had a VUS in NLRP3 (exon 4; c.A2176G; p.S726G). *Functional*: Monocytes isolated from genetic positive NLRP3 patients (P1 and P2) produced high level of IL-1ß when challenged with LPS and ATP did not amplify cytokine production, suggesting a defect in NLRP3 inflammasome when compared to healthy controls (Figure 1). In the group of genetically negative CAPS, NLPR3-IA was positive in 66% and in 50% of Schnitzler Syndrome. All NLRP3-IA results are described in Figure 1.

Conclusion: These results demonstrates the convenience of the NLRP3-IA test in the examination of suggestive CAPS patients or Schnitzler's Syndrome, especially for those with negative genetic test, to (1) confirm the diagnosis and to (2) guide the therapeutic choice.

	P1	P2	P3	P4	P5	P6	P7	P8
Age onset (<1 year)	Yes	Yes	No	Yes	No	Yes	Yes	Yes
Recurrent fever	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Cold/stress induced triggered episodes	Yes	No	No	Yes	No	No	Yes	No
sensorineural hearing loss	No	No	Yes	No	No	Yes	Yes	No
Neutrophilic urticarial rash	Yes	Yes	No	Yes	Yes	Yes	Yes	No
Musculoskeletal symptoms	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Abdominal Pain	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Chronic aseptic meningitis	No	Yes	Yes	No	No	No	No	Yes
Skeletal abnormalities	No	Yes	Yes	Yes	Yes	No	Yes	Yes
Raised inflammatory markers	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Genetic Findings (NLRP3, NLRP12 and NLRC4)	Heterozygous mutation in NLRP3 exon 3 - c.913G>C, p.Asp305His	Heterozygous mutation in NLRP3 exon 3 - c.926G>T, p.Gly309Val	Negative	Negative	Negative	Negative	Negative	Negative
Response to anti-IL1	Successfully treated with anti-IL1	Successfully treated with anti-IL1	Successfully treated with anti-IL1	Successfully treated with anti-IL1	Successfully treated with anti-IL1	Not treated	Not treated	Not treated
Final Diagnosis	CAPS	CAPS	CAPS	CAPS	CAPS	CAPS	CAPS-like	CAPS-like
Ratio (ATP +LPS)/LPS	2,55	1,29	1,01	1,11	1,34	0.94	10.94	20.86

Figure 1 - Serum IL-1B measurement in peripheral monocytes cells after ATP, LPS and ATP + LPS stimulus . All results are presented as mean and standard deviation (mean;SD) in pg/µL

Results	Genetically positive CAPS (n=2)	Genetically negative CAPS (n=4)	CAPS-LIKE (n=2)	Other Syndromes (n=11)	Schnitlzer (n=2)	Control (n=15)
Resting	11,35 ; 1,18	20,91 ; 24,01	11,90 ; 12,30	14,81 ; 15,55	25,46; 12,73	4,95; 5,21
ATP	13,55 ; 6,15	47,23 ; 36,14	21,61 ; 23,64	17,62 ; 11,8	24,18; 12,09	6,37; 6,80
LPS	575,25 ; 134,7	585,39 ; 765,44	486,28 ; 377,92	438,96 ; 528,40	1154,21; 661,50	80,20; 79,39
ATP + LPS	1047,19 ; 253,41	1035,35 ; 863,31	1759,99 ; 1165,87	869,52 ; 840,99	1877,09; 1774,19	234,05; 234,63
Ratio (ATP/LPS)/ LPS	1,92 ; 0,89	1,10 ; 0,17	10,98 ; 9,70	3,73 ; 3,47	5,43 ; 6,31	3,37 ; 1,61

1416 | Delay in diagnosis in adults patients with variable common immunodeficiency

Zanetti FA; Bialowas D; Pachani MADS; Souza TSD; Barros MT; Grecco O; Kalil J; Kokron CM; Marinho AKBB

Division of Clinical Immunology and Allergy, University São Paulo, São Paulo, Brazil

Background: Common Variable Immunodeficiency (CVID) represents a group of primary immunodeficiencies characterized by hypogammaglobulinemia and recurrent sinopulmonary infections associated with inflammation, autoimmunity, enteropathies, and neoplasms. The aim of the study was to evaluate the delay in the diagnosis of patients with CVID and different clinical presentations.

Method: Retrospective study reviewed the medical records of 123 patients with CVID (ESID diagnosis criteria) during the follow-up at the Primary Immunodeficiency Ambulatory. We analyzed the onset of alarm signals according to criteria developed by the Jeffrey Modell Foundation (USA) and the time it took to be diagnosed.

Results: From the 123 individuals diagnosed with CVID, 44.7% were men and 55.2% women, with a media age at diagnosis of 30.3 years in women and 30.4 in men. The average time between onset of symptoms and diagnosis of CVID was 13.9 years. Among the infectious manifestations presented, 73.9% of the individuals presented at least one pneumonia per year for more than 1 year, presenting as the most prevalent infection in the studied group. The second most prevalent disease were two or more sinusitis per year, present in 46.3% of individuals, and 27.6% had chronic diarrhea. From the 123 patients studied, 67 (54.5%) had bronchiectasis diagnosed by image diagnosis (chest computerized tomography). From those, 50 patients (75%) had manifestated recurrent pneumonia before definitive