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RESEARCH ARTICLE

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Cross-sectional analysis reveals autoantibody signatures associated with COVID-19 severity

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Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is associated with increased levels of autoantibodies targeting immunological proteins such as cytokines and chemokines. Reports further indicate that COVID-19 patients may develop a broad spectrum of autoimmune diseases due to reasons not fully understood. Even so, the landscape of autoantibodies induced by SARS-CoV-2 infection remains uncharted territory. To gain more insight, we carried out a comprehensive assessment of autoantibodies known to be linked to diverse autoimmune diseases observed in COVID-19 patients in a cohort of 231 individuals, of which 161 were COVID-19 patients (72 with mild, 61 moderate, and 28 with severe disease) and 70 were healthy controls. Dysregulated IgG and IgA autoantibody signatures, characterized mainly by elevated concentrations, occurred predominantly in patients with moderate or severe COVID-19 infection. Autoantibody levels often accompanied anti-SARS-CoV-2 antibody concentrations while stratifying COVID-19 severity as indicated by random forest and principal component analyses. Furthermore, while young versus elderly COVID-19 patients showed only slight differences in autoantibody levels, elderly patients with severe disease presented higher IgG autoantibody concentrations than young individuals with severe COVID-19. This work maps the intersection of COVID-19 and autoimmunity by demonstrating the dysregulation of multiple autoantibodies triggered during SARS-CoV-2 infection. Thus, this cross-sectional study suggests that SARS-CoV-2 infection induces autoantibody signatures associated with COVID-19 severity and several autoantibodies that can be used as biomarkers of COVID-19 severity, indicating autoantibodies as potential therapeutical targets for these patients.

KEYWORDS
autoantibodies, autoimmune diseases, COVID-19 severity, SARS-CoV-2 infection

1 | INTRODUCTION

Patients with severe Coronavirus disease 2019 (COVID-19), which is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), may present with systemic immune dysregulation of both the innate and adaptive immune responses that manifest mainly as cytokine storm syndrome,[1] hyperactivation of both myeloid and lymphoid cells,[1,2] and dysregulated humoral immune response.[3] Moreover, recent reports from our group and others[4-6] indicate that patients with COVID-19 have high levels of autoantibodies targeting immune-related proteins, cytokines (e.g., type I interferons or IFNs),[5] chemokines, and their receptors as well as complement factors,[4] G protein-coupled receptors and renin-angiotensin system-related molecules.[7-10]

The identified link between SARS-CoV-2 and altered autoantibody profiles suggests that similar to other viruses,[11-13] SARS-CoV-2 infection may result in a life-threatening disease with loss of self-tolerance.[7,8] A select group of autoantibodies observed in classical autoimmune diseases has been associated with COVID-19 severity, such as antinuclear antibodies (ANAs[14]) and antibodies

targeting ribosomal P proteins, chromatin proteins, and thyroid antigens.[3] Furthermore, high levels of antiphospholipid autoantibodies were linked to severe COVID-19, inducing neutrophil extracellular traps and venous thrombosis.[15] However, the full spectrum of autoantibodies observed in COVID-19 patients has not been fully characterized.

Thus, to better characterize the immunopathology of COVID-19, we investigated a broad-ranging spectrum of autoantibodies linked to autoimmune diseases induced by SARS-CoV-2 infection and their association with COVID-19 severity using a systems immunology approach.

2 | METHODS

2.1 | Study participants

A total of 231 adults from 5 states of the United States of America were enrolled in this cross-sectional study. Of these, 161 patients had COVID-19 symptoms (Supporting Information: Table 1) and a positive SARS-CoV-2 test by nasopharyngeal swab and polymerase chain reaction before receiving any SARS-CoV-2 vaccine. These patients participated in an online survey to determine the most common symptoms and outcomes of SARS-CoV-2 infection.[17,18] The SARS-CoV-2 positive cohort was classified as mild COVID-19 ($n = 72$; fever duration ≤ 1 day; peak temperature of 37.8°C), moderate COVID-19 ($n = 61$; fever duration ≥ 7 days; peak fever of ≥38.8°C), and severe COVID-19 groups ($n = 28$; severe symptoms and requiring supplemental oxygen therapy) according to the World Health Organization (WHO) severity classification.[19] Details of the survey study, symptoms, time since symptoms onset, and patient demographics were previously described[17,18] and can be found in Supporting Information: Table 1. We also included 77 randomly selected age- and sex-matched healthy controls who were SARS-CoV-2 negative and who neither presented any COVID-19 symptoms nor received the anti-SARS-CoV-2 vaccine. All healthy controls and all patients provided informed written consent to participate in the study, performed in accordance with the Declaration of Helsinki. The study was approved by the IntegReview institutional review board (Coronavirus Antibody Prevalence Study, CAPS-613). In addition, this study followed the reporting guidelines of Strengthening the Reporting of Observational Studies in Epidemiology (STROBE).

2.2 | Measurements of anti-SARS-CoV-2 antibody and autoantibodies linked to diverse autoimmune diseases

All collected sera were tested for immunoglobulin b(Ig)G anti-SARS-CoV-2 antibody (to spike and nucleoprotein combined) using the ZEUS SARS-CoV-2 ELISA Test System according to the manufacturer's instructions (ZEUS Scientific). Serum IgG ANA, extractable nuclear antigen, double-stranded DNA (dsDNA), actin, mitochondrial M2, and rheumatoid factor (RF) were measured using commercial ELISA kits obtained from INOVA Diagnostics. In a blinded fashion, IgG, and IgA antibodies against 52 different autoantigens were also measured by an in-house ELISA procedure at Immunosciences Lab., Inc. We assessed IgG autoantibodies because they are frequently involved in autoimmune diseases. In contrast, IgA autoantibodies were measured due to their involvement in mucosal damage during autoimmune and inflammatory diseases.[20] Furthermore, IgG and IgA autoantibodies commonly coexist during autoimmune diseases.[21] See Supporting Information: Table 2 for all autoantigens targeted by the autoantibodies. Details of the ELISA method used to investigate autoantibody and all R packages and bioinformatic tools used to analyze the data (as described below) obtained can be found in Supporting Information Methods.

2.3 | Differences in autoantibody levels

Box plots showing differences in autoantibodies from healthy controls and COVID-19 patient groups (mild, moderate, and severe) were generated using the R version 4.0.3 (The R Project for Statistical Computing. https://www.r-project.org/), RStudio Version 1.4.1106 (R-Studio. https://www.rstudio.com/), and the R packages ggpubr, lemon, and ggplot2. Statistical differences in autoantibody levels were assessed using the Kruskal Wallis test followed by the Dunn test as post hoc adjusted for false discovery rate (FDR). The association between autoantibody levels and age was investigated by spearman's correlation analysis.

2.4 | Autoantibody correlation signatures

Bivariate correlation analyses of autoantibodies for healthy controls and COVID-19 patient groups (mild, moderate, and severe) were performed using the corrgram, inlmisc, and psych R packages. Furthermore, hierarchical clustering heatmaps of autoantibody correlation signatures by Euclidian distance metric of Spearman's rank correlation coefficients were created using Morpheus (https://software.broadinstitute.org/morpheus/).

2.5 | Principal component analysis (PCA)

PCA with spectral decomposition[22,23] was performed to measure the autoantibodies' stratification power in distinguishing between COVID-19 (mild, moderate, and severe patients) and healthy controls. The analysis was performed using the R functions princomp and prcomp, through the factoextra package (PCA in R: prcomp vs princomp).

2.6 | Machine learning modeling and autoantibody ranking

We employed a random forest model to build a classifier to discriminate between controls and mild, moderate, and severe

COVID-19 patients. This approach aimed to identify the most significant classifiers of COVID-19 severity. We trained the random forest model using the functionalities of the R package random Forest (version 4.6.14). Five thousand trees were used, and follow-up analysis was conducted with the Gini decrease, mean minimum depth, and the number of nodes as criteria to determine variable (antibody) importance. The adequacy of the model as a classifier was evaluated through the out-of-bags error rate and the ROC curve. For cross-validation, we split the data set into training and testing sets, using 56% of the observations for training and 44% for testing.

3 | RESULTS

3.1 | Progressive increase of autoantibodies in COVID-19 patients according to disease severity

Here we investigated the serum levels of IgG and IgA autoantibodies targeting 58 and 52 self-antigens, respectively, that are linked to a variety of autoimmune diseases in COVID-19 patients (Figure 1). See Supporting Information: Table 1 for autoantibody levels, Supporting Information: Table 2 for their abbreviations and targets, and Supporting Information: Table 3 for autoantibody and disease association. Multistudy factor analysis (MSFA) (Supporting Information: Figure 1) revealed a progressive dysregulation in the number of latent factors from mild COVID-19 patients to those with moderate and severe disease. The two latter groups presented fewer latent factors when compared with control and mild COVID-19 groups, indicating by this statistical inference approach that the perturbation of normal autoantibody levels mainly occurs in patients with moderated and severe COVID-19.

We found significantly elevated levels of IgG (Supporting Information: Figure 2) and IgA (Supporting Information: Figure 3) autoantibodies targeting a total of 24 and 42 antigens, respectively, when comparing COVID-19 groups (mild, moderate, and severe) versus healthy controls (Figure 2A). Since autoantibodies have also been detected in healthy individuals at physiological levels,[24–28] we determined the significant differences of autoantibody levels across

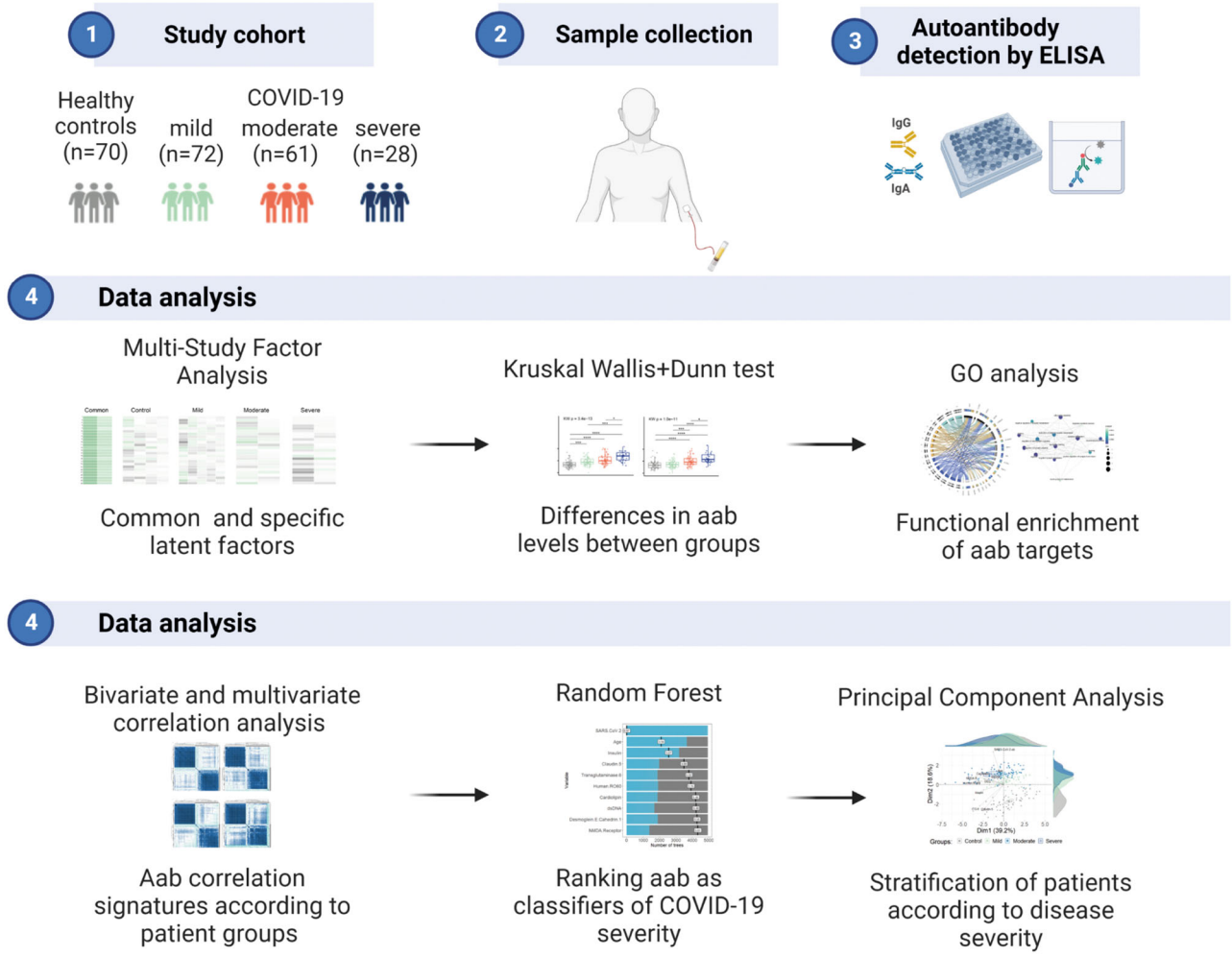

FIGURE 1 Cross-sectional investigation of autoantibody signatures. Schematic overview of study cohorts, statistics, and bioinformatics analyses to characterize the spectrum of autoantibodies induced by SARS-CoV-2 infection and their association with COVID-19 outcomes. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

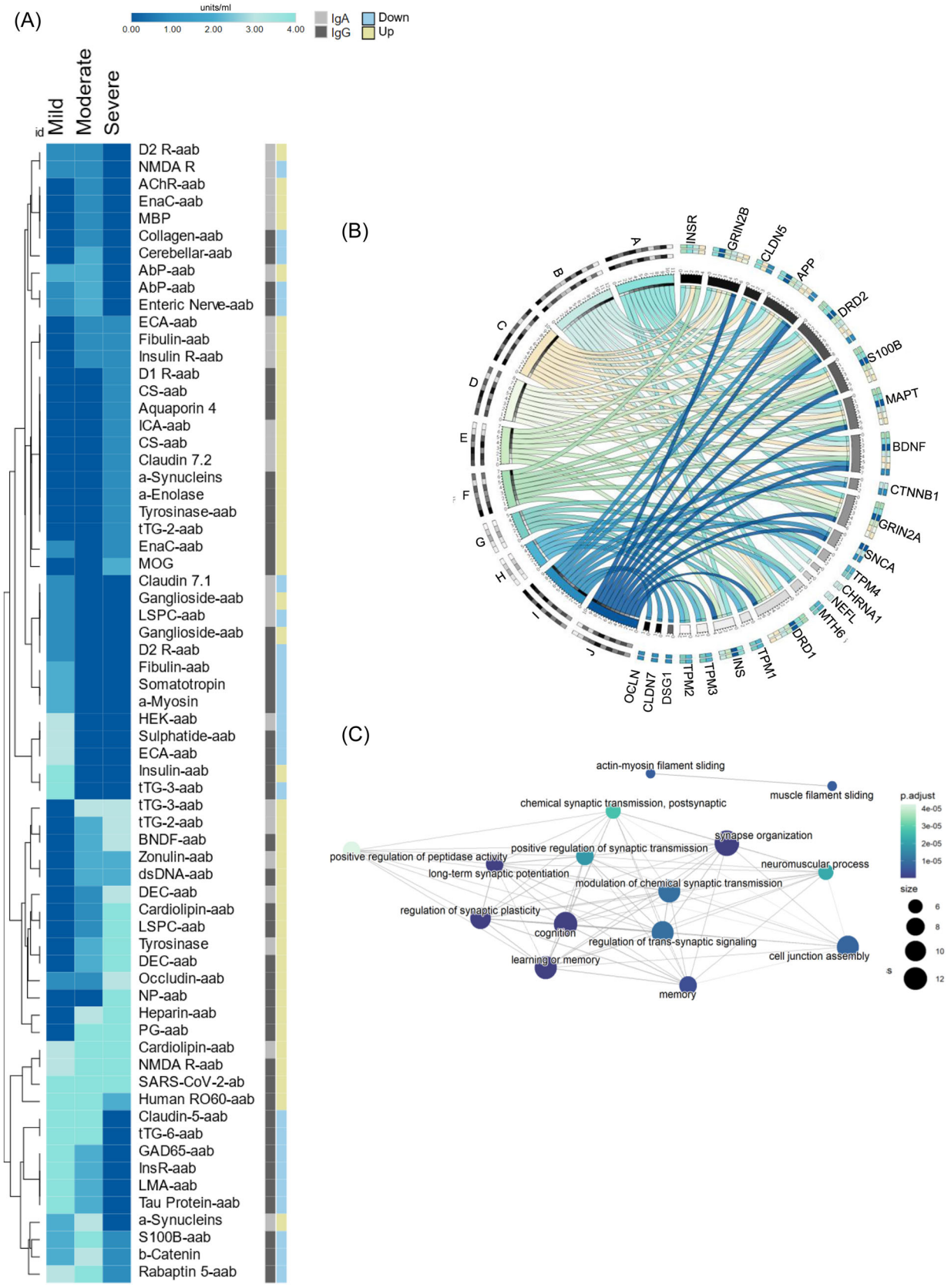

FIGURE 2 (See caption on next page)

the groups using Kruskal–Wallis test followed by Dunn test as post hoc adjusted for FDR, rather than the proportion of patients with autoantibodies versus healthy controls. Figure 2B,C display the functional associations of the autoantibody targets, which are represented by functional enrichment analysis (gene ontology or GO). These results indicate that, among others, COVID-19 patients have dysregulated levels of autoantibodies affecting neurological functions such as memory, learning, and cognition[29–31] (Supporting Information: Table 4).

In general, patients with mild COVID-19 exhibited only slight differences in the serum levels of autoantibodies compared to healthy controls. In contrast, patients with moderate COVID-19 exhibited more evident differences, with the highest autoantibody levels noted in the severe group. In agreement with the results obtained by MSFA, we observed a progressive increase of the autoantibodies targeting autoantigens associated with diverse autoimmune diseases (Supporting Information:Table 3; Supporting Information: Figure 2 and 3; and Figure 3A). In addition, we identified reduced levels of IgG autoantibodies targeting 24 autoantigens, such as those against amyloid-β peptide, cerebellar, claudin-5, collagen, D2 receptor, enteric nerve, epithelial cell antigen, fibulin, GAD65, insulin receptor, liver microsomal antigen, rabaptin-5, S100B, somatotropin, sulphatide, tau protein, transglutaminase 3, transglutaminase 6, α-myosin, and β-catenin as well as 4 IgA autoantibodies targeting claudin 7, human epidermal keratin, lung surfactant protein-C, and NMDA receptor, when comparing COVID-19 groups (mild, moderate, and severe) versus healthy controls. Thus, these data indicate a breakdown of physiological autoantibody levels in patients with COVID-19 that paralleled disease severity. Of note, the progressive perturbation of autoantibody levels was accompanied by increased serum concentration of anti-SARS-CoV-2 antibodies (Supporting Information: Figure 3).

Of note, despite the progressive increase of autoantibodies in COVID-19 patients according to disease severity, regression analysis revealed a reduction in the autoantibody levels according to the time of sample collection after COVID-19 symptom onset (Supporting Information: Figure 05 and 06). This result agrees with the previous observations that the timepoint of sample collection after COVID-19 symptom onset is relevant for interpreting autoantibody titers during acute infection.[3,4] Despite that, since the COVID-19 groups presented a similar average on the sample collection date, we excluded this variable as a potential confounder of patient subgroup comparisons.

3.2 | Autoantibody generation correlates with severe SARS-CoV-2 infection

Based on the concept that signatures of autoantibody correlations are associated with physiological and pathological immune homeostasis,[3,4,6] we investigated the correlation between signatures of the IgA and IgG autoantibodies and disease severity. Bivariate correlation analysis revealed (Figure 3 and Supporting Information: Table 5) that patients with mild COVID-19 exhibited few differences in the autoantibody correlation signatures compared to healthy controls. In contrast, patients with moderate COVID-19 started to show new positive and negative correlations between autoantibodies, while the severe group displayed the most disparate topological correlation pattern, increasingly with preferentially positive correlations between autoantibodies.

Taken together, this suggests the presence of multilayered factors that influence the autoantibody signatures, including disease severity that affect relationships among autoantibodies.

3.3 | Ranking autoantibodies as classifiers of COVID-19 severity

Next, we used random forest modeling[31] to rank the top 10 autoantibody classifiers of COVID-19 severity. Random forest is a machine learning approach widely used[32-39] and a powerful tool to rank the importance of different variables such as autoantibodies[40] across disease cohorts, enhancing our understanding of biological data.[41] That is, Random forest is a robust nonlinear and ensemble-based machine learning model[41] that predicts outcomes based on decision trees[31] and provides feature importance estimate.[42] Due to the assignment of feature importance values (by calculating the Gini importance) and the decision tree structure, this model is used to understand feature contribution to a classification, which is relevant for biological understanding.[41,42] We trained the model using 42 IgG and 24 IgA autoantibodies and 5000 trees for classification. Random forest classification of COVID-19 patients presented a

FIGURE 2 Autoantibodies and functional association of their targets. (A) Heatmap showing the hierarchical cluster of IgG and IgA autoantibodies significantly different when comparing each COVID-19 cohort (mild, moderate, and severe groups) with healthy controls. The bar, ranging from blue to light green, shows the range of significance from -log10 (p-value) = 0–4. Autoantibodies with increased levels (Up) or decreased levels (Down) are represented by yellow and blue colors. Rows were clustered using Euclidean distance between -log10 (p-values). (B) Circos plot illustrating the top 10 biological processes (BP) enriched by both IgA and IgG autoantibody targets. Supporting Information: Table 4 and Supporting Information: Figure 4 show all gene ontology (GO) categories (BPs and signaling pathways) enriched by the autoantibody targets. For this analysis, we selected autoantibody targets from significantly different autoantibodies among the groups. BPs are denoted by letters (A–J) (A: cognition; B: synapse organization; C: learning or memory; D: regulation of synaptic plasticity; E: long-term synaptic potentiation; F: memory; G: muscle filament sliding; H: actin-myosin filament sliding; I: cell junction assembly; J: modulation of chemical synaptic transmission). (C) Network of BPs enriched (adjusted $p < 0.05$) by the autoantibody targets. The edges connect related BPs. The circles' colors and size correspond to the adjusted p-value and the number of autoantibody targets enriching BPs, respectively.

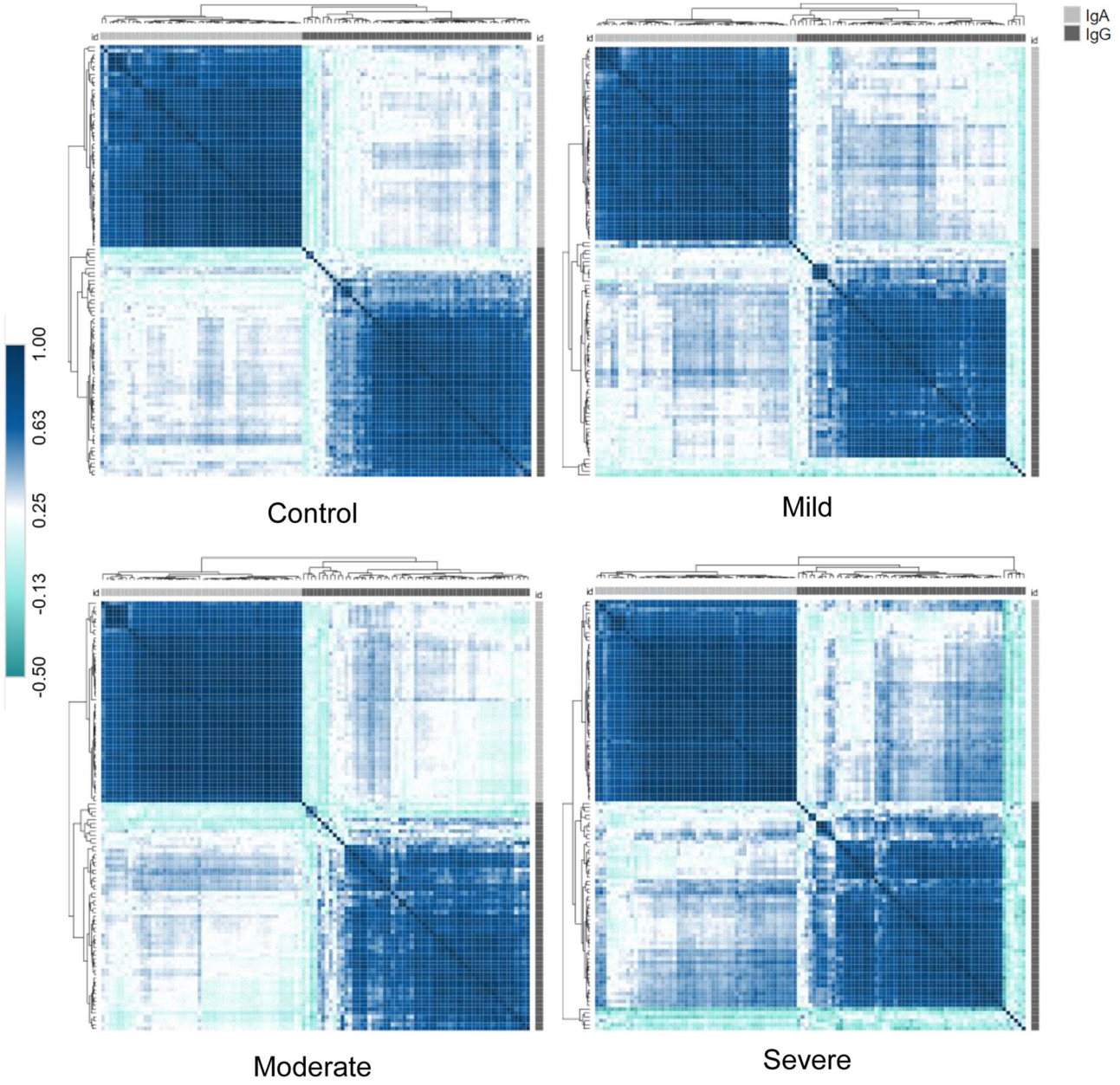

FIGURE 3 Hierarchical clustering of the autoantibody correlation signatures according to disease severity. Heatmap of significant different aab versus aab correlations: IgG and IgA autoantibodies in healthy control and COVID-19 groups. The color scale bar ranging from −0.5 to 1 represents Spearman's rank correlation coefficient. Rows and columns were clustered based on Euclidean distance. See Supporting Information: Table 5 for correlation matrices with the correlation coefficient values. Control (n = 70); mild (n = 72), moderate (n = 61), and severe (n = 28) COVID-19.

stable curve showing the highest error rate (out-of-bag OOB) for the severe COVID-19 group based on IgA or IgG autoantibodies. The OOB estimate of the error rate for all groups was 28.91% for IgA (Figures 4B) and 17.19% for IgG autoantibodies (Figure 4A). Based on these findings, the model was considered adequate to classify the COVID-19 patients by severity according to IgA and IgG autoantibodies, showing areas under the ROC curve of 0.87%, 0.78%, 0.80%, and 0.70% for the healthy control, mild, moderate and severe COVID-19 groups for IgA (Supporting Information: Figure B) and 1.00%, 0.97%, 0.89%, and 0.87% for IgG (Supporting Information: Figure A). In this context, autoantibodies tracked with anti-SARS-CoV-2 antibodies and age as essential classifiers of COVID-19 severity. Among them are IgG autoantibodies targeting insulin, claudin-5, tTG-6, human RO60, cardiolipin, dsDNA, DEC, NMDA R (Figure 4B), as well as IgA autoantibodies targeting cardiolipin, the NMDA R, HEK, AbP, α-synucleins, EnaC, claudin-7, ICA, and heparin (Figure 4D). Thus, IgA and IgG autoantibodies stratify COVID-19 according to disease severity.

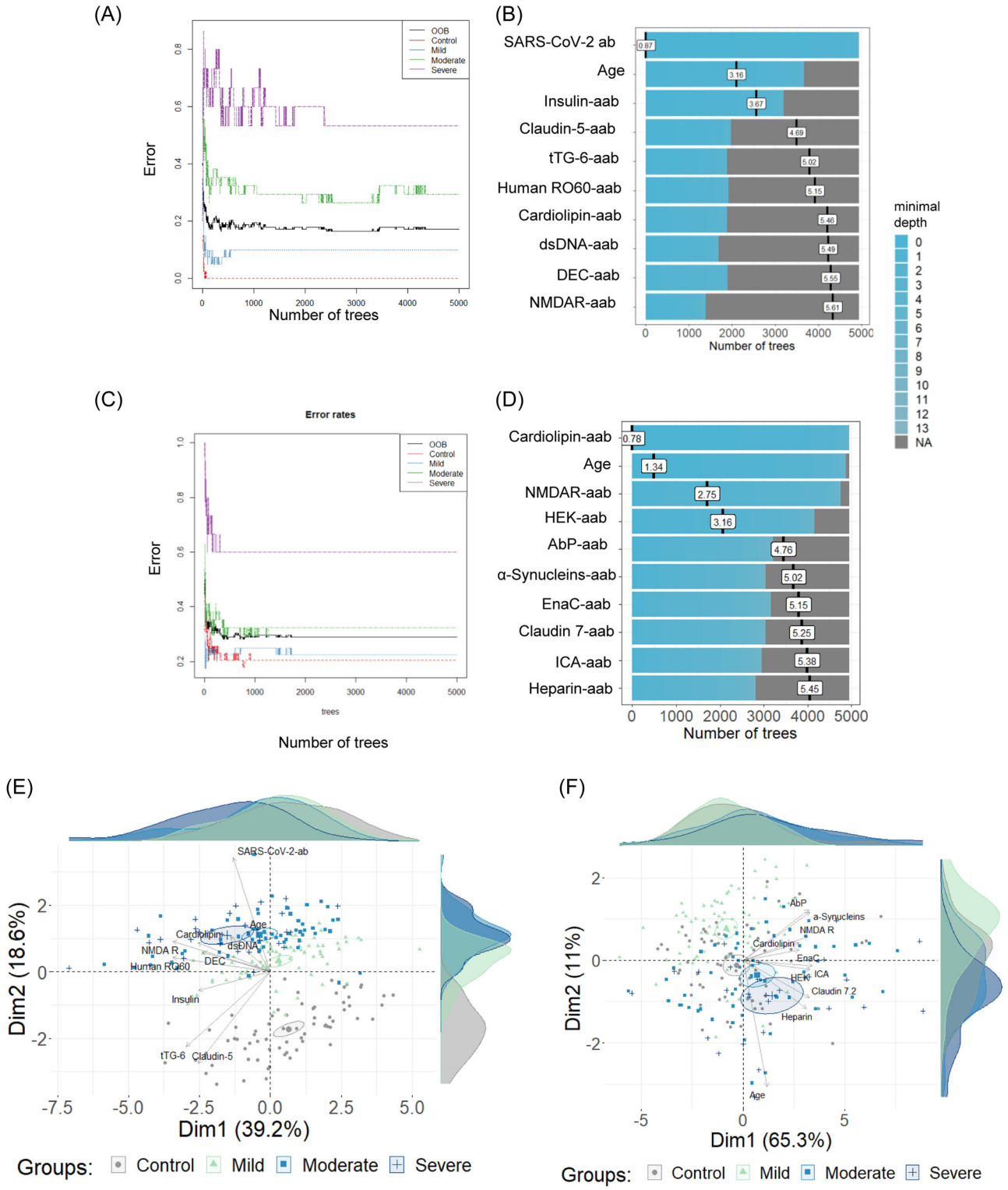

FIGURE 4 Ranking autoantibodies as predictors of COVID-19 severity. Stable curve showing the number of trees and error rates for (A) IgG and (C) IgA autoantibodies with out-of-bag (OOB) estimate of 17.19% and 28.91% error rate, respectively. Bar plots of the top 10 strongest (B) IgG (also anti-SARS-CoV-2 IgG antibodies and age) and (D) IgA autoantibodies classifiers of COVID-19 patients according to disease severity. The variables are shown according to minimal depth and number of trees. The color scale bar ranging from 0 to 13 represents the minimal and maximum minimal depth. The small dark vertical bars represent the mean of minimal depth for each variable. Principal component analysis (PCA) with spectral decomposition shows that (E) IgG and (F) IgA autoantibodies stratify COVID-19 patients according to disease severity, revealing the overlap between the moderate and severe cohorts. The PCA was carried out based on the top 10 most important variables as listed in b and d, respectively. Control ($n = 70$); mild ($n = 72$), moderate ($n = 61$), and severe ($n = 28$) COVID-19. See Supporting Information: Table 2 for full names and abbreviations of autoantibodies and their targets.

Furthermore, we performed PCA with a spectral decomposition approach[40,41] to analyze whether the top 10 ranked variables (IgA and IgG autoantibodies and age) as important classifiers of COVID-19 severity have stratification power. Following the random forest modeling, PCA showed the stratification of COVID-19 patients according to disease severity. (Figures 4E,F). While patients with mild COVID-19 and healthy controls presented with similar autoantibody patterns, moderate and severe COVID-19 groups clustered closely. Together, these results indicate that autoantibodies stratify COVID-19 according to disease severity.

3.4 | Elderly COVID-19 patients present higher autoantibody levels compared with younger individuals

Considering age as a significant predictor of severe outcomes in COVID-19, which was also indicated by Random forest,[44] we investigated the effect of age on the top IgG and IgA autoantibodies stratifying COVID-19 patients according to disease severity. We classified control and disease groups by age (young < 50 and elderly ≥ 50). We found that mainly severely affected elderly COVID-19 patients show an overall tendency to have elevated IgG autoantibody levels compared with younger patients. This age effect on autoantibodies was only observed for a few IgA autoantibodies (Figure 5A; Supporting Information: Figure A and 8b; Supporting Information: Table 6). We observed a continuous effect of age on IgG autoantibody levels, primarily in severe COVID-19 patients. Of note, spearman's correlation analysis indicated a frequent significant positive association between the levels of several autoantibodies and anti-SARS-CoV-2 antibodies with age. (Figures 5B,C; Supporting Information: Fig. A and 8B. Considering the relevance of these findings, we further explored the age effect on autoantibody levels in a follow-up study,[45] which will be published in detail elsewhere.

Furthermore, we asked whether sex also impacts the levels of autoantibodies during SARS-Co-2 infections. IgG anti-cardiolipin-aab was elevated in males from all three disease groups (but only significantly in mild and moderate COVID-19 cohorts) compared to females. Except for this, there were no significant sex differences in the top 10 IgG and IgA autoantibody classifiers of COVID-19 severity (Supporting Information: Figure 9).

4 | DISCUSSION

Evidence has been provided for strong, chronic inflammation promoting the release of self-antigens, high cytokine levels activating bystander T-cells,[46] and molecular mimicry[47–49] during severe COVID-19. This latter hypothesis has been explored by assessing the SARS-CoV-2 proteome, which revealed the existence of 21 viral peptides that show at least 90% homology with human proteins known to be involved in autoimmune diseases such as multiple sclerosis and rheumatoid arthritis.[47] These observations may explain, at least in part, why multiple serum autoantibodies linked to autoimmune diseases are dysregulated in COVID-19 patients. Our findings expand the number of autoantibodies that correlate with the severity of SARS-CoV-2 infection (Supporting Information: Table 2). Among them are those targeting molecules involved in neurological functions (e.g., cognition, learning, memory, and synaptic transmission) and glucose metabolism. That is, we described autoantibodies not yet reported in COVID-19 patients, such as those targeting neuronal antigens (e.g., α-synuclein, acetylcholine receptor, myeloid-β peptide, β-catenin, brain-derived neurotrophic factor, cerebellar antigen, chondroitin sulphate, dopamine receptors [D1 and D2], enteric nerve, ganglioside, glutamic acid decarboxylase, myelin basic protein, myelin oligodendrocyte glycoprotein, neurofilament proteins, NMDA receptor, rabaptin-5, somatotropin, S100B, tau protein, and transglutaminases 6) and non-neuronal antigens (e.g., α-enolase, α-myosin, claudin-5 and -7, collagen, desmoglein-E-cadherin 1, epithelial sodium channel α, fibulin, fibrinogen, human epidermal keratin, insulin receptor, islet cell antigen, occluding, platelet glycoprotein, transglutaminases 2 and 3, tyrosinase, and zonulin). Besides expanding the spectrum of autoantibodies previously associated (e.g., cardiolipin,[50] dsDNA,[50] epithelial cell antigen,[51] heparin,[52] human RO60,[50] liver microsomal antigen,[3] and lung surfactant protein C)[53] with COVID-19 our data also indicate that disease severity disrupts the physiological IgG and IgA autoantibody signatures. Our findings expand the number of autoantibodies that qualify as biomarkers for the severity of SARS-CoV-2 infection and are consistent with the notion that autoantibodies are natural components of human physiology and become dysregulated under pathological conditions.[24,26,33,54] Furthermore, while anti-cardiolipin antibodies were previously associated with the development of hyperinflammatory syndromes,[55] the high levels of autoantibodies targeting neuronal-associated molecules could explain why the respiratory symptoms of COVID-19 patients are often accompanied by short- and long-term neuropsychiatric symptoms and brain sequelae.[31] However, this possibility requires future investigation. It has been shown that SARS-CoV-2 can enter the brain by crossing the blood-brain barrier (BBB) because inflammatory cytokines induce BBB instability.[31,56,57] Our data indicate that the array of autoantibodies targeting neuronal molecules is an additional molecular layer that could contribute to the neurological manifestations[29,30] occurring in COVID-19 patients.

While we found no age effect on the levels of anti-SARS-CoV-2 antibodies, severe SARS-CoV-2 infection induces higher autoantibody titers in elderly patients compared with young patients. Thus, our data point to novel mechanisms involved in the risk intersection of immunosenescence and COVID-19,[44] suggesting that the SARS-CoV-2 infection increases the production of a broad spectrum of autoantibodies linked to autoimmune diseases, particularly in elderly patients. In this context, several age-associated factors, such as chronic inflammation in aging ("inflammageing"[58]), might promote the production of autoantibodies and the tendency to naturally progress to immune dysregulation of innate[59] and adaptive[60] immune cells. Therefore, our data support the idea that the induction of high levels of serum autoantibodies in elderly COVID-19 patients contributes to

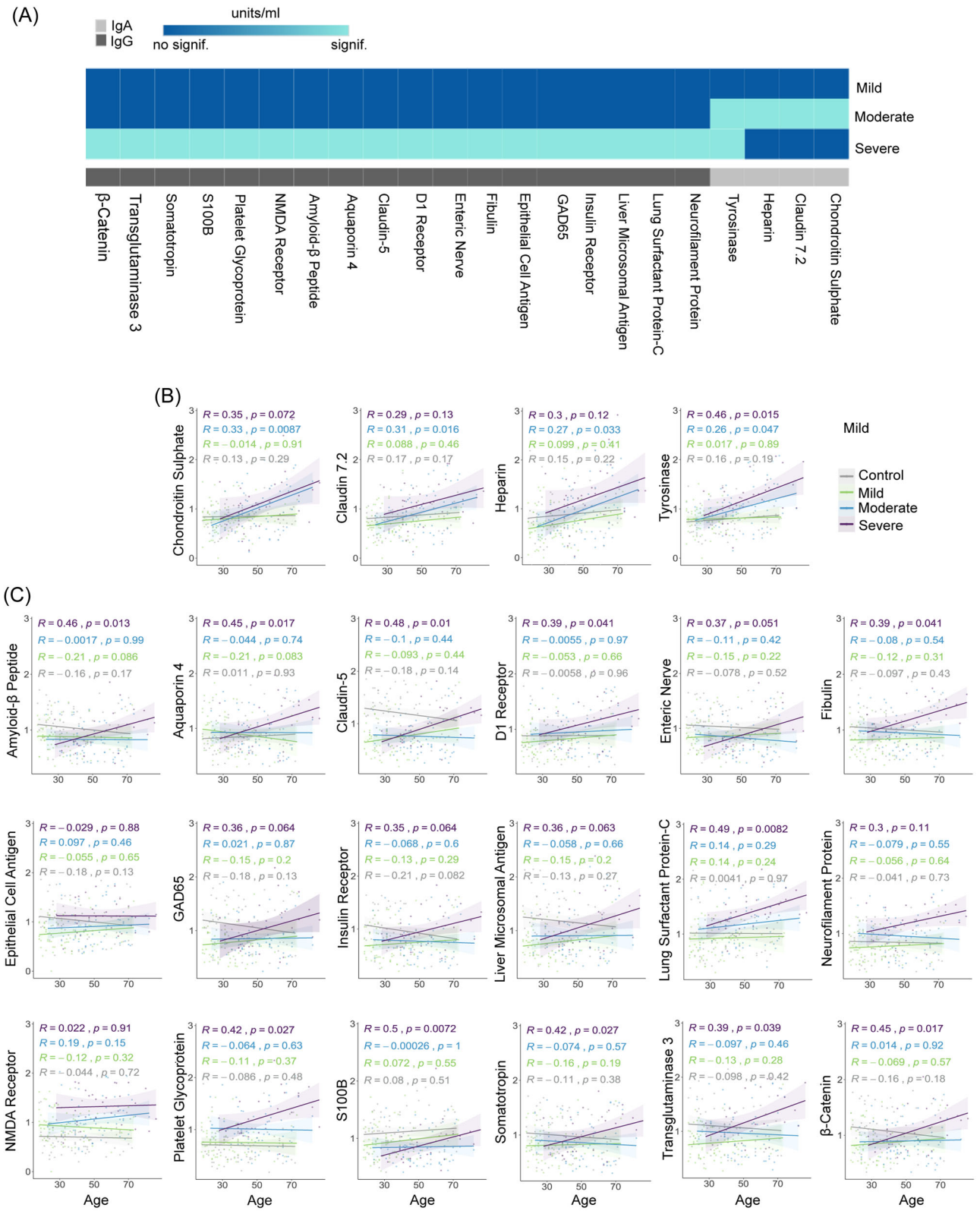

FIGURE 5 Age-dependent elevation of autoantibody levels in patients with severe SARS-CoV-2 infection. (A) Heatmap showing the hierarchical cluster of IgG and IgA autoantibodies significantly different when comparing young versus elderly controls or young versus elderly of each COVID-19 cohort. The colors blue and light green indicate autoantibodies with or without significant differences between the young versus elderly in each group (threshold for significance = $p < 0.05$). All autoantibodies with significant differences are shown in Supporting Information: Figure 8. Linear regression comparisons of (B) IgA and (C) IgG autoantibody levels by age. Regression lines, Spearman's rank correlation coefficient, and p-values are represented as gray (control), green (mild), light blue (moderate), and dark blue (severe COVID-19 patients). The shadow areas around the regression lines represent the 95% confidence interval.

the increased risk for adverse outcomes in older persons.[61] We further investigated this preliminary analysis of the effect of age on autoantibodies from COVID-19 patients in a new manuscript, which will be published elsewhere. Of note, the age impact on autoantibody levels was mainly on IgG but not IgA autoantibodies. Other differences between IgG and IgA autoantibodies are their correlation pattern and classification power of COVID-19 severity, as shown by Random Forest results. The reason for these differences between IgG and IgA autoantibody during SARS-CoV-2 infection remains unknown, requiring future investigation.

Noteworthy, autoantibodies are present in healthy individuals at physiologic levels,[23-26,32,53] which are conserved among species,[25] form network signatures,[23] and may represent a unique opportunity to expand our view of the immune system. Thus, high and low autoantibody levels may play an essential role in pathophysiological conditions, as we have previously described.[23] However, the physiologic function of autoantibodies remains poorly investigated. Serum autoantibodyome analysis revealed that healthy individuals share common autoantibodies.[61] Furthermore, autoantibodies are protective against developing type 1 diabetes and psoriasis.[23] Of note, a limitation of our study is that we did not address how some of the autoantibodies measured in our study could contribute to pathophysiologic functions. For instance, it has been shown that autoantibodies play homeostatic roles, binding cellular antigens and helping the clearance of apoptotic cells.[62] Our group also recently reported that overexpression of extracellular receptors increases the production of autoantibodies with chemotactic properties.[23] Therefore, we hypothesize that the expression of cellular receptors can physiologically modulate the levels of autoantibodies, representing a possible mechanism by which SARS-CoV-2 infection induces autoantibody signatures associated with COVID-19 severity.

Other limitations of our work that need consideration. For instance, our study did not have longitudinal data to analyze the kinetics of the IgG and IgA autoantibodies from disease onset to convalescence or post-acute COVID-19 syndrome. Our study cohort did not include asymptomatic patients. Moreover, we cannot exclude the possibility that some of our patients had high levels of autoantibodies before SARS-CoV-2 infection or that autoantibodies to some antigens (e.g., heparin) could have been induced by anticoagulant therapy with heparin. In addition, we did not assess alterations in the number of circulating immune cells, such as B lymphocytes, and their association with the serum levels of autoantibodies. In addition, future studies are required to clarify the role of viruses, the influence of different viral subtypes, host genetics, and the potential effects of vaccination on the production of autoantibodies. On the other hand, our work raises new questions, such as whether the dysregulated levels of autoantibodies remain after COVID-19 remission and whether these levels remain elevated in patients with the post-COVID-19 syndrome. Likewise, since similar autoantibodies have been noticed in previous studies, it will be essential to validate our findings in additional cohorts to provide a comprehensive view of the pattern of autoantibodies linked to autoimmune diseases in COVID-19 patients.

In conclusion, this work provides a comprehensive view of the spectrum of autoantibodies induced by SARS-CoV-2 infection, demonstrating the dysregulation of new autoantibodies triggered during SARS-CoV-2 infection. That is, this study identified several previously unreported autoantibodies induced by SARS-CoV-2 infection that can potentially classify COVID-19 patients according to the disease severity. Hence, the data presented here expand the critical intersection of COVID-19 and autoimmunity[64,65] while pointing to new potential therapeutical targets.

AUTHOR CONTRIBUTIONS

Yehuda Shoenfeld, Avi Z. Rosenberg, Israel Zyskind, Aristo Vojdani, and Elroy Vojdani conceived the project; Yehuda Shoenfeld, Avi Z. Rosenberg, Israel Zyskind, and Gilad Halpert designed the study. Jonathan I. Silverberg, Avi Z. Rosenberg, Israel Zyskind, and Miriam T. Lattin diagnosed, recruited, or followed up the patients. Aristo Vojdani and Elroy Vojdani performed the experiments. Avi Z. Rosenberg, Dana A. Hill, Amanda Thornton, Gilad Halpert, Jason Zimmerman, Jonathan I. Silverberg, Avi Z. Rosenberg, Israel Zyskind, Miriam T. Lattin, Elroy Vojdani, Yael Bublil Lavi, Howard Amital, and Yehuda Shoenfeld coordinated the serum collection and databank. Gabriela C. Baiocchi, Alexandre H. C. Marques, Igor S. Filgueiras, Dennyson L. M. Fonseca, Desireé Rodrigues Plaça, Paula P. Freire, Myungjin Kim, Roberta De Vito, and Otavio Cabral-Marques performed data and bioinformatics analyses. Gabriela C. Baiocchi, Aristo Vojdani, and Otavio Cabral-Marques wrote the manuscript. Gabriela C. Baiocchi, Jonathan I. Silverberg, Harald Heidecke, Yuri Ostrinski, Avi Z. Rosenberg, Vera L. G. Calich, Niels O. S. Camara, Roberta De Vito, Israel Zyskind, Aristo Vojdani, Lena F. Schimke, Lasse M. Giil, Hans D. Ochs, and Otavio Cabral-Marques edited the manuscript; Gabriela C. Baiocchi, Aristo Vojdani, Avi Z. Rosenberg, Yuri Ostrinski, Lena F. Schimke, Israel Zyskind, Alexandre H. C. Marques, Elroy Vojdani, Dana A. Hill, Amanda Thornton, Lasse M. Giil, Yael Bublil Lavi, Jonathan I. Silverberg, Jason Zimmerman, Niels O. S. Camara, Vera L. G. Calich, Harald Heidecke, Gabriela Riemekasten, Howard Amital, Otavio Cabral-Marques, and Yehuda Shoenfeld, provided scientific insights.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Supporting Information Material of this article.

All data present in this manuscript are provided as Supporting Information Files, and all R packages used are available at the following link: https://github.com/gabrielacbaiocchi/Autoantibodies-linked-to-autoimmune-diseases-associate-with-COVID-19-outcomes.git.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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