Severe COVID-19 is associated with elevated serum IgA and antiphospholipid IgA-antibodies

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Summary:

Our retrospective cohort study in Liechtenstein and Switzerland found that severe COVID-19 is significantly associated with elevated total IgA and IgA antiphospholipid antibodies. These data suggest that a vigorous IgA response to SARS-CoV-2 may trigger autoimmunity with systemic symptoms.

ABSTRACT

Background: Severe coronavirus disease 2019 (COVID-19) frequently entails complications that bear similarities to autoimmune diseases. To date, there is little data on possible IgA-mediated autoimmune responses. Here, we aim to determine whether COVID-19 is associated with a vigorous total IgA response and if IgA antibodies are associated with complications of severe illness. Since thrombotic events are frequent in severe COVID-19 and resemble hypercoagulation of antiphospholipid syndrome (APS), our approach focused on antiphospholipid antibodies (aPL).

Methods: In this retrospective cohort study clinical data and aPL from 64 patients with COVID-19 were compared from three independent tertiary hospitals (one in Liechtenstein, two in Switzerland). Samples were collected from April 9th to May 1st, 2020.

Results: Clinical records of 64 patients with COVID-19 were reviewed and divided into a cohort with mild illness (mCOVID) (41%), a discovery cohort with severe illness (sdCOVID) (22%) and a confirmation cohort with severe illness (scCOVID) (38%). Total IgA, IgG and aPL were measured with clinical diagnostic kits. Severe illness was significantly associated with increased total IgA (sdCOVID, P=0.01; scCOVID, p-value<0.001), but not total IgG. Among aPL, both cohorts with severe illness significantly correlated with elevated anti-Cardiolipin IgA (sdCOVID and scCOVID, p-value<0.001), anti-Cardiolipin IgM (sdCOVID, P=0.003; scCOVID, P<0.001), and anti-Beta2 Glycoprotein-1 IgA (sdCOVID and scCOVID, P<0.001). Systemic lupus erythematosus was excluded from all patients as a potential confounder.

Conclusions: Higher total IgA and IgA-aPL were consistently associated with severe illness. These novel data strongly suggest that a vigorous antiviral IgA-response, possibly triggered in the bronchial mucosa, induces systemic autoimmunity.

Key words: COVID-19; immunoglobulin A; autoimmunity; antiphospholipid syndrome; thromboembolisms

INTRODUCTION

The novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a global pandemic with wide-ranging health and socio-economic implications. After SARS and the Middle East Respiratory Syndrome, it represents the third known spillover of a severe coronavirus-associated disease from animals to humans in the last twenty years [1-3]. SARS-CoV-2 enters human cells by attachment to and subsequent internalization of Angiotensin-converting enzyme 2 receptors that are highly expressed by type-II pneumocytes in the deep bronchial system [4], where IgA immunoglobulins produced in the bronchial-associated lymphoid tissue (BALT) are the main line of humoral defense [5]. Indeed, specific IgA against the SARS-CoV-2 spike protein have been shown to appear early in infected patients [6]. However, despite the important role of IgA in mucosal immunity, the rate of total IgA generated by that response and its role in COVID-19 severity remains unexplored.

Autopsies have shown acute respiratory distress syndrome (ARDS) and sepsis to be the most common complications in critically-ill COVID-19 patients [7]. A large case series from Northern Italy that assessed lung histologies of deceased COVID-19 patients present consistent diffuse alveolar damage and necrosis of pneumocytes [8]. A distinctive factor for COVID-19 was a marked presence of diffuse thrombosis of the peripheral small vessels. This is in line with reports of frequent thromboembolisms of patients with severe COVID-19 that occur despite the prophylactic in-hospital use of low weight molecular heparins [9, 10]. While the reasons remain unclear, a recent report describes a case series of severe COVID-19 patients with stroke and elevated levels of antiphospholipid (aPL) antibodies compatible with antiphospholipid syndrome (APS) [11]. APS is an acquired

autoimmune disease that is mediated by autoantibodies directed against phospholipid-binding proteins that leads to hypercoagulability. The most common trigger factor of APS is systemic lupus erythematosus (SLE), followed by lung infections with mycobacteria or viruses [12]. These cases suggest that COVID-19 leads to virally triggered APS in severe COVID-19 patients. To our knowledge, complete aPL profiling in mild and severe cases of COVID-19 has never been undertaken. The aim of this study was to explore, whether patients with severe COVID-19 have elevated total IgA as an immediate immune response and aPL compatible with APS.

METHODS

Sources and Ethical Statement:

The multicenter cohort study was conducted at the Landesspital Liechtenstein (LLS) in Vaduz, Liechtenstein, the Kantonsspital St. Gallen (KSSG) in St. Gallen, Switzerland, and the University Hospital Zurich (USZ) in Zurich, Switzerland, in accordance with the Declaration of Helsinki guidelines. The collection of patient data and blood samples was approved by the respective local ethics committees of the participating study centers (Project-IDs 2020-00676, 2020-00821, and 2020-00646). All participants agreed to the hospitals' general consent policies allowing further use of clinical data and biologic material (LLS and KSSG) or signed an informed consent (USZ). Where signing of informed consent was not possible due to severe illness and to prevent surface contamination, consent was sought verbally or from the next of kin, which had been approved by the respective local ethics committee.

Patient data and sample collection:

Collection of patient data and samples (serum or plasma) was conducted from April 9, 2020, to May 1, 2020. All samples were obtained within two weeks of symptom onset. Patients with SARS-CoV-2 infection were included that had been confirmed by either real-time reverse transcriptase-polymerase chain reaction (RT-PCR) of nasopharyngeal swab samples or serology. The methods of RT-PCR used to detect SARS-CoV-2 depended on the study center and were performed as previously described [13, 14]. For confirmation with serology SARS-CoV-2 antibodies were analyzed by two independent antibody tests: a lateral flow immunochromatographic assay (LFIA, gold nanoparticle-based, SGIT flex Covid 19) (Sugentech, Daejeon, South Korea) and an electro-chemiluminescence immunoassay (ECLIA, Elecsys Anti-SARS-CoV-2) (Roche International Diagnostics AG, Rotkreuz, Switzerland). Seropositivity was defined as a positive IgM signal in the LFIA in the acute phase that was confirmed by IgG in the ECLIA after 3-4 weeks.

Patients were categorized into the following cohorts: 1. mild illness without requirement of hospitalization (mCOVID) which were collected at the LLS, 2. a discovery cohort of patients with severe illness (sdCOVID) obtained at the USZ, and 3. an independent confirmation cohort of patients with severe illness (scCOVID) from the KSSG. Mild illness was defined as uncomplicated upper respiratory tract infection with unspecific symptoms or uncomplicated pneumonia, while severe illness for the sdCOVID and scCOVID cohorts required hospitalization and included severe pneumonia, ARDS, and septic shock [15].

Immunoglobulins and aPL testing:

aPL antibodies were determined by fluorescence enzyme immunoassay on a Phadia 250 analyzer (Thermo Fisher Diagnostics AG, Steinhausen, Switzerland) using EliATM Cardiolipin as well as EliATM Beta 2-Glycoprotein 1 assays for IgG, IgA, and IgM isotypes (all Thermo Fisher Diagnostics AG, Steinhausen, Switzerland). Total IgA and IgG were

determined on a Cobas c501 analyzer (Roche International Diagnostics AG, Rotkreuz, Switzerland) with a nephelometric assay (IgA-2, Tina-quant IgA Gen.2, and IgGu2, Tina-quant IgG Gen.2) (Roche International Diagnostics AG, Rotkreuz, Switzerland). IgG against SS-A/Ro, SS-B/La, dsDNA, Sm, chromatin and RNP were simultaneously determined by bead-based suspension array principle on a Bioplex 2200 System (Biorad Laboratories, Cressier, Switzerland). Coefficients of variations, as determined by commercially available control materials were 4.5% for total IgA, <2.0% for total IgG, 4.6% for aPL antibodies, and 3.7% for lupus-antibodies. Measurements were performed at the Labormedizinisches Zentrum Dr. Risch, Vaduz, Liechtenstein.

Statistical methods:

Point estimates of autoantibody levels were described using the mean and the standard error of the mean. Differences in autoantibody levels between cohorts were assessed using the nonparametric Mann-Whitney test. All *P*-values were adjusted for multiple hypothesis testing using the false discovery rate (FDR) method. Statistical significance was defined at the level of FDR <0.05. All analyses were performed using R software, version 3.5.0 (R Project for Statistical Computing, Vienna, Austria). Furthermore, we generated hierarchical clustering (spearman clustering distance and a complete clustering method) on the median centered values of antibody levels. Missing values were replaced with the respective mean value across the remaining patients.

RESULTS

Patient characteristics:

We collected a total of 64 serum or plasma samples from patients with SARS-CoV-2 infection (one sample per patient). The median age of all patients was 62 years (interquartile range [IQR], 46-74) and 32 (60%) were male. The mCOVID cohort comprised 26 (41%) patients with a median age of 57 (IQR, 45-63) years. 9 (35%) of mCOVID patients were male. 38 (59%) patients fulfilled the criteria for severe COVID-19. The median age of all severely ill patients was 70 (IQR, 58-76) years and, as opposed to mCOVID, they were predominantly male (28 [74%]). Of those, the sdCOVID cohort included 14 (22%) patients with a median age of 64 (ICR 56-70) years and the scCOVID cohort 24 (38%) patients with a median age of 73 (64-80) years. For SARS-CoV-2 diagnosis, 59 (92%) of 64 patients were screened by RT-PCR and 7 (11%) patients by serology.

4/26 (15%) patients in the mCOVID cohort had co-morbidities predisposing to severe COVID-19, of which the most frequent was hypertension (3/26 [12%]). In contrast, 26 (93%) severely ill patients had at least one co-morbidity, of which the most common were hypertension (26 [68%]), cardiovascular disease (25 [66%]) and diabetes mellitus (13/38 [34%]). Of note, 13 (34%) of patients with severe COVID-19 developed thromboses during hospitalization, which did not correlate with d-dimer levels (P=0.48, two-sided t-test). Patient characteristics and laboratory values are listed in Table 1.

Antibody results:

Severely ill COVID-19 patients had significantly higher total IgA titers compared to mCOVID patients (sdCOVID, mean 2.94 g/l, SD ±0.46, P=0.01; scCOVID mean 3.04 g/l, SD ±0.19, P<0.001), but not higher total IgG (sdCOVID mean 7.69 g/l, SD ±0.55, P=0.09; scCOVID not measured). They also had significantly higher anti-Cardiolipin IgA (sdCOVID mean 6.38 U/ml, SD ±0.96, P<0.001; scCOVID mean 4.86 U/ml, SD ±0.84, P<0.001), anti-Beta2

Glycoprotein-1 IgA (sdCOVID mean 8.50 U/ml, SD ±3.86, *P*<0.001; scCOVID mean 4.71 U/ml, SD ±2.17, *P*<0.001), and anti-Cardiolipin IgM (sdCOVID mean 4.01 U/ml, SD ±0.88, *P*=0.003; scCOVID mean 10.35 U/ml, SD ±5.48, *P*<0.001), as shown in Figure 1. With two other aPL antibodies we found a significant difference only in the sdCOVID but not the scCOVID cohort: anti-Cardiolipin IgG (sdCOVID mean 8.23 U/ml, SD ±4.02, *P*=0.02; scCOVID mean 2.42, SD ±0.54, *P*=0.09) and anti-Beta2 Glycoprotein-1 IgG (sdCOVID mean 1.57 U/ml, SD ±0.23, *P*=0.002; scCOVID mean 1.58 U/ml, SD ±0.85, *P*=0.15). No significant difference was found among anti-Beta2 Glycoprotein-1 IgM among the cohorts (sdCOVID mean 1.07 U/ml, SD ±0.25, *P*=0.16; scCOVID mean 2.00 U/ml, SD ±0.72, *P*=0.16), as shown in Figure 2. Hierarchical clustering analysis further demonstrates common elevations of antibody titers among patients with mild and severe COVID-19 (see Figure 3). We could not detect a correlation between sex and IgA-aPL (anti-Cardiolipin IgA male mean 4.46 U/ml, SD±3.70, female mean 3.47 U/ml, SD±3.31, *P*=0.26; anti-Beta2 Glycoprotein-1 IgA male mean 3.89 U/ml, SD±8.65, female mean 4.67 U/ml, SD±10.9, *P*=0.76), as shown in Supplementary Figure 1. Also, there was no correlation between age and IgA-aPL (anti-Beta2 Glycoprotein-1 IgA R=0.10, *P*=0.45; anti-Cardiolipin IgA R=0.19, *P*=0.12), presented in Supplementary Figure 2.

Since APS is commonly triggered by SLE serology screening was performed for all patients, as described in the methods section. One patient from the scCOVID cohort had elevated anti-La IgG (7.4 U/ml, normal < 1 U/ml) but no other lupus-specific antibodies. None of the other patients were positive for any SLE-associated antibodies.

DISCUSSION

In this study we measured total IgA and IgG, as well as aPL in COVID-19 patients of similar age and compared the results of mildly ill with severely ill patients from independent tertiary care health care centers. For severely ill patients we established a discovery and

investigation cohort. Our novel finding shows a marked elevation of total IgA that is significantly associated with severe COVID-19, which to our knowledge has not been reported before. There is no significant association with total IgG. These data support our hypothesis, that a strong, IgA-driven immune response possibly emerges from the BALT when SARS-CoV-2 affects the deeper respiratory system [16]. In line with literature, about a third of severely ill patients developed thromboses [10] that did not correlate with d-dimer levels [17, 18]. These thromboses may be explained by an elevation of total IgA and aPL IgA antibodies, which we found to be significantly associated with severe illness. This correlation could neither be seen for total IgG, nor for aPL IgG antibodies. While an association of elevated aPL and severe COVID-19 has been suggested [19], the elevation of total IgA together with IgA-aPL when comparing mild and severe COVID-19 is novel and draws the missing link between immune response and hypercoagulation, as it strongly suggests induction of IgA-dominated APS. APS is most commonly triggered by SLE [12], which was excluded in all patients through serological screening [20]. Another potential trigger of APS is infection, including pneumonia, that leads to autoimmunity via molecular mimicry [21-23]. In the case of COVID-19 such a mechanism may be mediated by pulmonary surfactant, as it is rich in phospholipid-binding proteins [24]. Surfactant is produced by type-II pneumocytes, which express high levels of ACE-2 receptors and are a primary target of SARS-CoV-2 [8, 25]. Pneumocyte necrosis leads to surfactant leakage, exposing phospholipid proteins to the immune system. It is feasible that peptide commonalities between SARS-CoV-2 and surfactant proteins induce APS. Indeed, a high coverage of such peptide commonalities has recently been demonstrated by Kanduc D. et al., who found that almost half of the immunoreactive epitopes on the spike glycoprotein of SARS-CoV-2 share pentapeptides on human surfactant-related proteins [26]. In practice, preliminary results of the COVID-19 therapy (RECOVERY) trial demonstrate a significant benefit of dexamethasone therapy for severely ill patients [27], further suggesting an important role of immune exacerbation in COVID-19 related deaths. The effects of aPL could be further enhanced by toll-like receptor-4, which is upregulated in patients with SARS [28] and has been shown to enhance

hypercoagulation [29]. Importantly, these data support a link between IgA and Kawasaki disease-like multisystem inflammatory syndrome in children (MIS-C). MIS-C is a novel COVID-19 related disease that shows significant overlaps with classical Kawasaki disease (KD) but has a later onset and tends to be more severe [30, 31]. Interestingly, classical KD is most commonly associated with respiratory viruses [32] and rare severe KD has been shown to be associated with organ deposits of IgA-producing plasma cells [33, 34]. This suggests that elevated total IgA may have a causal role in MIS-C.

Study limitations:

Main limitations of the study are its cohort sizes and retrospective design. Furthermore, the study does not include longitudinal measurements of antibodies. Due to an inconsistent availability of data on past history of thrombosis, pro-inflammatory cytokines and clinical outcome, these factors could not be considered. Furthermore, C-reactive protein and d-dimer levels were only available from the severely ill cohorts. Nevertheless, the inclusion of cohorts from independent health care centers, as well as a discovery and a confirmation cohort of patients with severe COVID-19 from different hospitals for additional validation are major strengths of the study.

In conclusion, we present a novel significant association between severe COVID-19, elevated total IgA and IgA-aPL. These findings imply that autoantibodies may hold a causal role in severe COVID-19 and its systemic complications. The data strongly suggest that COVID-19 is a potent inductor of autoimmunity and recommend further studies with larger cohorts, longitudinal sampling and mechanistic exploration.

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Disclosures:

J.P. is founder and shareholder of Apeiron (Vienna, Austria) developing soluble ACE-2 as a COVID-19 therapy. J.P. has no direct conflict of interest related to the paper or data in the paper. All other authors have no conflicts of interest to declare.

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Table 1. Overview of patient characteristics

Patient characteristics	Mild illness (*n*=26)	Severe illness, Discovery cohort (*n*=14)	Severe illness, Confirmation cohort (*n*=24)
Sex - No. (%)			
Male	9 (35)	8 (57)	20 (83)
Female	17 (65)	6 (43)	4 (17)
Age (median, IQR) - yr	57 (45-63)	64 (56-70)	73 (64-80)
Comorbidities[a] - No. (%)			
No	22 (85)	2 (14)	0 (0)
Yes	4 (15)	12 (86)	24 (100)
Hypertension	3 (12)	10 (71)	16 (67)
Cardiovascular disease	0 (0)	6 (43)	19 (79)
Cerebrovascular disease	0 (0)	0 (0)	6 (25)
Diabetes	1 (4)	5 (36)	8 (33)
Liver disease	0 (0)	0 (0)	5 (21)
Cancer	0 (0)	0 (0)	9 (38)
Kidney disease	0 (0)	5 (36)	7 (29)
Thromboembolisms, No. (%)			
Yes	0 (0)	4 (29)	9 (38)
No	26 (100)	10 (71)	15 (63)
Immunosuppression, No. (%)[b]			
Yes	0 (0)	5 (36)	5 (21)
No	26 (100)	9 (64)	19 (79)
Laboratory parameters (median, IQR)			
C-reactive protein (mg/l)[c]	n/a	228 (95-303)	201 (161-262)
Patients contributing to this calculation (n, %)	0 (0)	14 (100)	24 (100)
D-dimers (mg/l)[d]	n/a	1.69 (1.01-2.41)	1.94 (0.99-12.03)
Patients contributing to this calculation (n, %)	0 (0)	10 (71)	21 (88)

[a]Comorbidities only consider diagnoses that are known risk factors for developing severe COVID-19, as listed.
[b]Immunosuppression is defined as systemically administered prednisone ≥7.5mg/day (or equivalent), other systemic immunosuppressive drugs, such as calcineurin inhibitors, and chemotherapy.

[c,d] Maximum values of C-reactive protein and D-dimers during hospitalization. Only patients with values available were included (n provided in consecutive line).
Percentages adding to >100 are due to number rounding.

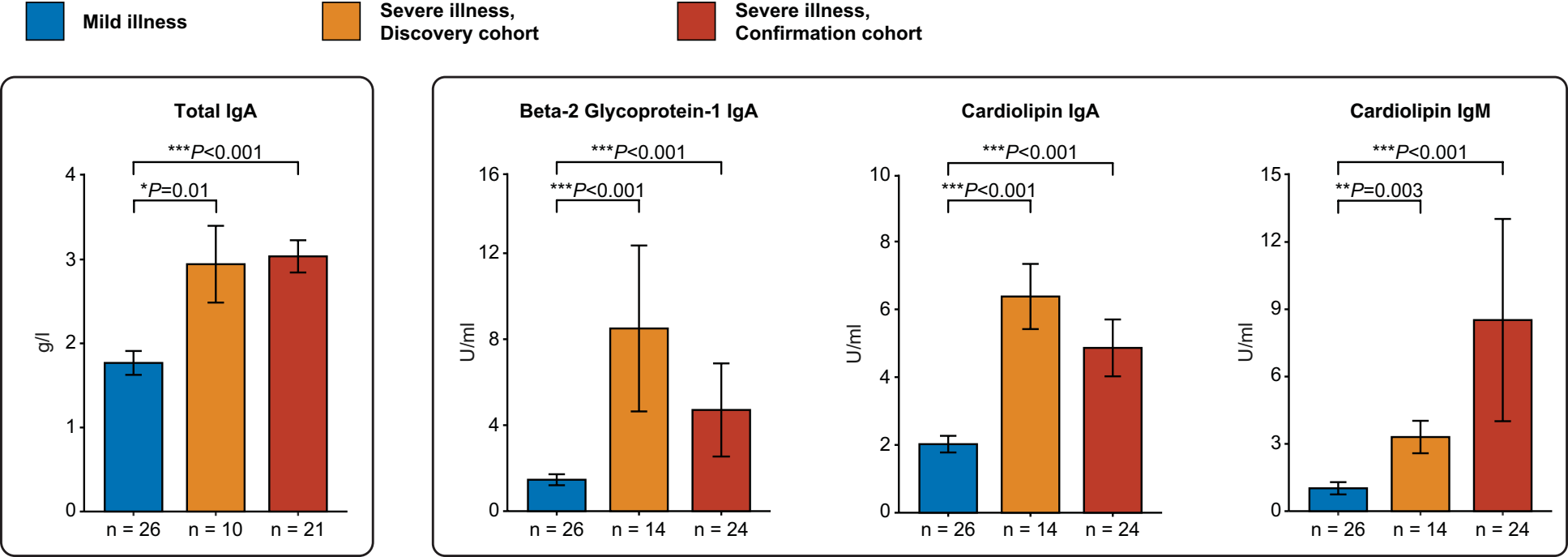
Abbreviations: IQR, interquartile range; n/a, not available; mg/l, milligrams per liter; No., number; yr, years.

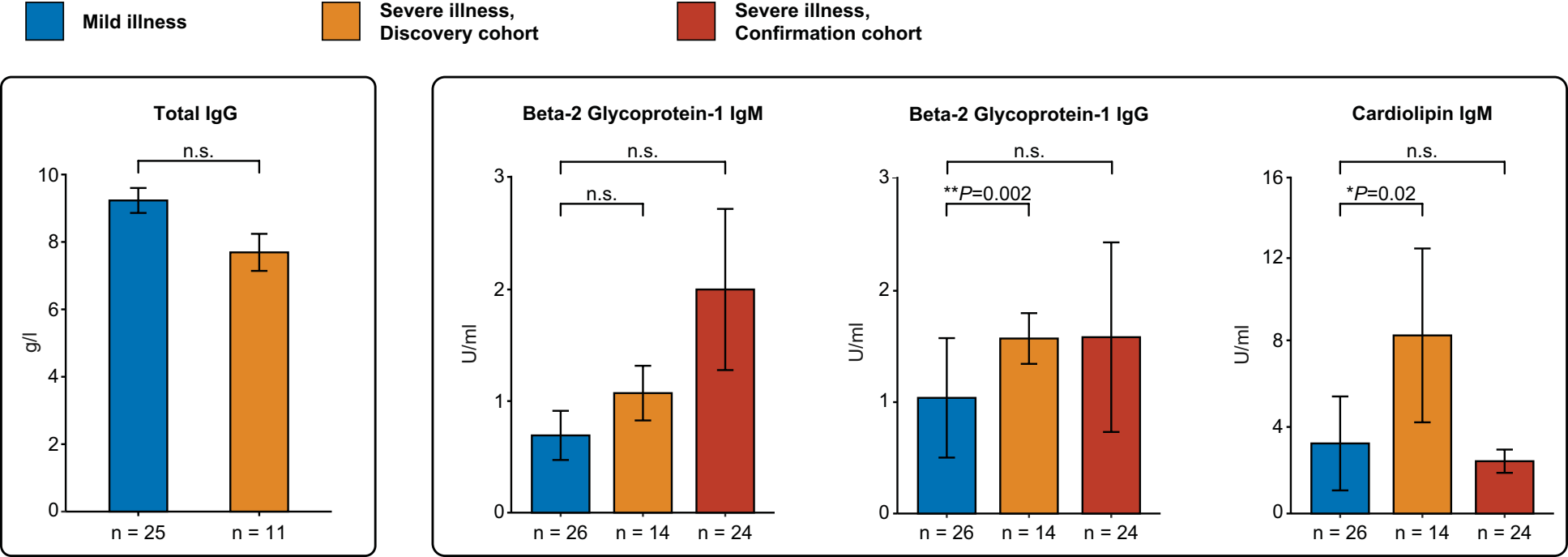
FIGURE LEGENDS

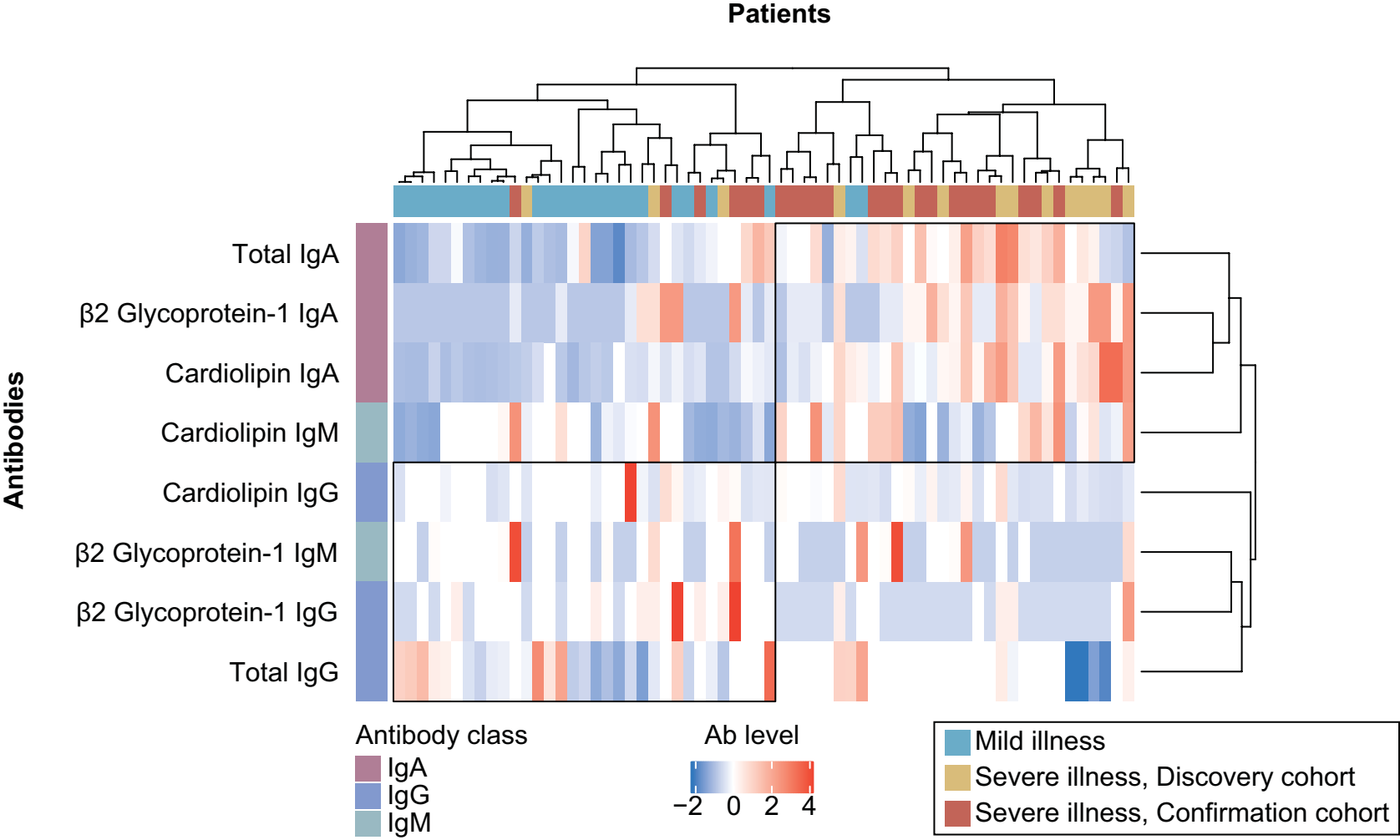
Figure 1. Antibodies significantly associated with severe COVID-19 in both cohorts. Plot titles indicate the respective protein targets and type of immunoglobulin. Y-axes reflect measured results and units. X-axes display patient counts.

Figure 2. Antibodies with partial or no significant association with severe COVID-19. Plot titles indicate the respective protein targets and type of immunoglobulin. Y-axes reflect measured results and units. X-axes display patient counts.

Figure 3. Hierarchical clustering of antibody levels across patients. The heatmap depicts common shifts of antibody levels among patients with mild illness and severe COVID-19. Rows show antibodies and columns patients. All antibody levels are median centered and are displayed as fold relative compared to the median, with higher titers colored red and lower titers blue. For missing results, the mean value of the respective antibody/immunoglobulin was used. The scale bar (bottom center) denotes the x-fold levels.


Mild illness
Severe illness, Discovery cohort
Severe illness, Confirmation cohort
Total IgA
***P<0.001
*P=0.01
g/l
0
1
2
3
4
n = 26
n = 10
n = 21
Beta-2 Glycoprotein-1 IgA
***P<0.001
***P<0.001
U/ml
0
4
8
12
16
n = 26
n = 14
n = 24
Cardiolipin IgA
***P<0.001
***P<0.001
U/ml
0
2
4
6
8
10
n = 26
n = 14
n = 24
Cardiolipin IgM
***P<0.001
**P=0.003
U/ml
0
3
6
9
12
15
n = 26
n = 14
n = 24


Mild illness
Severe illness, Discovery cohort
Severe illness, Confirmation cohort
Total IgG
g/l
n.s.
n = 25
n = 11
Beta-2 Glycoprotein-1 IgM
U/ml
n.s.
n.s.
n = 26
n = 14
n = 24
Beta-2 Glycoprotein-1 IgG
U/ml
n.s.
**P=0.002
n = 26
n = 14
n = 24
Cardiolipin IgM
U/ml
n.s.
*P=0.02
n = 26
n = 14
n = 24


Patients
Antibodies
Total IgA
β2 Glycoprotein-1 IgA
Cardiolipin IgA
Cardiolipin IgM
Cardiolipin IgG
β2 Glycoprotein-1 IgM
β2 Glycoprotein-1 IgG
Total IgG
Antibody class
IgA
IgG
IgM
Ab level
−2 0 2 4
Mild illness
Severe illness, Discovery cohort
Severe illness, Confirmation cohort