CLINICAL SCIENCE

Anticardiolipin and other antiphospholipid antibodies in critically ill COVID-19 positive and negative patients

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ABSTRACT

Background Reports of severe COVID-19 being associated with thrombosis, antiphospholipid antibodies (APLA), and antiphospholipid syndrome have yielded disparate conclusions. Studies comparing patients with COVID-19 with contemporaneous controls of similar severity are lacking.

Methods 22 COVID-19+ and 20 COVID-19– patients with respiratory failure admitted to intensive care were studied longitudinally. Demographic and clinical data were obtained from the day of admission. APLA testing included anticardiolipin (aCL), anti-β2glycoprotein 1 (β2GP1), antiparainuclear antibodies (ANAs) and antibodies to cytokines by a commercially available multiplexed array. Analysis of variance was used for continuous variables and Fisher’s exact test was used for categorical variables with α=0.05 and the false discovery rate at q=0.05.

Results APLAs were predominantly IgG aCL (48%), followed by IgM (21%) in all patients, with a tendency towards higher frequency among the COVID-19+ patients. aCL was not associated with surrogate markers of thrombosis but IgG aCL was strongly associated with worse disease severity and higher ANA titres regardless of COVID-19 status. An association between aCL and anticytokine autoantibodies tended to be higher among the COVID-19+ patients.

Conclusions Positive APLA serology was associated with more severe disease regardless of COVID-19 status.

INTRODUCTION

Antiphospholipid antibodies (APLAs) are biomarkers of a spectrum of clinical features observed in antiphospholipid syndrome (APS). Features of APS include venous and arterial thrombosis involving multiple organs and having various presentations. APLAs that are components of APS criteria include IgG and/or IgM anticardiolipin (aCL), anti-β2-glycoprotein 1 (anti-β2GP1) and the lupus anticoagulant (LAC). Other non-criteria APLA such as antiphosphatidylserine/prothrombin (PS/PT) complex, anti-PT and antitrombin 1 and β2-GP1 have also found a diagnostic niche in APS.

One of the salient features of COVID-19 is the development of thrombotic events associated with severe morbidity and mortality. In the context of systemic inflammation and dysregulated immunity, some reports have linked APLA to these thromboses, severe COVID-19 and release of neutrophil extracellular traps. However, APLAs are also described in a variety of other infectious diseases and critically ill patients have high rates of thromboembolism that were not linked to APS or APLA (critically reviewed in ref. 15). Therefore, the association of COVID-19 with APS and their potential pathogenic role has not been clearly demonstrated due to the lack of contemporaneous COVID-19 negative controls. Here, we compare the prevalence and clinical correlations of APLA in patients with severe COVID-19 as compared with contemporaneous non-COVID-19 patients with similar clinical characteristics.

METHODS

Informed consent was obtained from all patients or their legal surrogates. Inclusion criteria were age ≥18 years, admission to intensive care unit (ICU)
Table 1  Patient demographics, clinical and autoantibody status

| Cohort          | All | COVID+ | COVID− |
|-----------------|-----|--------|--------|
|                 | N   | 42     | 22     | 20     |
| Age             | Mean (CI) | 58.2 (62.7 to 54.1) | 60.9 (66.6 to 55.3) | 55.7 (62 to 48.7) |
| Sex             | N male (%) | 29/42 (69) | 17/22 (77) | 12/20 (60) |
| Censored?       | N (%) | 5/42 (12) | 4/22 (18) | 1/20 (5) |
| No of days before censoring | Mean (CI) | 39.4 (59.4 to 19.4) | 44.3 (66.2 to 22.3) | 20 (NA) |
| Days from symptom onset to ICU | Mean (CI) | 6 (8.3 to 3.7) | 7.5 (9.9 to 5.2) | 4.2 (8.5 to 0) |
| APACHE II on ICU admission | Mean (CI) | 25.3 (27.6 to 22.9) | 23.7 (27 to 20.4) | 27 (30.5 to 23.5) |
| Mean of SOFA score for first 3 days | Mean (CI) | 9.6 (10.7 to 8.5) | 9.3 (11 to 7.7) | 9.9 (11.6 to 8.3) |
| Mean of SOFA score for first 7 days | Mean (CI) | 8.9 (10.1 to 7.8) | 9.1 (11 to 7.3) | 8.7 (10.3 to 7.2) |
| ICU days (censored) | Mean (CI) | 14.1 (17.3 to 10.8) | 14.2 (20.5 to 7.8) | 14 (16.9 to 11.1) |
| Death in ICU    | N (%) | 13/42 (31) | 7/22 (32) | 6/20 (30) |
| Mechanical ventilation days (censored) | Mean (CI) | 14.4 (18.9 to 10) | 16.8 (25.1 to 8.6) | 11.8 (14.9 to 8.7) |
| Total days of ventilation rescue measures | Mean (CI) | 2.9 (4.3 to 1.4) | 4.4 (7 to 1.8) | 1.2 (2 to 0.4) |
| Therapeutic anticoagulation used | N (%) | 8/42 (19) | 3/22 (14) | 5/20 (25) |
| Mean platelet count | Mean (CI) | 239 (269 to 209) | 264 (313 to 214) | 212 (245 to 179) |
| Mean platelet to neutrophil ratio | Mean (CI) | 35.2 (42 to 28.4) | 38.7 (48.4 to 29) | 31.4 (41.6 to 21.2) |
| aCL IgG         | N (%) | 20/42 (48) | 13/22 (59) | 7/20 (35) |
| aCL IgM         | N (%) | 9/42 (21) | 7/22 (32) | 2/20 (10) |
| Anti-β2GP1 IgG | N (%) | 0 | 0 | 0 |
| Anti-β2GP1 IgM | N (%) | 0 | 0 | 0 |
| Anti-domain 1 β2GP1 IgG | N (%) | 0 | 0 | 0 |
| Anti-PS/PT IgG | N (%) | 0 | 0 | 0 |
| Anti-PS/PT IgM | N (%) | 1/42 (2) | 1/22 (5) | 0 |

The data were censored on 31 May 2020. Days from symptom onset were self-reported by the patients or their representatives. The SOFA score was performed daily for all patients; the average was calculated for the first 3 and 7 days in the ICU for each patient, and the mean of those averages are reported. For patients who underwent tracheostomy, mechanical ventilation days are counted until successfully weaned from ventilatory support for 24 hours. Rescue measures included use of paralytics, proning and inhaled NO (counted additively if more than one intervention used in the same day). The clinical outcomes were measured for up to 3 months. All the serologies were tested longitudinally and are reported for the first 10 days from admission to the ICU (for standardisation among patients). There was no statistically significant difference between COVID+ and COVID− patients for all variables, using ANOVA for continuous variables and Fisher’s exact test for categorical variables at α=0.05, followed by the false discovery rate adjustment at q=0.05.

RESULTS

The demographic and clinical parameters of 22 COVID-19 positive (COVID+) and 20 COVID-19 negative (COVID−) patients (table 1) included an average of 14.1-day stays in ICU and 31% mortality, but no statistically significant differences between the two cohorts, including the lack of significant differences in the number of thrombotic events requiring therapeutic anticoagulation, platelet counts or platelet counts normalised to the neutrophil counts (to index for severity) (table 1). None of the patients had a history of antecedent APS, systemic lupus erythematosus (SLE) or other conditions associated with APS, nor were there significant differences in other past medical history between COVID+ and COVID− patients (online supplemental table 1).

Frequency, development and distribution of aCL

Forty-eight per cent of all the ICU cohort had a positive IgG aCL test (table 1); interestingly, fewer patients had elevated titres of IgM aCL (n=9, 21%), with only two patients having IgM without IgG. Although more COVID-19+ had aCL antibodies, the difference was not statistically significant (table 1); aCL titres were slightly higher among the COVID-19− (not statistically significant, (online supplemental table 2) and online supplemental figure 1). Longitudinally testing for anti-β2GP1 and anti-PS/PT for IgG and IgM, as well as domain 1 anti-β2GP1 IgG revealed only one patient (COVID-19+) with positive serology for any of these autoantibodies. This patient seroconverted to IgM anti-PS/PT at days 5−7 of ICU hospitalisation. Table 2 shows the temporal development of the aCL IgG and IgM antibodies stratified by COVID-19 status. Late appearing (beyond 10 days after admission) aCL antibodies were not included in the statistical

with acute respiratory failure. Exclusion criteria were inability to ascertain the primary outcome or determine a baseline blood sample, and SARS-CoV2 infection in the 4 weeks prior to admission. COVID-19 status was determined with PCR of nasopharyngeal swabs and/or endotracheal aspirates. Follow-up was 3 months post-ICU admission or hospital discharge. Primary outcome was death in the ICU. Secondary outcomes were in hospital-death, ICU utilisation metrics, organ dysfunction measures and severity scores. Clinical data and serum samples were collected longitudinally at days 0, 1, 3, 5, 7 and 10; after day 10 or ICU discharge. aCL, anti-β2GP1 and anti-PS/PT were tested for IgG and IgM, as well as IgG anti-domain 1 β2-GP1; all by ELISA or chemiluminescence (Inova Diagnostics, San Diego, California, USA). Analysis of variance was used for continuous variables and Fisher’s exact test was used for categorical variables at α=0.05, followed by a false discovery rate adjustment at q=0.05. Detailed methods are available (online supplemental file), including methods for detection of anti-nuclear autoantibodies (ANA) by HEP-2 immunofluorescence assay (IFA) (Inova Diagnostics) and antigen-specific autoantibodies (TheraDiag, Paris, France) and anticytokine autoantibodies (Millipore, Oakville, Ontario, Canada) using addressable laser bead immunoassays.

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This trend remained when analysing the COVID+ and COVID− IFA ANA at a dilution of 1:160, it was significantly associated with IgG positivity was not associated with the presence of HEp-2 potential relationship to APS autoantibodies. Although aCL tended to associate with COVID−19+, they did not associate with the presence of other antigen-specific autoantibodies, although they had a strong association with certain anticytokine autoantibodies, which are reported to neutralise interferon-γ, anti-IL10 and anti-IL-17F were the most prevalent. When analysing the aCL IgG positive according to their COVID−19 status, the COVID+ had significantly higher levels of anticytokine autoantibodies than the COVID− (online supplemental table 4). aCL IgG was not associated with antigen-specific autoantibodies, including SLE and myositis-related autoantibodies (not shown).

### DISCUSSION

In the year since the onset of the SARS-CoV2 pandemic, there has been a remarkable surge in publications about one disease, COVID−19, chronicling the clinical onset and outcomes, and a host of biomarkers purported to have related pathophysiological significance (reviewed in references 17 18). The key observation of this study is that patients with positive IgG aCL showed a trend towards more severe disease regardless of whether they were COVID+ and COVID−. That is, while COVID+ patients showed non-significant trends towards worse respiratory outcomes when compared with COVID−, aCL status had an independent association with disease severity, and did not modulate the outcomes differentially based on COVID status. The pathologic significance of aCL seropositivity is unclear since there were no major differences in platelet counts or thrombotic events in the two cohorts. Others have reported a high prevalence of aCL autoantibodies among COVID+ patients, but these studies lacked contemporaneous COVID− control groups of similar disease severity.6 19 19 20

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### Epidemiology

#### Table 2 Development of ACL IgG and IgM over time

| Cohort          | All | aCL detected on admission | aCL developed within 10 days | Late appearing aCL | aCL negative on admission |
|-----------------|-----|--------------------------|----------------------------|-------------------|-------------------------|
| aCL IgG positive | COVID+ | 4                        | 9                          | 2                 | 0                       |
| aCL IgM positive | COVID+ | 3                        | 4                          | 0                 | 1                       |
| aCL IgG positive | COVID− | 1                        | 6                          | 2                 | 1                       |
| aCL IgM positive | COVID− | 1                        | 1                          | 1                 | 1                       |

Late aCL was not included in the statistical analyses to avoid survival and availability bias, and is shown here for qualitative assessment.

#### Table 3 Association between ACL IgG and disease severity, platelet counts and need for anticoagulation

| Cohort          | All | aCL IgG positive | aCL IgG negative |
|-----------------|-----|-----------------|-----------------|
| Age Mean (CI)    | 58.2 (62.7 to 54.1) | 55.9 (62.9 to 49) | 60.7 (66.4 to 55) |
| Sex N male (%)   | 29/42 (69) | 13/20 (65) | 16/22 (73) |
| Days from symptom onset to ICU Mean (CI)   | 6 (8.3 to 3.7) | 8.7 (12.8 to 4.6) | 3.4 (5.4 to 1.5) |
| APACHE II on ICU admission Mean (CI)       | 25.3 (27.6 to 22.9) | 25.7 (28.5 to 22.9) | 24.9 (28.8 to 20.9) |
| Mean of SOFA score for first 3 days Mean (CI) | 9.6 (10.7 to 8.5) | 10.6 (12.2 to 9.1) | 8.7 (10.3 to 7) |
| Mean of SOFA score for first 7 days Mean (CI) | 8.9 (10.1 to 7.8) | 10 (11.7 to 8.4) | 8.9 (9.5 to 6.4) |
| ICU days (censored) Mean (CI)               | 14.1 (17.3 to 10.8) | 16.6 (21.9 to 11.3) | 12.1 (16.5 to 7.6) |
| Death in ICU N (%)                           | 13/42 (31) | 8/20 (40) | 5/22 (23) |
| Mechanical ventilation days (censored) Mean (CI) | 14.4 (18.9 to 10) | 18.2 (25.5 to 10.8) | 11.1 (16.4 to 5.7) |
| Total days of ventilation rescue measures Mean (CI) | 2.9 (4.3 to 1.4) | 3.6 (5.6 to 1.5) | 2.3 (4.4 to 0.1) |
| Therapeutic anticoagulation used N (%)       | 8 | 4/20 (20) | 4/22 (18) |
| Mean platelet count Mean (CI)               | 239 (269 to 209) | 268 (321 to 216) | 212 (246 to 179) |
| Mean platelet to neutrophil ratio Mean (CI) | 35.2 (42 to 28.4) | 34.8 (45.2 to 24.3) | 35.6 (45.4 to 28.9) |

See table 1 for details on the variables shown. There were no statistically significant differences between aCL IgG positive and aCL IgG negative patients for all variables, using ANOVA for continuous variables and Fisher’s exact test for categorical variables at α=0.05, followed by the false discovery rate at q=0.05.

aCL, anticardiolipin antibodies; ANOVA, analysis of variance; APACHE, Acute Physiology and Chronic Health Evaluation (score); ICU, intensive care unit; SOFA, sequential organ failure assessment (score).
admission to the development of IgG aCL (table 3). Our findings highlight the importance of longitudinal monitoring of acutely ill patients. It seems plausible that disparate conclusions in the literature with respect to the significance of APLAs in COVID-19 may relate to arbitrary sampling times and lack of longitudinal follow-up in the setting of dynamic inflammatory diseases.

While some reports have included LAC in their analyses, we did not because LAC is known to be an unreliable biomarker in severe illnesses where C reactive protein, anticoagulant use and other factors confound its detection. In this study, we used the anti-PS/PT test regarded by some as a surrogate for LAC (reviewed in reference 3). However, only one patient developed anti-PS/PT 5–7 days after admission. Further, our observation that no patient had antibodies to β2-GPI (an APS criteria antibody) or to domain 1 β2-GPI (reportedly higher specificity for APS) argues against the presence of APS in our cohort. In addition, aCL in isolation and/or the depletion of β2-GPI reactivity has been associated with the loss of pathogenic thrombosis formation (reviewed in reference 3). In a study of 37 COVID+ acute respiratory disease vs 31 prepandemic (not COVID+) patients, Frapard et al reported that 37 patients with COVID-19 exhibited more thrombotic events as compared with 31 prepandemic controls but the occurrence of APLA in the two groups was similar. Using APLA assays similar to ours, Borghi et al reported a low prevalence of APLA in COVID+ sera, where the most common target was IgG β2-GPI (15.6%). In addition, the primary β2GPI antibody targets were in domains 2–4 which are less specific for APS. In agreement with our study, Bertin et al20 concluded that APLA were not associated with major thrombotic events.

The main limitation of our study is the small sample size, although studies using somewhat larger COVID-19 cohorts have reached similar conclusions. The strengths of our study include its prospective, contemporaneous COVID+ cohort with similar severity of disease. Importantly, we tested a broad APLA serological panel longitudinally, providing a more robust assessment of its true prevalence and incidence than in other reported studies; this is particularly relevant for such acutely ill patients with dynamic clinical courses. Finally, our use of an extensive serological panel allowed us to better characterise the broad phenotype associated with aCL.

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Competing interests MJF is the Director of MitogenDX. MJF is a consultant for and received speaking honoraria from Inova Diagnostics Inc (San Diego, California, USA) and Werfen International (Barcelona, Spain). All the other authors have no disclosures to declare.

Patient and public involvement statement Patients and public were not involved in the design of the study. During the initial phases of the study, we obtained feedback from the patients and their substitute decision makers. Their concerns, questions and preferences were incorporated into improved processes for consent and collection of biological samples. The consent forms have checkboxes with optional aspects of the study, to accommodate different patient preferences. The results of the study will be disseminated in lay versions by St. Michael’s Hospital public relations and communications departments for the benefit of the public.

Patient consent for publication Not required.

Ethics approval This is an observational cohort study of patients admitted to St Michael’s Hospital (Toronto, ON, Canada), as approved by the Research Ethics Board (REB# 20-078).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as online supplemental information. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Online materials and methods

Study design. This report is part of the COLOBILI study – Coronavirus Longitudinal Biomarkers in Lung Injury, being conducted at St. Michael’s Hospital (Toronto, ON, Canada). This is an observational cohort study that includes analysis of biological samples. The study was approved by the Research Ethics Board of St. Michael’s Hospital (REB# 20-078). The inclusion criteria were all patients above age 18 years admitted to the Medical-Surgical or Trauma-Neuro intensive care units (ICU) with acute respiratory distress, suspected to have COVID-19. COVID-19 status was determined according to diagnostic PCR of nasopharyngeal swabs and/or endotracheal aspirates as described in detail below. The exclusion criteria were refusal to participate, inability to ascertain mortality status during the first 2 weeks of the study, failure to obtain a blood sample on either day 0 or 1, or individuals known to have had COVID-19 in the 4 weeks prior to admission in any setting. Patients were followed for up to 3 months in hospital or hospital discharge, whichever occurred first. The primary outcome was death in the ICU; secondary outcomes included death outside the ICU, ICU utilization metrics, and organ dysfunction measures and scores. Clinical data and blood samples were collected longitudinally immediately upon admission, as available, defined as day 0, and on the morning of days 1, 3, 5, 7 and 10; after day 10 or ICU discharge, they were sampled every 2 weeks. The study started on March 26th, 2020, and the first patient was recruited on March 29th, 2020. The study is ongoing; the last patient from the cohort presented in this manuscript was recruited on May 17th, 2020, and the data was censored for analysis on May 31st, 2020. No COVID-19 treatments were given to the patients beyond the standard of care since at the time there was no evidence of efficacy for any such treatments. Informed consent was obtained from the patients or their legal representatives; in case that was not possible, the patients were enrolled using a deferred consent model and kept in the study until they regained capacity, or a surrogate decision maker was identified.

Data and sample collection. Demographics, clinical data and clinical laboratory were collected from the patients’ paper and electronic medical records, with auditing performed reciprocally by research coordination team members and curated by UT. To standardize handling and processing, blood samples were collected in EDTA tubes between 8:00 and 12:00 AM and kept on ice for up to 60 minutes until their processing in a dedicated translational research station located inside the ICU. They were then immediately frozen at -20 °C on site, and transferred to -80 °C for storage within 48 hrs. All procedures were performed by dedicated research
personnel. Nasopharyngeal samples were obtained from all patients by bedside nurses and analyzed by the clinical laboratory using either the Altona RealStar SARS-CoV-2 RT-PCR Kit 1.0 or Cepheid GeneXpert Xpert Xpress SARS-CoV-2 assay. Endotracheal tube aspirates were analyzed using the Seegene Allplex 2019-CoV Assay. All patients had a nasopharyngeal PCR performed; intubated patients had an endotracheal aspirate sent as well. Further PCR tests were repeated by the clinical and infection control teams at their discretion if there was suspicion of a false negative result based on clinical observations or to confirm negativity. All patients in the PCR negative cohort had at least two negative tests performed acutely, except one patient who had only one test done acutely. To analyze longitudinal trends, only patients with 3 or more longitudinal sampling times were included in the study. To mitigate bias, five patients with shorter ICU admissions were included; 2 had early deaths and 3 had early discharges.

Experimental procedures. Plasma samples were stored and managed under a standard operating procedure which included shipping on dry ice and storage at -80C until assay performance by Mitogen Diagnostics Laboratory (MitogenDx, Calgary, AB, Canada). Anti-cardiolipin, anti-β2-GP1 and anti-PS/PT complex were tested by ELISA for IgG and IgM antibodies and for IgG anti-domain 1 β2-GP1 by chemiluminescence immunoassay (Inova Diagnostics, San Diego, CA USA). For all of the anti-phospholipid antibodies listed above, the manufacturer’s cutoffs were utilized and previously validated for routine diagnostic testing (MitogenDx: https://https://mitogendx.com/); 20 Units (U) for anti-cardiolipin, anti-β2-GP1 and anti-PS/PT complex and 20 chemiluminescence units (CU) for the anti-domain 1 β2-GP1 immunoassay. The anti-PS/PT assay is approved for use in serum and EDTA plasma, as it includes calcium protein stabilizers and calcium to overcome any chelating effect of EDTA. A HEP-2 indirect immunofluorescence assay (IFA) was used to detect anti-cellular antibodies (also referred to as anti-nuclear antibodies (ANA) – see “nomenclature” below) (NOVA Lite HEP-2, Inova Diagnostics, San Diego, CA) at a serum dilution of 1:80 and read on an automated instrument (Nova View, Inova Diagnostics) which interpolates fluorescence intensity to an end point titer. IFA staining patterns were classified according to the International Consensus on Autoantibody Patterns (ICAP, https://anapatterns.org/index.php), and considered positive at a dilution ≥1:160. All samples were also tested for systemic autoimmune disease-related autoantibodies by a FIDIS Connective13 addressable laser bead immunoassay (ALBIA) (TheraDiag, Paris, France) detecting antibodies to Sm/U1-U6 ribonucleoprotein (RNP), U1-RNP, SSA/Ro60, SSB/La, Ro52/Tripartite Motif Protein 21 (TRIM21), histones, and ribosomal P, read on a Luminex 200.
system using the MLX-Booster software. A cut-off of >40 units was considered positive. Anti-
dsDNA positivity and titers were detected by a chemiluminescence test (Inova Diagnostics, San
Diego, USA). A cut-off of <27 chemiluminescence units was considered within normal range, 27-
35 was indeterminate, and >35 was positive. All samples were also tested for autoantibodies
associated with autoimmune inflammatory myopathies using a multiplexed solid phase line
immunoassay: Ro-52/TRIM21, OJ, EJ, PL-12, PL-7, SRP, Jo-1, PM-75, PM-100, Ku, SAE1, NXP2,
MDA5, TIF1γ, Mi-2α, Mi-2β (Euroimmun AG, Luebeck, Germany), and anti-NT5c1A by ALBIA4.
The following anti-cytokine antibodies were assayed using an ALBIA (Millipore, Oakville, ON,
Canada; HCYTAAB-17K-15) read on a Luminex 200 system: BAFF, GMCSF, IFN-β, IFN-γ, IL-1a, IL-6,
IL-8, IL-10, IL-12p40, IL-15, IL-17a, IL-17f, IL-18, IL-22 and TNF-α. The manufacturer’s thresholds
were 500 for positive and 1000 for high-positive (arbitrary units). All tests were performed
according to the manufacturer’s instructions.

Nomenclature. There is considerable heterogeneity in the nomenclature of autoimmune assays
in the literature and clinical practice; therefore, we used the most contemporary nomenclature.
Autoantibodies is a general term that encompasses the autoimmune humoral responses
assayed. The HEp-2 IFA, although including anti-cytoplasmic and anti-mitotic cell antibodies, are
commonly referred as anti-nuclear antibodies (ANA)1, and we have adopted that usage for
clarity. The AAB test results that identified specific, named antigens (see details above), were
called collectively antigen-specific autoantibodies. We have further separated them into
myositis-related and non-myositis-related AAB. Anti-cytokine autoantibodies are referred to
directly.

Data analysis. All the data was organized and analyzed by UT. The data was censored on May
31st, 2020; only 5 patients had censored data for the primary outcome, death in the ICU within 3
months. Given the elapsed time until censoring, the risk of right-censoring bias is low. ANOVA
was used for continuous variables and Fisher’s exact test was used for categorical variables at
α=0.05, adjusted for multiple comparisons as indicated in the text using the false discovery rate
at q=0.05. All statistical and graphical analyses were performed on JMP Pro (version 15.2.1; SAS
Institute Inc, Cary, NC, USA).
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Anti-cardiolipin and other anti-phospholipid antibodies in critically ill COVID-19 positive and negative patients

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**Supplemental Table 1: Premorbid Clinical Characteristics and Therapeutics**

|                          | All (N) | COVID+ (N) | COVID- (N) |
|--------------------------|---------|------------|------------|
| Respiratory PMH          | 18      | 8          | 10         |
| Cardiovascular PMH       | 19      | 11         | 8          |
| Renal PMH                | 7       | 6          | 1          |
| Type 2 Diabetes          | 20      | 12         | 8          |
| Hypertension             | 24      | 14         | 10         |
| Other comorbidities      | 37      | 18         | 19         |
| Premorbid steroid used   | 3       | 1          | 2          |
| Premorbid immunomodulatory medication use | 2 | 1 | 1 |
| Premorbid ACEi/ARB use   | 15      | 10         | 5          |

**Abbreviations:** ACEi, Angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; PMH, past medical history.

**Legend:** “Other comorbidities” include autoimmune diseases: Myasthenia gravis among COVID+, and autoimmune hemolytic anemia, rheumatoid arthritis and multiple sclerosis among the COVID-. No statistically significant difference between COVID+ and COVID- patients for all variables were detected using ANOVA for continuous variables and Fisher’s exact test for categorical variables at α=0.05.
Supplemental Table 2: distribution of aCL titers among the aCL positive

|       | N  | Mean | SD  | Median | Range        | IQR           |
|-------|----|------|-----|--------|--------------|---------------|
| COVID | 13 | 47.5 | 21.2| 38.1   | 22.1 – 96.5  | 31.2 – 63.1 (31.9) |
| COVID | 7  | 36.1 | 23.3| 27.4   | 21.7 – 87.3  | 22.9 – 39.6 (16.7) |

Abbreviations: aCL, anti-cardiolipin antibodies; IQR, interquartile range (25%-75%); SD, standard deviation.

Legend: Descriptive statistics of aCL titers in aCL positive patients were stratified into COVID+ and COVID-. N is the number of patients included in each group. These differences were not statistically significant (ANOVA, significance threshold at α=0.05).

Supplemental Figure 1: aCL titers according to COVID status

Legend: This is a graphical representation of the data shown in supplemental Table 2, where aCL absorbance units (AU) are displayed according to COVID-19 status.
Supplemental Figure 2: ANA positivity and titers in aCL positive and aCL negative patients

Legend:

Left panel: ANA positivity vs aCL antibodies. The Y axis is the proportion of ANA positivity (red are negative (N), blue are positive (Y)). These differences are not statistically significantly (Fisher’s exact test, significance threshold at α=0.05).

Right Panel: Same as left panel, but ANA positive patients are subdivided into high and low titers. The Y axis is the proportion of ANA titers: red are negative, green are low (1:160-1:320), and blue are high (>1:320) titers. These differences are statistically significantly (Fisher’s exact test, p=0.03).

Abbreviations: aCL, anti-cardiolipin antibodies; ANA, anti-nuclear antibodies.
Supplemental Table 3: Association between aCL IgG and high-titer anti-cytokine autoantibodies

| Cohort    | All        | aCL positive | aCL negative |
|-----------|------------|--------------|--------------|
| N         | 42         | 20           | 22           |
| ALL       | 16/42 (38%)| 13/20 (65%)  | 3/22 (14%)   |
| anti-GMCSF| 1/42 (2%)  | 1/20 (5%)    | 0/22 (0%)    |
| anti-IFN-γ| 7/42 (17%) | 6/20 (30%)   | 1/22 (5%)    |
| anti-IL-1a| 1/42 (2%)  | 0/20 (0%)    | 1/22 (5%)    |
| anti-IL-6 | 5/42 (12%) | 3/20 (15%)   | 2/22 (9%)    |
| anti-IL-10| 5/42 (12%) | 5/20 (25%)   | 0/22 (0%)    |
| anti-IL-12p40| 2/42 (5%)| 2/20 (10%)  | 0/22 (0%)    |
| anti-IL-17a| 2/42 (5%) | 2/20 (10%)  | 0/22 (0%)    |
| anti-IL-17f| 4/42 (10%)| 4/20 (20%)  | 0/22 (0%)    |
| anti-IL-22| 1/42 (2%)  | 0/22 (0%)    | 1/22 (5%)    |

**Legend:** The table reports the number of patients with high titers of anti-cytokine antibodies at any time during the first 10 days of ICU admission. “ALL” represents all patients having a high titer of anti-cytokine antibody of any type during that period. The numbers of the specific anti-cytokine antibodies sum to more than “ALL” since some patients had more than one high titer anti-cytokine antibody. Once adjusted for multiple comparisons, there were no statistically significant differences between aCL positive and aCL negative for any of the results (Fisher’s exact test at α=0.05 followed by the false discovery rate at q=0.05). The following anti-cytokine AAB did not show high levels in any of the patients: anti-BAFF, anti-IFN-β, anti-TNF-α, anti-IL8, anti-IL-15 and anti-IL-18.
Supplemental Table 4: Association between aCL IgG and anti-cytokine autoantibodies per COVID-19 status

|                | Anti-cytokine autoantibody titers |                  |            |
|----------------|----------------------------------|------------------|------------|
|                | Positive                         | High-positive    |            |
| COVID+ aCL IgG | 12/13, 92%*                      | 9/13, 69%        |            |
| COVID- aCL IgG | 4/7, 57%                         | 4/7, 57%         |            |

Abbreviations: aCL, anti-cardiolipin antibodies.

Legend: The asterisk (*) represents a significant association between aCL IgG and anti-cytokine autoantibodies (Fisher’s exact test, p=0.006, adjusted for multiple comparisons).