

SUPPLEMENTAL MATERIAL AND DATA

COVID-19 associated autoimmunity as a feature of severe respiratory disease - a Bayesian analysis.

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Supplemental background

The immune response in SARS-CoV2 infection shows evidence of immune dysregulation in patients with severe clinical manifestations[1]. While there is continued debate about the nature of the heightened inflammatory responses in severe COVID-19 patients [2,3] there is mounting evidence that some clinical and serological features bear remarkable similarities to systemic autoimmune conditions [4-6]. In addition, some COVID-19 patients continue to develop *de novo* clinical signs and symptoms reminiscent of autoimmune diseases during convalescence [7]. Potential mechanisms leading to autoimmunity in COVID-19 include increased release of self-antigens as a result of tissue damage, neutrophil activation and NETosis[8], molecular mimicry from homologous sequences of SARS-COV2 with human proteins[9] and activation of autoreactive immune cells due to cytokinemia [3]. A significant limitation of studies that have reported the emergence of autoantibodies in COVID-19[4,6,10] has been the lack of control groups with similar clinical characteristics and the lack of longitudinal data monitoring the development of autoimmunity over time.

While it is reported that a spectrum of autoantibodies emerge in COVID-19 perhaps the most well-known example of autoantibodies emerging in acute disease are anti-phospholipid antibodies (APLA) [4,5]. We and others have shown that APLA are found in COVID-19 but they were associated with severity but not with increased thrombotic events [11,12]. Nevertheless, in acute respiratory illnesses such as COVID-19 there are examples, among them are myasthenia gravis and Lambert-Eaton myasthenic syndrome [13] and atypical Guillain-Barre syndrome [14] where autoantibodies appear to be pathogenic. Other autoantibodies of special interest include anti-IFN and their association with non-TB mycobacterial infections [15], flares of interstitial pneumonia in systemic lupus erythematosus (SLE) [16], or paraneoplastic conditions [17]. Interestingly, there are reports of CNS autoantibodies in COVID-19 patients with neurological symptoms [18], yet these studies suffer from the lack of proper controls as

the reports on respiratory presentations. Prior to the SARS-CoV2 pandemic, there were two key reports on the emergence of autoantibodies during critical disease, including severe respiratory disease [19] [20]. The null hypothesis of our study was that, when observed longitudinally, severe COVID-19 patients would have similar autoantibody prevalence as a control cohort of patients with acute respiratory failure.

Supplementary Discussion

We analyzed disease severity and presence of autoantibodies over time in 22 COVID⁺ and 20 COVID⁻ ICU patients with respiratory distress of similar severity, enrolled contemporaneously. We used HEp2 IFA to typify ANAs, as well as solid-phase multi-analyte arrays and bead-based multiplexed immunoassays to typify specific autoreactive antigens such as those observed in autoimmune diseases such as systemic lupus erythematosus, Sjögren syndrome, systemic sclerosis, autoimmune inflammatory myopathies, and others [4-6].

Overall, close to 15,000 data points were collected between demographic variables and laboratory and clinical variables over time. We observed high prevalence and titers of autoantibodies among the COVID⁺ patients in our study (68% had ANA), in agreement with some previous reports[21,22]. Yet, surprisingly, we also observed high prevalence and titers of autoantibodies in the COVID⁻ patient cohort (60% had ANA). In another cross-sectional study[4], the majority of positive sera had reactivity to only single nuclear antigens, whereas most of our patients showed multiple reactivities (supplemental figures 1 and 2), suggesting a relatively widespread loss of humoral tolerance in some of our patients. We found no major differences in either the autoantigen specificity, temporal dynamics, or titers in COVID⁺ vs. COVID⁻ cohorts. These data suggest that autoantibody production may be a feature of immune

dysfunction associated with respiratory failure rather than specifically with a SARS-CoV-2 driven immune pathology. Importantly, specific ANA patterns (AC19 and/or AC20) were broadly correlated with disease severity measures, suggesting a causal relationship, or at least a common factor “driving” autoreactive B cell responses in critically ill patients.

A previous study reported that approximately 10% of patients with life-threatening COVID-19 pneumonia had neutralizing IgG autoantibodies against a spectrum of interferon proteins, which blocked SARS-CoV-2 infection *in vitro*[23]. Of note, these autoantibodies were not found in asymptomatic or mild SARS-CoV-2 infection, but they were not studied in similarly ill, COVID- patients. Our results show that matched COVID⁻ controls have a similar incidence of anti-interferon- γ and anti-interferon- β autoantibodies as COVID⁺ patients. While we did not examine whether the anti-cytokine autoantibodies (acAAB) in our study had neutralizing activity, we did not find that the presence of these autoantibodies differed between COVID⁺ and COVID⁻. The small sample size and the large absolute difference for anti-IL-6, anti-IL-10 and anti-IL-17f raise the possibility of a type-2 error. We addressed this concern using Bayesian analysis, which confirmed the results of the null-hypothesis statistical testing.

Our data and analysis support that, humoral autoimmunity, as described in detail using an extensive panel of ANA, spAAB and acAAB, longitudinally, does not seem to be a particular characteristic of COVID⁺ patients but rather of critically ill patients with respiratory failure. The collection of data over time significantly increases the reliability of our results by a) reducing the biases of different, arbitrary sampling times between different patients, b) providing test-retest internal validity, and c) characterizing the development of autoimmunity in a physiological context - over time. Although there is no direct evidence to assume *a priori* that COVID⁺ have more autoimmunity than COVID⁻, some

94 clinicians may consider the immune characteristics of severe COVID-19 enough reason to warrant such
95 assumptions. Our analysis suggests that, in that case, the expected differences would be in the order of
96 a modest 15-20% (main figure 1, supplemental table 4). These results provide boundaries to the
97 autoimmunity phenomenon of COVID⁺ patients and provide the context of high prevalence of
98 autoimmunity among critically ill patients with respiratory failure in general. Nevertheless, there are
99 some suggestions in our data that, within this context, COVID-19 patients may have a tendency for
100 particular autoimmune phenotypes, as hinted by the large absolute difference of anti-IL6, anti-IL10 and
101 anti-IL17f autoantibodies. Larger studies are needed to assess this possibility, as well as the pathogenic
102 role of autoantibodies in both COVID⁺ and COVID⁻ patients, which is yet to be demonstrated.

103
104 Given that all of our patients (both COVID⁺ and COVID⁻) had respiratory failure, it may be that the
105 association between autoantibody production is related to lung injury and/or lung endothelial
106 pathology, a known feature of acute lung injury[24] regardless of etiology. The activation of both innate
107 immune and adaptive responses to abnormal danger-associated molecular patterns released into the
108 circulation following the initial lung injury may contribute to the loss of humoral tolerance[25-28].
109 Another potential cause of autoantibody formation may be related to immune activation associated
110 with the biotrauma of ventilator associated lung injury, in the context of loss of pulmonary
111 compartmentalization[29]. This could explain some of the autoantibodies that developed during the
112 course of hospitalization (supplemental figure 2). Both of these mechanisms are compatible with
113 previous findings of newly developed autoantibodies in sepsis, particularly ARDS [19] [20]. Indeed,
114 perhaps the most salient result of our study is the high prevalence of broad autoimmune serology
115 among the critically ill, a phenomenon that is underappreciated except perhaps for APLA [12]. These
116 results stand in stark contrast to the known prevalence of about 5% in the general population.

Our study's main shortcoming is the small sample size. We addressed this by employing a Bayesian analysis to explore the boundaries of the possible states of nature underlying our results. Another shortcoming is the need to consolidate the different autoimmune serologies under the broad categories of ANA, spAAB and acAAB, due to the small sample size and to avoid multiple testing. Nonetheless, we do present these results in detail, providing a qualitative assessment, and performed analyses of specific features when the sample size allowed for it, such as for the AC19 and/or AC20 patterns, or the ANA titers.

In conclusion, in some patients, severe COVID-19 is characterized by immune dysregulation with clinical features of systemic autoimmunity. We present evidence that humoral autoimmunity is a common feature of critically ill patients with respiratory deterioration. Our study urges caution in the interpretation of autoantibody test results among COVID⁺ patients without relevant controls. Further studies are needed to determine whether transient or sustained production of autoantibodies in the setting of acute illness is associated with the development of systemic autoimmunity.

Supplemental Table 1: Patient Demographic, Clinical and Autoantibody Status

	Cohort	All	COVID ⁺	COVID ⁻
	<i>N</i>	42	22	20
Age	<i>Mean (CI)</i>	58.2 (62.7-54.1)	60.9 (66.6-55.3)	55.7 (62-48.7)
Sex	<i>N male (%)</i>	29/42 (69%)	17/22 (77%)	12/20 (60%)
Censored?	<i>N (%)</i>	5/42 (12%)	4/22 (18%)	1/20 (5%)
Number of days before censoring	<i>Mean (CI)</i>	39.4 (59.4-19.4)	44.3 (66.2-22.3)	20 (NA)
Days from symptom onset to ICU	<i>Mean (CI)</i>	6 (8.3-3.7)	7.5 (9.9-5.2)	4.2 (8.5-0)
APACHE II on ICU admission	<i>mean (CI)</i>	25.3 (27.6-22.9)	23.7 (27-20.4)	27 (30.5-23.5)
Mean of SOFA score for 1st 3 days	<i>mean (CI)</i>	9.6 (10.7-8.5)	9.3 (11-7.7)	9.9 (11.6-8.3)
Mean of SOFA score for 1st 7 days	<i>mean (CI)</i>	8.9 (10.1-7.8)	9.1 (11-7.3)	8.7 (10.3-7.2)
ICU days (censored)	<i>mean (CI)</i>	14.1 (17.3-10.8)	14.2 (20.5-7.8)	14 (16.9-11.1)
Death in ICU	<i>N (%)</i>	13/42 (31%)	7/22 (32%)	6/20 (30%)
Mechanical ventilation days (censored)	<i>Mean (CI)</i>	14.4 (18.9-10)	16.8 (25.1-8.6)	11.8 (14.9-8.7)
Total days of ventilation rescue measures	<i>Mean (CI)</i>	2.9 (4.3-1.4)	4.4 (7-1.8)	1.2 (2-0.4)
ANA (admission and up to 10d)	<i>N (%)</i>	27/42 (64%)	15/22 (68%)	12/20 (60%)
ANA titer	<i>N (%) 0 to 80</i>	15/42 (36%)	7/22 (32%)	8/20 (40%)
	<i>N (%) 160 to 320</i>	16/42 (38%)	9/22 (41%)	7/20 (35%)
	<i>N (%) >320</i>	11/42 (26%)	6/22 (27%)	5/20 (25%)
Highest ANA titer	<i>mean (CI)</i>	655 (888-422)	677 (1021-334)	629 (986-273)
AC4 and/or AC5 pattern	<i>N (%)</i>	26/42 (62%)	13/22 (59%)	13/20 (65%)
AC19 and/or AC20 pattern	<i>N (%)</i>	15/42 (36%)	10/22 (45%)	5/20 (25%)
spAAB	<i>N (%)</i>	16/42 (38%)	9/22 (41%)	7/20 (35%)
Myositis-related spAAB	<i>N (%)</i>	13/42 (31%)	6/22 (27%)	7/20 (35%)
Ever high-positive acAAB?	<i>N (%)</i>	16/42 (38%)	11/22 (50%)	5/20 (25%)
Ever positive acAAB?	<i>N (%)</i>	22/42 (52%)	14/22 (64%)	8/20 (40%)

Abbreviations: AAB, autoantibody; AC, International Consensus on Autoantibody Patterns anti-cellular pattern nomenclature; acAAB, anti-cytokine autoantibodies; ANA, anti-nuclear antibodies; APACHE, Acute Physiology and Chronic Health Evaluation (score); CI, 95% confidence interval; ICU, intensive care

unit; IQR, 25—75% interquartile length; SOFA, sequential organ failure assessment; spAAB, antigen-specific autoantibodies. For more details on the nomenclature used for the autoimmune assays please refer to the online materials and methods.

Legend:

The data was censored on May 31st. Days from symptom onset were self-reported by the patients or their representatives. ANA were determined with immunofluorescence using Hep-2 cells and classified using the International Consensus on Autoantibody Patterns system. The table reports the highest value found during longitudinal sampling up to day 10 of ICU admission, for standardization among patients. Titres of 0 to 80 are considered negative and titres above 320 are considered high. 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 that underwent tracheostomy, mechanical ventilation days are counted until successfully weaned from ventilatory support for 24 hrs. Rescue measures included use of paralytics, proning and NO (counted additively if more than one intervention used in the same day). The clinical outcomes were measured for up to 3 months. 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 $\alpha=0.05$, followed by the false discovery rate at $q=0.05$.

Interpretation

The demographic and clinical characteristics, including past medical history are shown in Table 1 (see also supplemental Table 3). No statistically significant clinical differences were noted between the two patient groups. Importantly, both patient groups experienced comparable disease severity[30,31] as measured in ICU days, mechanical ventilation days and mortality rates, as well as surrogate severity scores. Age, sex and ethnicity were not correlated with the presence of autoantibodies (data not shown).

Supplemental Table 2 – High titer anti-Cytokine autoantibodies

Ever high positive	All	COVID ⁺	COVID ⁻
	N=42	N=22	N=20
ALL	16/42 (38%)	11/22 (50%)	5/20 (25%)
anti-GMCSF	1/42 (2%)	1/22 (5%)	0/20 (0%)
anti-IFN- γ	7/42 (17%)	3/22 (14%)	4/20 (20%)
anti-IL-1a	1/42 (2%)	1/22 (5%)	0/20 (0%)
anti-IL-6	5/42 (12%)	4/22 (18%)	1/20 (5%)
anti-IL-10	5/42 (12%)	4/22 (18%)	1/20 (5%)
anti-IL-12p40	2/42 (5%)	1/22 (5%)	1/20 (5%)
anti-IL-17a	2/42 (5%)	2/22 (9%)	0/20 (0%)
anti-IL-17f	4/42 (10%)	4/22 (18%)	0/20 (0%)
anti-IL-22	1/42 (2%)	0/22 (0%)	1/20 (5%)

Abbreviations: GMCSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin.

Legend:

The table reports the number of patients with high titers of acAAB at any time during the first 10 days of ICU admission, with the percent positive in parentheses. “ALL” represents all patient having a high titer of acAAB of any type during that period. The numbers of the specific acAAB sum to more than “ALL” since some patients had more than one high titer acAAB. Once adjusted for multiple comparisons, there were no statistically significant differences between COVID⁺ and COVID⁻ for any of the results (Fisher’s exact test at $\alpha=0.05$ followed by the false discovery rate at $q=0.05$). The following acAAB 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.

Interpretation:

50% of COVID⁺ patients had high titers of acAAB, as compared to 25% for COVID⁻. This difference was driven mainly by anti-interleukin (IL)-6, anti-IL-10 and anti-IL-17f autoantibodies but it did not reach statistical significance. When analyzing all positive results of acAAB (as opposed to only the high titers),

183 the difference between COVID⁺ and COVID⁻ remained at ~25% (supplemental table 1). Interestingly,
184 COVID⁻ patients had a similar incidence of anti-interferon- γ and anti-interferon- β autoantibodies as
185 COVID⁺. AcAAB were not associated with the presence of spAAB or ANA (not shown).

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Supplemental Table 3: Premorbid Clinical Characteristics and Therapeutics

	All (N)	COVID+ (N)	COVID- (N)
	42	22	20
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 $\alpha=0.05$

Supplemental Table 4: Smallest prior bias for COVID⁺ > COVID⁻ producing a posterior distribution with a 95% high density interval that does not include zero

Autoantibody	Prior bias COVID ⁺ vs COVID ⁻	Mean Difference	95% HDI (range, %)
ANA	35% (76.5 vs. 51.5)	20%	1.9 – 41.2
spAAB	20% (27.0 vs 7.0)	15%	1.7 – 31.9
Ever high-positive acAAB	15% (45.5 vs 30.5)	20%	0.1 – 40.2

Abbreviations: acAAB, anti-cytokine autoantibodies; AAB, autoantibodies; ANA, anti-nuclear antibodies; HDI, high density interval; spAAB, antigen-specific autoantibodies.

Legend:

Exploratory analysis was performed with of varying levels of prior bias, assuming COVID⁺ patients have a higher incidence of autoimmune serology than COVID⁻ patients. The bias was increased in 5% increments. The 2nd column shows the threshold level where the 95% HDI of the posterior distribution of COVID⁺ - COVID⁻ does not enclose zero, with the exact biases used for each group in parenthesis. The 3rd column shows the mean difference between the COVID⁺ and the COVID⁻ for that distribution, and the 4th column shows the range of the 95% HDI obtained. Values have been rounded to the nearest 5%.

Interpretation of supplemental table 4 and Bayesian analysis

To better understand the ramifications of our data as well as confirm our results, we performed a Bayesian analysis, which is more informative than null hypothesis statistical testing [32]. In Figure 1 of the manuscript, we show the distribution of posterior probabilities for the difference between the COVID⁺ and COVID⁻ cohorts who were positive for ANA, spAAB and aC-AAB. Since all the 95% credible HDI enclose zero, our data is compatible with the null hypothesis, confirming the results from the null hypothesis statistical testing. Importantly, this analysis used a non-informative prior; that is, we did not assume any previous knowledge of what the true state of nature is. This is fitting since there little is known on the prevalence of autoimmune phenomena among critically ill patients [5] and, to our knowledge, this is the first report on autoimmunity among severe COVID-19 patients that has a control group of similarly ill, critical patients. We then performed an exploratory prior sensitivity analysis assigning speculated, *a priori* differences between the COVID⁺ and COVID⁻ populations. Figure 1 of the main manuscript shows that, for our results to be compatible with the existence of a significant difference, it would be necessary to assume a prior probability of the COVID⁺ patients having a prevalence that is at least 15% higher than the COVID⁻ patients for acAAB, 20% higher for spAAB, and 35% higher for ANA. The resulting posterior mean differences would be in the order of 15 to 25% more prevalence among COVID⁺ than COVID⁻ (summarized in supplemental table 4, above).

Supplemental Figure 1: Anti-nuclear antibodies

1a: Association between anti-nuclear antibodies and outcomes

	APACHE II	Rescue days	MV days	ICU days	ICU death	SOFA 3d	SOFA 7d
ANA Y/N							
ANA titre							
AC19 and/or AC20 Y/N							

1b: Characterization of autoantibodies

COVID-									COVID+								
StudyID	Max(AC1)	Max(AC3)	Max(AC10)	Max(AC21)	Max(AC24)	Max(AC4/5)	Max(AC19/20)	Max(AC7/26/27/28)	StudyID	Max(AC1)	Max(AC3)	Max(AC10)	Max(AC21)	Max(AC24)	Max(AC4/5)	Max(AC19/20)	Max(AC7/26/27/28)
4						640	160		1		2560				1280	2560	
8						320			2			160			160	160	
9			160			160		320	5						160		
13	320				640			2560	6							160	
16						160			7				160		320		1280
19			1280	320		640	320		10			160			160	160	160
24						320			18			160					
36						640			20						640	160	
37					160	160			34						160		
41						160	160		39				160	1280	640		
48						320			44	160							
49					320				50				160	640	160		
55						640	320		52					1280	1280		
60	160			320		1280			53			640					640
63					320	320	160		54						640	320	160
									56						160		
									59			160			1280	320	

Abbreviations: ANA, anti-nuclear antibodies; AC, International Consensus on Autoantibody Patterns anti-cellular pattern nomenclature; APACHE, Acute Physiology and Chronic Health Evaluation (score); Max, end point titer; ICU, intensive care unit; MV, mechanical ventilation; SOFA, sequential organ failure assessment (score). For more details on the nomenclature used for the autoimmune assays please refer to the online materials and methods.

Legend

A) Association between anti-nuclear antibodies and outcomes. Refer to the legend of supplemental table 1 for details on the variables. AC19 is shown together with AC20 given their similarity, as is the common usage. The variables were compared in a pairwise fashion using ANOVA for continuous variables and Fisher's exact test for categorical variables at $\alpha=0.05$, followed by the false discovery rate at $q=0.05$; green squares indicate lack of association, red squares indicate statistically significant associations. Y/N denotes dichotomization into whether they were present "yes or no" (i.e. titer above 1:80). Similar to supplemental table 1, the ANA represent the results during longitudinal sampling up to day 10 of ICU admission, for standardization among patients. The clinical outcomes were measured for up to 3 months. The association between AC19 and/or AC20 with APACHE II had an FDR corrected p-value of 0.0008; the association with ICU days an FDR corrected p-value of 0.0143. B) Characterization of autoantibodies. Heatmap representation of the highest ANA titers during the first 3 months of admission. Includes more patients than in supplemental table 1 and supplemental figure 1a since some autoantibodies developed after 10 days. This may introduce bias since not all patients had prolonged hospitalizations but is a better representation of the dynamics and spectrum of ANA detected. Only patients with titers > 1:80 are included. AC4 is shown together with AC5, and AC19 is shown together with AC20, given their similarity, as is the common usage. AC7, 26, 27 and 28 are shown together given their rarity individually.

Interpretation:

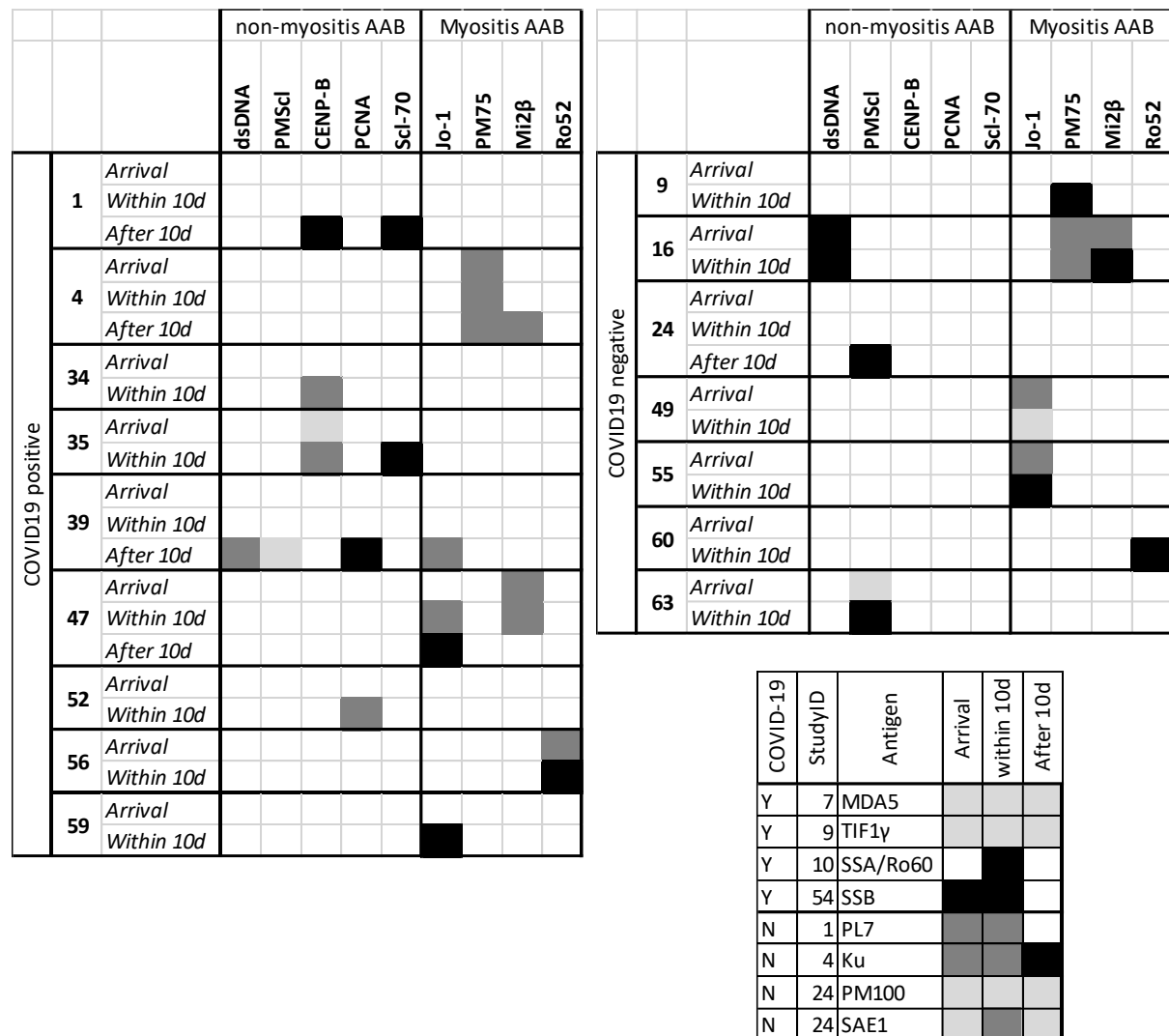
The presence of ANA in general were not significantly associated with disease severity, although there was a positive correlation with disease severity that did not reach statistical significance. Nevertheless, the presence of ICAP AC19 (cytoplasmic dense fine speckled) and/or AC20 (cytoplasmic fine speckled) IFA patterns, specifically, were consistently associated with worse severity of illness scores in both

288 patient groups. Neither the prevalence nor the IFA staining patterns were different between the COVID⁺
289 and COVID⁻ groups, although some patients demonstrated unique IFA patterns.

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Supplemental Figure 2: Evolution of specific autoantibody serology and titres over time.



Abbreviations: AAB, autoantibodies; CENP-B, centromere protein; dsDNA, double stranded DNA; Jo-1, histidyl tRNA synthetase; Ku, a component of the DNA-dependent protein kinase complex; Mi2β, a component of the nucleosome remodeling-deacetylase complex; MDA, melanoma differentiating antigen 1; PCNA, proliferating cell nuclear antigen; PL7, threonyl-tRNA synthetase; PM75/100, polymyositis-scleroderma (PM/Scl) overlap antigens part of the nucleolar PM/Scl complex; Ro52: tripartite motif 21 antigen; SAE1, component of the small ubiquitin like modifier activating enzyme heterodimer; Scl-70, DNA topoisomerase I; SSA/Ro60, Sjögren syndrome antigen A; SSB, Sjögren syndrome antigen B. For more details on the nomenclature used for the autoimmune assays please refer to the online materials and methods.

Legend:

The left panel shows the specific autoantibody serology of COVID+ patients, the right panel shows the specific autoantibody serology of COVID- patients, and the lower panel shows specific autoantibody that were detected on only one patient, shown separately for clarity. The titers were classified into low, medium and high levels using cutoffs established by the laboratory, shown in light grey, dark grey and black, respectively; white indicates negative. Arrival indicates the first sample obtained, either on the day of admission or the next morning. Within 10 d indicates during the longitudinal sampling, up to day 10 of ICU stay. After 10 d indicates later samples, collected every two weeks; not all patients had samples after 10 days. The following specific autoantibodies were not detected in any samples: histones, Sm/U2-U6 RNP, U1-RNP, ribosomal P, topoisomerase I, Mi2- α , MDA5, NXP2, PL7, PL12, SRP, EJ, OJ, HMGCR or NT5C1 A/Mup44 and SAR1.

Interpretation:

We compared the incidence of spAAB directed against autoantigens typically associated with systemic autoimmune diseases and myositis in critically ill COVID+ and COVID- patients. Patients who had a single, transient, low-titer spAAB result were not classified as having a positive test. Supplemental figure 2 shows that the presence of spAAB was similar in both COVID+ and COVID-. In addition, there was no difference between the temporal development of spAAB between the two groups, nor was there a correlation between the emergence of autoantibodies and SARS-CoV-2 seroconversion in the COVID+ group (not shown). Of note, most of these autoantibodies persisted beyond 10 days, with the longest measurement available up to 54 days of hospitalization. There was no correlation between spAAB with COVID-19 status (supplemental Table 1) or any of the measures of disease severity.

Online materials and methods

Study design. This report is part of the COVID-19 chapter of the Longitudinal Biomarkers in Lung Injury study group, being conducted at St. Michael's Hospital (Toronto, ON, Canada). This is an observational, prospective 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 failure, 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 (i.e. due to repatriation or lost to follow up – no patient in the study so far), 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. Data from these same 42 patients have been reported in another publication dealing with a separate subject (anticardiolipin antibodies and coagulation risk among COVID-19 patients [12]). 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. 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 and had a clear alternative diagnosis. Of note, 4 patients in the COVID⁺ cohort had an initial negative PCR test and were retested due to high clinical suspicion. In order 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). A HEp-2 indirect immunofluorescence assay (IFA) was used to detect anti-cellular antibodies (also referred to as anti-nuclear antibodies (ANA) – see

396 “nomenclature” below) [33] (NOVA Lite HEp-2, Inova Diagnostics, San Diego, CA) at a serum dilution of
397 1:80 and read on an automated instrument (Nova View, Inova Diagnostics) which interpolates
398 fluorescence intensity to an end point titer[34]. IFA staining patterns were classified according to the
399 International Consensus on Autoantibody Patterns (ICAP, <https://anapatterns.org/index.php>)[35]. All
400 samples were also tested for systemic autoimmune disease-related autoantibodies by a FIDIS
401 Connective13 addressable laser bead immunoassay (ALBIA, TheraDiag, Paris, France) detecting
402 antibodies to Sm/U2-U6 ribonucleoprotein (RNP), U1-RNP, SSA/Ro60, SSB/La, Ro52/Tripartite Motif
403 Protein 21 (TRIM21), histones, and ribosomal P, read on a Luminex 200 system using the MLX-Booster
404 software. A cut-off of >40 units was considered positive. Anti-dsDNA positivity and titers were detected
405 by a chemiluminescence test (Inova Diagnostics, San Diego, USA). A cut-off of <27 chemiluminescence
406 units was considered within normal range, 27-35 was indeterminate, and >35 was positive. All samples
407 were also tested for autoantibodies associated with autoimmune inflammatory myopathies using a
408 multiplexed solid phase line immunoassay: Ro-52/TRIM21, OJ, EJ, PL-12, PL-7, SRP, Jo-1, PM-75, PM-100,
409 Ku, SAE1, NXP2, MDA5, TIF1 γ , Mi-2 α , Mi-2 β (Euroimmun AG, Luebeck, Germany), and anti-NT5c1A by
410 addressable laser bead immunoassay[36]. The following anti-cytokine antibodies (acAAB) were assayed
411 using an addressable laser bead immunoassay (Millipore, Oakville, ON, Canada; HCYTAAB-17K-15) read
412 on a Luminex 200 system: BAFF, GMCSF, IFN- β , IFN- γ , IL-1 α , IL-6, IL-8, IL-10, IL-12p40, IL-15, IL-17a, IL-
413 17f, IL-18, IL-22 and TNF- α . The manufacturer’s thresholds were 500 for positive and 1000 for high-
414 positive (arbitrary units). All tests were performed according to the manufacturer’s instructions.

415 **Nomenclature.** There is considerable heterogeneity in the nomenclature of autoimmune assays in the
416 literature and clinical practice; therefore, we used the most contemporary usages. Autoantibodies is a
417 general term that encompasses all of the autoimmune humoral responses assayed. The HEp-2 IFA,
418 although including anti-cytoplasmic and anti-mitotic cell antibodies, are commonly referred as anti-
419 nuclear antibodies (ANA), and we have adopted that usage for clarity. Their classification uses the

different patterns identified as AC0, AC1, AC2, etc. according to the International Consensus on Autoantibody Patterns (ICAP: <https://anapatterns.org/index.php>)[37]. The autoantibody test results that identified specific, named antigens (see details above), were called collectively specific autoantibodies (spAAB). We have further separated them into myositis-related and non-myositis-related autoantibodies while acAAB are referred to directly.

Data analysis. All the data was organized by UT and analyzed by UT and ADB. 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 $\alpha=0.05$, adjusted for multiple comparisons as indicated in the text using the false discovery rate at $q=0.05$. Bayesian analysis: To evaluate the difference in the proportion of individuals who were positive for autoantibodies in COVID⁺ vs. COVID⁻ patients a Gibbs Markov chain Monte Carlo sampling algorithm was employed. For each variable, posterior parameter estimation in both groups was derived via the Bernoulli likelihood function, incorporating a uniform beta prior ($\alpha = 1, \beta = 1$). The simulations were run across 4 chains, each with 5000 iterations and a 1% burn-in (50 iterations removed per chain). Chain convergence was measured using the Gelman convergence statistic, with the accepted convergence value of all chains set at <1.01 [38]. Monte Carlo standard error was also calculated on all simulations to measure chain accuracy [39]. Briefly, for each iteration of the simulation, a credible parameter value was obtained for both groups: the parameters (θ) represented the probability of occurrence (success) for each variable, which in the current case is represented by autoantibody positivity. A posterior distribution of difference scores was also calculated ($\theta_2 - \theta_1$). From this posterior distribution, the mean difference value and 95% High Density Interval (HDI) were derived; the HDI was calculated according to the shortest credible interval method to account for potentially slight asymmetries in the distribution [32] Posterior predictive checks were employed; simulated data derived from the model parameter estimates were qualitatively

evaluated against the observed data parameters to evaluate fit. Results were considered significant for each variable if the 95% HDI of the difference between COVID⁺ vs. COVID⁻ patients did not enclose zero. Finally, an exploratory prior sensitivity analysis was conducted to estimate the effect different priors would have on the posterior distribution of ANA, spABB and ever high-positive acAAB; the priors represent varying pre-existing assumptions of the prevalence differences between COVID⁺ vs. COVID⁻ patients. Starting at 0% difference, beta priors were adjusted to represent the difference in positivity for both groups. A κ level equivalent to the sample size of each group (COVID⁺, $\kappa = 22$; COVID⁻, $\kappa = 20$), was used to allocate equal weight for the prior to the observed data. This avoids the introduction of arbitrary assumptions by conserving the structure of the observed data. The initial starting point was derived from the combined prevalence of each variable's positivity in all participants ($n = 42$, supplemental table 1). Prior bias was then allocated symmetrically. For example, the combined proportion of all patients who were ANA positive was 64%; a 10% difference skewed towards the COVID⁺ group was evaluated by adding 5% to COVID⁺ and subtracting 5% from COVID⁻ patients, resulting in a 69% vs 59% assumed prior prevalence of ANA. This was done in 5% increments until significant posterior distributions were found for all variables studied, up to a maximum of 40%. For clarity, we would like to reiterate that β priors are always weighted before they are multiplied by the link likelihood function. When performing the sensitivity analysis with serial priors, the κ weight was adjusted to ensure the prior never received more than 0.5 weighting on the posterior simulation draws. This was, again, in order not to overwhelm the posterior probabilities with priors assumptions, as they are exploratory. All statistical and graphical analyses were performed on R (RStudio, version 1.3.1093, Boston, United States) and JMP Pro (version 15.2.1; SAS Institute Inc, Cary, NC, USA).

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