

## ARTICLE

# COVID-19 and Immunological Dysregulation: Can Autoantibodies be Useful?

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Coronavirus disease 2019 (COVID-19) is often associated with interstitial pneumonia. However, there is insufficient knowledge on the presence of autoimmune serological markers in patients with COVID-19. We analyzed the presence and role of autoantibodies in patients with COVID-19-associated pneumonia. We prospectively studied 33 consecutive patients with COVID-19, 31 (94%) of whom had interstitial pneumonia, and 25 age-matched and sex-matched patients with fever and/or pneumonia with etiologies other than COVID-19 as the pathological control group. All patients were tested for the presence of antinuclear antibodies (ANAs), anti-antiphospholipid antibodies, and anti-cytoplasmic neutrophil antibodies (ANCA). Clinical, biochemical, and radiological parameters were also collected. Fifteen of 33 patients (45%) tested positive for at least one autoantibody, including 11 who tested positive for ANAs (33%), 8 who tested positive for anti-cardiolipin antibodies (immunoglobulin (Ig)G and/or IgM; 24%), and 3 who tested positive for anti- $\beta$ 2-glycoprotein antibodies (IgG and/or IgM; 9%). ANCA reactivity was not detected in any patient. Patients that tested positive for auto-antibodies had a significantly more severe prognosis than other patients did: 6 of 15 patients (40%) with auto-antibodies died due to COVID-19 complications during hospitalization, whereas only 1 of 18 patients (5.5%) who did not have auto-antibodies died ( $P = 0.03$ ). Patients with poor prognosis (death due to COVID-19 complications) had a significantly higher respiratory rate at admission (23 breaths per minute vs. 17 breaths per minute;  $P = 0.03$ ) and a higher frequency of auto-antibodies (86% vs. 27%;  $P = 0.008$ ). In conclusion, auto-antibodies are frequently detected in patients with COVID-19 possibly reflecting a pathogenetic role of immune dysregulation. However, given the small number of patients, the association of auto-antibodies with an unfavorable prognosis requires further multicenter studies.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✔ Coronavirus disease 2019 (COVID-19) is a pandemic and all researchers are committed to characterizing it in order to find the appropriate therapy, waiting for a resolutive vaccine.

### WHAT QUESTION DID THIS STUDY ADDRESS?

✔ This study evaluates the presence and clinical significance of non-organ specific auto-antibodies in the setting of patients with COVID-19.

### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

✔ Our experience shows a high frequency of auto-antibodies in patients with COVID-19 pneumonia and their presence seems to be associated with a poor prognosis.

### HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

✔ Our study needs to be confirmed in a large multicenter experience in order to better define the clinical significance of the auto-antibody positivity in this setting and to understand the possible role of immune dysregulation.

With almost 20 million cases and > 700,000 fatalities to date,<sup>1</sup> coronavirus disease 2019 (COVID-19) threatens healthcare and economic systems worldwide. This pandemic represents the worst pandemic since the 1918 Spanish flu. Initially described in China in December 2019,<sup>2</sup> COVID-19 is caused by a beta-coronavirus, severe acute

respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 shares genetic and clinical similarities with two other coronaviruses, SARS-CoV and Middle East respiratory syndrome-CoV, which were responsible for epidemics in 2003 and 2012, respectively. The overall mortality of COVID-19 ranged from 4.3% to 14.6% in preliminary Chinese

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studies.<sup>3-5</sup> The World Health Organization reports a worldwide mortality of 3.7%.<sup>1</sup> However, up to 29% of critically ill patients die in the hospital, and 50% of patients who receive invasive respiratory support are likely to die during treatment.<sup>6</sup> The predominant cause of death is severe lung failure due to bilateral interstitial pneumonia; acute and organizing diffuse alveolar damage, and SARS-CoV-2 persistence in the respiratory tract are the predominant histopathologic findings of postmortem examinations and the leading causes of death.<sup>7</sup> Multi-organ involvement is also observed.

Because of the rapid spread of SARS-CoV-2, physicians have not always been able to follow evidence-based medicine and standardized protocols. Further, the necessity of treating patients with a high risk of mortality has led many practitioners to make common sense-driven treatment decisions. Based on experience from the 2003 SARS and 2012 Middle East respiratory syndrome epidemics and the work of some centers, it was possible to establish some cardinal points on the pathogenesis and treatment of COVID-19:

1. The most common symptoms of COVID-19 are fever, fatigue, and respiratory symptoms, including cough, sore throat, and shortness of breath. Diarrhea and gastrointestinal symptoms are also reported, with 50% of patients having positive results of reverse transcriptase polymerase chain reaction (RT-PCR) tests in feces samples.<sup>8</sup>
2. Anywhere from 1.6% to 56.5% of patients may be asymptomatic.<sup>9-13</sup> Combined with the long incubation period (from 2–14 days), this may account for the high level of contagion and global spread.
3. COVID-19 pneumonia is associated with lung damage, and acute respiratory distress syndrome and robust interferon immunosuppression with lymphopenia are part of the virally induced immunosuppression.<sup>8</sup> This loss of “front-line” antiviral defenses may activate a “second wave” of more tissue-aggressive immunity, including exaggerated interleukin-6 (IL-6) production with a secondary cytokine storm and tissue damage.<sup>14</sup> This cytokine storm may play a major role in the pathogenesis of the second phase of COVID-19, initiating viral sepsis and inflammation-induced lung injury that leads to other complications, including acute respiratory distress syndrome, organ failure, and death.<sup>15</sup>
4. Several studies have reported COVID-19-related coagulopathy correlating with severe viral infection.<sup>5,16,17</sup> Elevated D-dimer has been described as an independent biomarker for poor prognosis.<sup>17</sup> In a recent report, persistently elevated D-dimer levels during hospitalization correlated with poor prognosis, intensive care unit (ICU) admission, and death.<sup>18</sup>

Based on these assumptions, some have evaluated immunological biomarkers, such as lupus anticoagulant and antiphospholipid antibodies (APLs) to explore the mechanisms underlying this COVID-19-associated coagulopathy. Harzallah and colleagues reported the presence of lupus anticoagulant in at least 50% of their patients; 5 patients also had either anticardiolipin or anti- $\beta$ 2-glycoprotein I

antibodies.<sup>19</sup> In another case report,<sup>20</sup> Zhang and colleagues described 3 cases of multiple cerebral infarctions in patients with severe COVID-19; all 3 patients were positive for anticardiolipin immunoglobulin (Ig)A and anti- $\beta$ 2-glycoprotein I IgM and IgG. All three patients had a history of cardiovascular disease, and two had previously experienced strokes, although it is unclear whether they discontinued antiplatelet/anticoagulant therapy upon admission. APLs have been described in a variety of viral infections<sup>21</sup>; these are usually transient and not associated with thrombotic complications.

However, there is insufficient knowledge on the presence of other autoimmune serological markers in patients with COVID-19. The only study that has evaluated antinuclear antibodies (ANAs) in patients with COVID-19 described the presence of ANAs in 50% of the cases.<sup>22</sup> The authors also described the presence of anti-52 kDa SSA/Ro antibodies and anti-60 kDa SSA/Ro antibodies in 20% and 25% of cases, respectively. No studies have evaluated the presence of antineutrophil cytoplasmic antibodies (ANCA). The aim of our study was to evaluate the presence of auto-antibodies, such as APLs, ANAs, and ANCA in patients with COVID-19 and examine their frequency and clinical significance in COVID-19 pneumonia.

## METHODS

### Ethical approval

This study was approved by the local ethics committee. Oral or written consent was obtained from the patients.

### Patients and methods

From March 30, 2020, to May 10, 2020, we prospectively enrolled 47 consecutive patients referred to our hospital for suspected SARS-CoV-2 infection. All patients were evaluated at admission by clinical and respiratory parameters (blood pressure, heart rate, respiratory rate, and arterial blood gas). Complete laboratory panels were obtained from all patients (white blood cells, hemoglobin, platelets, transaminases, bilirubin, urea, creatinine, D-dimer, international normalized ratio, partial thromboplastin time, immunoglobulins, lactate dehydrogenase, IL-6, RCP, and ferritin), and every patient underwent high-resolution lung computed tomography. Fourteen patients were excluded from the study due to repeated negativity of SARS-CoV-2 RT-PCR results, and 33 patients were included for analysis.

Clinical, laboratory, and radiological characteristics and treatment and outcome data were inserted daily in a dedicated database from the electronic medical records.

We also included 25 age-matched and sex-matched patients with fever and/or pneumonia with etiologies other than COVID-19 as the pathological control group.

### SARS-CoV-2 infection diagnosis

Throat and nose swab specimens were collected for the extraction of SARS-CoV-2 RNA from patients suspected of infection. After collection, throat and nose swabs were placed in a tube with 150  $\mu$ L of virus storage solution, and RT-PCR was performed by the Microbiology Department.

### Autoimmune serological tests

All samples were tested by indirect immunofluorescence assay on HEp-2 cells (Euroimmun, Lübeck, Germany) with a screening dilution of 1:80 for the detection and characterization of ANAs. Samples were tested for the most common ANAs and for antibodies to extractable nuclear antigens (ENAs) by immunoblot (Euroimmun) that is specific and validated for the following reactivities: Sm and RNP/Sm; RNP70, A, and C; SSA-Ro52; SSA-Ro60; SSB; Scl-70; PM-Scl; Jo-1; CENP-B; PCNA; dsDNA; nucleosomes; histones; ribosomal P protein; and M2.

Detection of ANCA was performed by indirect immunofluorescence assay on ethanol-fixed and formalin-fixed human granulocytes (Euroimmun) with a screening dilution of 1:20. Detection of antibodies to proteinase 3 and myeloperoxidase was performed using FEIA (Thermo Fisher). Anti- $\beta$ 2-glycoprotein 1 and anticardiolipin antibodies (IgG and IgM) were also assessed using FEIA.

### Treatment

All patients received hydroxychloroquine at a loading dose of 400 mg b.i.d. for the first day and 200 mg b.i.d. for the following 4 days. All patients received subcutaneous enoxaparin, 24 (72.7%) at 4,000 IU per day and 9 (27.2%) at 8,000 IU per day. Twenty-four patients (72.7%) received antibiotic therapy (ceftriaxone, azithromycin, or piperacillin/tazobactam, depending on severity, allergies, and other diseases accompanying COVID-19). Seventeen patients (51.5%) received methylprednisolone at different dosages (from 0.25 mg/kg/day to 1 mg/kg/day).

Ten patients (30.3%) were treated with the anti-IL6R monoclonal antibody tocilizumab (two subcutaneous administrations).

### Statistical analysis

Categorical variables were compared using  $\chi^2$  and Fisher's exact test when appropriate. Comparison of continuous variables was performed using the Mann-Whitney test. A *P* value < 0.05 was considered significant. Statistical analysis was performed using both GraphPad InStat 3.0a for Macintosh (GraphPad Software, San Diego, CA) and SPSS for Windows 10.0.07 (SPSS, Chicago, IL).

## RESULTS

### Patient characteristics

The clinical and laboratory parameters at baseline are shown in **Table 1**. Sixteen (48.4%) of the 33 patients were women, and 17 (51.5%) were men. The median age of the patients was 70 years. Twenty-six (78.7%) of 33 patients were admitted to the hospital and discharged after recovery, and 7 (21.2%) died during hospitalization.

### Autoimmune antibody positivity

Fifteen of 33 patients (45.4%) had at least one autoimmune reactivity: 11 (33.3%) had ANA reactivity, 8 (24.2%) had anticardiolipin antibody (IgG and/or IgM) reactivity, and 3 (9.1%) had anti- $\beta$ 2-glycoprotein I antibody (IgG and/or IgM) reactivity. ANCA reactivity was not detected in any patient. Of the ANA-positive cases, 4 (36.3%) had nucleolar staining, 4 (36.3%) had speckled staining, 2 (18.1%)

**Table 1** Clinical and laboratory characteristics of patients at baseline

	Patients with COVID-19 (n = 33)
Female sex	16 (48.4%)
Age, years, median (range)	70 (22–90)
pH median (range)	7.49 (7.40–7.58)
pO <sub>2</sub> , mmHg, median (range)	71 (51–89)
pCO <sub>2</sub> , mmHg, median (range)	33 (18–40)
Lactate, mmol/L, median (range)	1 (0.5–1.4)
P/F median (range)	338 (229–426)
A-a O <sub>2</sub> gradient, mmHg, median (range)	41 (19–73.2)
SBP, mmHg, median (range)	120 (100–180)
DBP, mmHg, median (range)	70 (53–90)
HR, bpm, median (range)	86 (55–115)
Oxygen saturation % median (range)	96 (85–99)
Respiratory rate, minutes, median (range)	20 (8–32)
Temperature, °C, median (range)	36.8 (36–39.5)
Interstitial pneumonia on chest HRCT	31 (94%)
Deaths related to COVID-19	7 (21%)
WBC × 10 <sup>9</sup> /L median (range)	6.17 (2.71–15.69)
Lymphocytes × 10 <sup>9</sup> /L median (range)	1.05 (0.25–3.93)
Hb gr/dL median (range)	11.9 (7–16)
PLT × 10 <sup>9</sup> /L median (range)	211 (70–528)
ALT U/L median (range)	26 (8–174)
LDH U/L median (range)	242 (144–1151)
Creatinine mg/dL median (range)	0.93 (0.44–2.3)
CRP mg/dL median (range)	4 (0.18–23)
IL-6 pg/mL, ULN < 5.9, median (range)	20 (2.6–175)
Ferritin ng/mL median (range)	318 (36–6488)
IgG mg/dL median (range)	926 (419–1470)
IgA mg/dL median (range)	203 (21–571)
IgM mg/dL median (range)	70 (28–256)
D-dimer $\mu$ g/mL, ULN < 0.45, median (range)	0.98 (0.22–8.35)
aPTT ratio median (range)	1.02 (0.7–1.58)
INR ratio median (range)	1.10 (0.98–1.34)

ALT, alanine transferase; aPTT, activated partial thromboplastin time; COVID-19, coronavirus disease 2019; CRP, C reactive protein; DBP, diastolic blood pressure; Hb, hemoglobin; HR, heart rate; HRCT, high resolution computed tomography; Ig, immunoglobulin; IL-6, interleukin 6; INR, international normalized ratio, LDH, lactate dehydrogenase; PLT, platelet; SBP, systolic blood pressure; ULN, upper limit of normal; WBC, white blood cells.

had indeterminate staining, and 1 (9.1%) had homogenous staining (**Table 2**). The median titer of ANA reactivities was 1:640 (range 1:160–1:5120). Of the APL-positive cases, we observed anticardiolipin IgG positivity in five cases, anticardiolipin IgM positivity in six cases, anti- $\beta$ 2-glycoprotein IgG positivity in two cases, and anti- $\beta$ 2-glycoprotein IgM positivity in two cases, with some overlap (**Table 3**).

There were three patients with seropositivity in the control group. Two patients tested positive for ANAs with a speckled pattern (titer 1:80), and one patient was positive for anticardiolipin IgG (34 GPL/mL). The frequency of autoantibody positivity in the COVID-19 group was significantly higher than that in the control group (45% vs. 12%; *P* = 0.03).

**Table 2 Autoimmune serological markers**

	Patients with COVID-19 (n = 33)
ANAs (all patterns)	11 (33.3%)
Nucleolar	4 (36.3%)
Speckled	4 (36.3%)
Homogeneous	1 (9%)
Undetermined	2 (18.1%)
APLs (all antibodies) <sup>a</sup>	8 (24.2%)
Anticardiolipin IgG	3 (37.5%)
Anticardiolipin IgM	5 (62.5%)
Anti-β2-glycoprotein IgG	2 (25%)
Anti-β2-glycoprotein IgM	2 (25%)

Data are presented as n (%).

ANAs, anti-nuclear antibodies; APLs, antiphospholipid antibodies; COVID-19, coronavirus disease 2019; Ig, immunoglobulin.

<sup>a</sup>APL-positive patients presented with positivity of one or more APLs (see Table 3).

**Correlation of autoantibody positivity with clinico-pathologic features and outcome**

The autoantibody-positive subgroup had higher lactate values than the autoantibody-negative subgroup (1 mmol/L vs. 0.86 mmol/L; *P* = 0.03). Additionally, the autoantibody-positive subgroup had a significantly more severe prognosis; 6 of 15 patients in this subgroup (40%) died due to COVID-19 complications, whereas only 1 of 18 patients (5.5%) in the autoantibody-negative subgroup died (*P* = 0.03; Table 4).

Nine of 33 patients (27.2%) enrolled in the study were admitted to the ICU, 2 of whom died in the ICU. Both of the patients who died were seropositive for ANAs.

We ultimately divided our patients into those with a good prognosis and those with a poor prognosis (death due to COVID-19 complications). The subgroup with a poor prognosis had a significantly higher prevalence of auto-antibodies than the subgroup with a good prognosis (86% vs. 27%; *P* = 0.008). Furthermore, patients with a poor prognosis had a significantly higher respiratory rate (23 breaths per minute vs. 16 breaths per minute; *P* = 0.03) upon admission and higher IL-6 and serum C-reactive protein levels, although these differences were not significant (Table 5).

The raw data of each patient (both study and control populations) are reported in the **Supplementary Excel File**.

**Table 3 Autoantibody profiles of APL-positive patients**

	ACL IgG	ACL IgM	Ab2GP IgG	Ab2GP IgM
Patient 1	68	81	–	–
Patient 2	–	56	–	65
Patient 3	74	49	–	–
Patient 4	–	55	–	–
Patient 5	–	83	48	76
Patient 6	–	79	–	–
Patient 7	–	–	91	–
Patient 8	–	–	–	56

Positivity > 40 GPL or MGL/mL for ACL and > 40 U/mL for Ab2GP.

ACL, anti-cardiolipin antibody; Ab2GP, anti-β2-glycoprotein antibody; Ig, immunoglobulin.

**Table 4 Autoantibodies-positive patients vs. autoantibodies-negative patients**

	ANA and/or APL + (n = 15)	ANA/APL – (n = 18)	<i>P</i> value
Female sex	7 (44%)	9 (50%)	0.7
Age, years, median (range)	71 (48–96)	69 (22–88)	0.3
pH median (range)	7.50 (7.40–7.58)	7.46 (7.40–7.51)	0.08
pO <sub>2</sub> , mmHg, median (range)	62 (51–82)	72 (57–89)	0.3
pCO <sub>2</sub> , mmHg, median (range)	33 (21–40)	34 (28–39)	0.4
Lactate mmol/L median (range)	<b>1 (0.9–1.4)</b>	<b>0.86 (0.5–1.4)</b>	<b>0.02</b>
P/F median (range)	295 (229–390)	387 (242–426)	0.9
A-a O <sub>2</sub> gradient mmHg median (range)	43 (27–63)	41 (19–73.2)	0.8
SBP, mmHg, median (range)	130 (100–180)	120 (100–155)	0.1
DBP, mmHg, median (range)	70 (60–90)	80 (53–85)	0.9
HR bpm median (range)	96 (70–110)	85 (55–115)	0.06
Oxygen saturation % median (range)	96 (87–99)	97 (85–99)	0.5
Respiratory rate, minutes, median (range)	20 (8–28)	17 (14–32)	0.4
Temperature, °C, median (range)	37 (36–39.5)	36.6 (36–38)	0.3
WBC × 10 <sup>9</sup> /L median (range)	6.02 (2.8–16)	6.3 (2.7–14.5)	0.9
Lymphocytes × 10 <sup>9</sup> /L median (range)	0.82 (0.25–3.93)	1.21 (0.25–10)	0.3
Hb gr/dL median (range)	13 (10.3–16)	11.6 (7–15)	0.1
PLT × 10 <sup>9</sup> /L median (range)	169 (81–311)	239 (70–528)	0.1
ALT U/L median (range)	21 (8–54)	30 (15–174)	0.1
LDH U/L median (range)	244 (144–1151)	243 (178–481)	0.9
Creatinine mg/dL median (range)	0.9 (0.5–2.3)	0.94 (0.44–1.49)	0.1
CRP mg/dL median (range)	3.6 (0.19–23)	4.8 (0.18–17.8)	0.8
IL-6 pg/mL (ULN < 5.9) median (range)	35.8 (9–175)	18.85 (2.7–173)	0.7
Hospitalization duration (days)	19 (3–28)	17 (5–19)	0.6
D-dimer μg/mL (< 0.45) median (range)	0.54 (0.34–3.92)	1.53 (0.19–8.35)	0.07
aPTT ratio median (range)	1 (0.7–1.51)	1.07 (0.7–1.58)	0.4
INR ratio median (range)	1.09 (1–1.34)	1.10 (0.8–131)	0.9
Death (%)	<b>6 (40%)</b>	<b>1 (5.5%)</b>	<b>0.03</b>

ALT, alanine transferase; IL-6, interleukin 6; ANA, antinuclear antibodies; APL, antiphospholipid antibodies; aPTT, partial thromboplastin time; CRP, C reactive protein; DBP, diastolic blood pressure; Hb, hemoglobin; HR, heart rate; INR, international normalized ratio; LDH, lactate dehydrogenase; PLT, platelet; SBP, systolic blood pressure; WBC, white blood cell. Bold indicates statistically significant results.

**DISCUSSION**

In our single-center study, 45.4% (15 of 33) of patients with COVID-19 had reactivity to at least one autoantibody. Specifically, 33.3% (11 patients) of the patients had ANA reactivity, characterized in one patient by anti-histone



**Table 5 Outcome of the hospitalization: Recovery vs. death**

	Patients discharged (n = 26)	Patients dead (n = 7)	P value
Female sex	10 (38.4%)	4 (57.1%)	0.7
Age, years, median (range)	69 (22–96)	74 (59–90)	0.1
pH median (range)	7.49 (7.40–7.58)	7.48 (7.47–7.50)	0.6
pO <sub>2</sub> mmHg median (range)	70 (55–89)	66 (51–77)	0.3
pCO <sub>2</sub> mmHg median (range)	33 (18–40)	37 (27–38)	0.3
Lactate mmol/L median (range)	1 (0.5–1.4)	1.10 (0.9–1.4)	0.07
P/F median (range)	327 (229–427)	314 (242–366)	0.9
A-a O <sub>2</sub> gradient mmHg median (range)	40 (18–73.2)	32 (26–63)	0.6
SBP, mmHg, median (range)	120 (100–180)	135 (100–180)	0.1
DBP, mmHg, median (range)	70 (53–90)	75 (60–90)	0.9
HR bpm median (range)	85 (55–115)	75 (60–90)	0.07
Oxygen saturation % median (range)	97.5 (87–99)	95 (85–98)	0.06
Respiratory rate, minutes, median (range)	<b>16 (8–32)</b>	<b>23 (16–28)</b>	<b>0.04</b>
Temperature, °C, median (range)	36.6 (36–39.5)	37.1 (36.2–38.4)	0.3
WBC × 10 <sup>9</sup> /L median (range)	5.9 (2.7–15.6)	9.0 (2.8–16)	0.4
Lymphocytes × 10 <sup>9</sup> /L median (range)	1.19 (0.25–10)	0.72 (0.47–3.93)	0.1
Hb gr/dL median (range)	11.8 (7–15.2)	13 (9.4–14)	0.5
PLT × 10 <sup>9</sup> /L median (range)	199 (70–528)	314 (86–488)	0.1
ALT U/L median (range)	30 (15–174)	18 (13–31)	0.1
LDH U/L median (range)	240 (178–1151)	301 (144–503)	0.7
Creatinine mg/dL median (range)	0.9 (0.4–1.5)	1.03 (0.5–2.3)	0.1
CRP mg/dL median (range)	3.6 (0.19–23)	4.8 (0.18–17.8)	0.8
IL-6 pg/mL (ULN < 5.9) median (range)	22.8 (2.7–175)	26 (13.8–173)	0.7
Ferritin ng/mL median (range)	304 (59–2371)	320 (36–6488)	0.5
Hospitalization duration (days)	19 (3–28)	17 (5–19)	0.6
D-dimer µg/mL (< 0.45) median (range)	0.89 (0.22–8.35)	1.07 (0.35–1.93)	0.6
aPTT ratio median (range)	0.97 (0.7–1.58)	1.05 (0.8–1.13)	0.9
INR ratio median (range)	1.1 (0.98–1.34)	1.09 (1.1.18)	0.9
ANA and/or APL	<b>7 (26.9%)</b>	<b>6 (85.7%)</b>	<b>0.008</b>
APLs	<b>4 (15.4%)</b>	<b>4 (57.1%)</b>	<b>0.03</b>
ANA	7 (26.9%)	4 (57.1%)	0.1

ALT, alanine transferase; ANA, antinuclear antibodies; APL, antiphospholipid antibodies; aPTT, activated partial thromboplastin time; CRP, C reactive protein; DBP, diastolic blood pressure; Hb, hemoglobin; HR, heart rate; IL-6, interleukin 6; INR, international normalized ratio; LDH, lactate dehydrogenase; PLT, platelet; SBP, systolic blood pressure; ULN, upper limit of normal; WBC, white blood cell. Bold indicates statistically significant results.

antibodies on specific ENAs. A recent study from China<sup>22</sup> including 21 patients documented a slightly higher prevalence of ANA positivity (50% vs. 33%). However, we examined ANA positivity by indirect immunofluorescence, whereas that study examined reactivity by ANA immunoblot. Furthermore, the studies enrolled 2 different subsets of patients with COVID-19. In the Chinese study, all patients were in severe (38.1%) or critical (61.9%) condition and were in the ICU of the Huangshi Central Hospital. Conversely, we selected patients admitted from the Emergency Department to a General Medicine Unit or to a Subintensive Care Unit. Nine patients (27.2%) were later admitted to the ICU, only four of whom died. We only observed seven deaths (21.2%) in this study; this is in line with the overall in-hospital mortality rates from larger studies (24%<sup>23</sup> and 28%<sup>17</sup>). Furthermore, the aforementioned Chinese study<sup>22</sup> described a subgroup of patients with anti-ENA positivity (specifically anti-SSA Ro52 and anti-SSA Ro60), which we did not observe in our study. This discrepancy among reactivities could be attributable to the

low numbers of patients enrolled in the two studies and to the different genetic backgrounds of the populations, which could lead to different antibody phenotypes. It is of interest that the prevalence of auto-antibodies was higher in critical cases of COVID-19 than in less severe cases in both of these studies.

The nucleolar pattern of ANAs is often associated with the interstitial pneumonia that characterizes the clinical course of systemic sclerosis.<sup>24</sup> Samples from four of our patients showed nucleolar staining of ANAs, and these patients also had a radiological definitive diagnosis of interstitial pneumonia, but none had reactivity against O Scl-7. Such reactivity usually represents the target antigen in diffuse systemic sclerosis.

We observed an overall frequency of APLs of > 21% (7 cases), which is at least 2-fold more than that reported by Harzallah and colleagues in a series of 56 patients with COVID-19.<sup>14</sup> Furthermore, the presence of APLs in this study was significantly associated with poor prognosis. Unlike Tang *et al.*,<sup>16</sup> who identified coagulopathy as an

independent negative prognostic factor, we did not observe any difference in these parameters among patients with poor prognosis and those with good prognosis. This difference could be due, at least in part, to the different selection criteria and is also likely affected by the lower number of cases in our series (183 vs. 33). However, we did observe a significant association between autoreactivities and poor prognosis in our series; all but one of our severely ill patients had at least one autoreactivity (Table 5).

The majority of post-infectious APLs differ immunohistochemically from those seen in patients with autoimmune disease.<sup>25</sup> Infection-induced APLs have been traditionally regarded as transient phenomena and are generally not associated with clinical features of anti phospholipid syndrome (APS). However, this classification has been challenged by reports describing thrombotic events following infection and in particular by the association of the most aggressive form of APS, catastrophic APS, with infectious triggers.<sup>26,27</sup> None of our patients experienced thrombotic complications, which suggests that our findings are compatible with an epiphenomenon of infection-induced immune dysregulation. Therefore, our findings should be evaluated as part of the complex viral-host interaction rather than an independent autoimmune phenomenon.

It is of particular interest that none of our patients had ANCA reactivity. ANCAs are reactive against multiple antigens in the cytoplasmic and perinuclear regions in neutrophils and monocytes. The most prevalent ANCAs in ANCA-associated vasculitis target myeloperoxidase and proteinase 3 and are strongly associated with small vessel vasculitis.<sup>28</sup> It is believed that, in genetically predisposed patients, the first neutrophil immune response may lead to antigen presentation and chronic immune dysregulation in small vessel vasculitis.<sup>28</sup> However, the immunopathology of vasculitis is still unclear. Several models have suggested that T-lymphocytes may play a leading role in the loss of tolerance and development of autoimmunity. Additionally, some studies have evaluated the cytokine profile in these patients, showing that the cytokines IL-6, IL-10, TNF- $\alpha$ , IFN- $\gamma$ , and IL-17A are increased in patients with adeno-associated virus infection.<sup>29,30</sup> The lack of expression of these markers in our study may be because of the small number of cases. Additionally, our serological tests may have been performed too early to detect antigen presentation and the subsequent rise in autoreactivity titers. By contrast, it is possible that the initial viral immunosuppression may decrease the first neutrophil response and inhibit ANCA production.

Our study has some limitations. It would be inappropriate and risky to attribute clinical significance to the high frequency of autoreactivities among patients with a poor prognosis because of the low number of patients, the possibility of an insignificant and transient epiphenomenon,<sup>31</sup> and the possibility that, at least in some cases, the death can be attributed, at least in part, to a concomitant disease rather than COVID-19.

However, we believe that because no standardized treatment for COVID-19 has been identified, it would be useful to characterize the immunopathology of SARS-CoV-2 in order to develop biological models that could shed light on possible

future strategies. Our results need to be confirmed by a large multicenter study in order to define the role of self-reactivity and autoimmunity in the context of this new viral infection.

**Supporting Information.** Supplementary information accompanies this paper on the *Clinical and Translational Science* website ([www.cts-journal.com](http://www.cts-journal.com)).

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