



ARTICLE

SARS-CoV-2 infection as a trigger of autoimmune response

Maria C. Sacchi¹ | Stefania Tamiazzo¹ | Paolo Stobbione² | Lisa Agatea³ |
Piera De Gaspari⁴ | Anna Stecca³ | Ernesto C. Lauritano⁵ | Annalisa Roveta⁶ |
Renato Tozzoli⁷ | Roberto Guaschino¹ | Ramona Bonometti⁵

¹Autoimmunology and Analysis

Laboratory Unit, "SS Antonio e Biagio e Cesare Arrigo" Hospital, Alessandria, Italy

²Rheumatology Unit, "SS Antonio e Biagio e Cesare Arrigo" Hospital, Alessandria, Italy

³Laboratory Department, Affiliated to Euroimmun, Padova, Italy

⁴Neuroimmunology Laboratory, Pediatric Research Institute, Città della Speranza Foundation, Padova, Italy

⁵Emergency Medicine Unit, "SS Antonio e Biagio e Cesare Arrigo" Hospital, Alessandria, Italy

⁶IRFI (Infrastruttura Ricerca Formazione Innovazione) "SS Antonio e Biagio e Cesare Arrigo" Hospital, Alessandria, Italy

⁷Endocrinology Unit, S. Giorgio Hospital, Pordenone, Italy

Correspondence

Maria C. Sacchi, Autoimmunology and Analysis Laboratory Unit, "SS Antonio e Biagio e Cesare Arrigo" Hospital, Alessandria, Italy.

Email: csacchi@ospedale.al.it

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Abstract

Currently, few evidences have shown the possible involvement of autoimmunity in patients affected by coronavirus disease 2019 (COVID-19). In this study, we elucidate whether severe acute respiratory syndrome coronavirus disease 2 (SARS-CoV-2) stimulates autoantibody production and contributes to autoimmunity activation. We enrolled 40 adult patients (66.8 years mean age) admitted to Alessandria Hospital between March and April 2020. All the patients had a confirmed COVID-19 diagnosis and no previously clinical record of autoimmune disease. Forty blood donors were analyzed for the same markers and considered as healthy controls. Our patients had high levels of common inflammatory markers, such as C reactive protein, lactate dehydrogenase, ferritin, and creatinine. Interleukin-6 concentrations were also increased, supporting the major role of this interleukin during COVID-19 infection. Lymphocyte numbers were generally lower compared with healthy individuals. All the patients were also screened for the most common autoantibodies. We found a significant prevalence of antinuclear antibodies, antineutrophil cytoplasmic antibodies, and ASCA immunoglobulin A antibodies. We observed that patients having a *de novo* autoimmune response had the worst acute viral disease prognosis and outcome. Our results sustain the hypothesis that COVID-19 infection correlates with the autoimmunity markers. Our study might help clinicians to: (a) better understand the heterogeneity of this pathology and (b) correctly evaluate COVID-19 clinical manifestations. Our data explained why drugs used to treat autoimmune diseases may also be useful for SARS-CoV-2 infection. In addition, we highly recommend checking patients with COVID-19 for autoimmunity markers, mainly when deciding on whether to treat them with plasma transfer therapy.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Recent data sustain the idea that autoimmune phenomena exist in patients with coronavirus disease 2019 (COVID-19), but other investigations are necessary to define the possible link between severe acute respiratory syndrome coronavirus disease 2 (SARS-CoV-2) infection and autoimmune disease onset.

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WHAT QUESTION DID THIS STUDY ADDRESS?

☑ In this monocentric study, we demonstrated how SARS-CoV-2 infection could be associated with an autoimmune response and development of autoantibodies.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Patients with COVID-19 having an increased level of inflammatory markers and strong autoantibodies positivity (i.e., antinuclear antibodies and antineutrophil cytoplasmic antibodies) presented the worst clinical outcome.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ These results suggest that the drugs normally used to treat autoimmune diseases should also be considered during SARS-CoV-2, improving public health. In addition, before starting a transfer plasma therapy, it is important to also evaluate the autoimmunity conditions of the patients with COVID-19. Transferring antibodies or trying to neutralize them should be done with precaution. It is possible that the risk of developing or increasing the autoimmune response may enhance.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) currently represents the dominating pandemic emergency, due both to the lack of specific therapies and to the high virulence of its pathogen. Patients affected by COVID-19 are characterized by different manifestations. Although some subjects present flu-like symptoms, such as fever, cough, fatigue, and dyspnea, others may develop a dire clinical picture, defined by severe acute respiratory syndrome (SARS-CoV-2), sepsis, and multiorgan failure: in the latter case, the risk of death increases. Based on the clinical features, SARS-CoV-2 infection can be divided in two stages: (1) the replicative stage and (2) the adaptive autoimmunity stage. These two phases may explain the heterogeneity of COVID-19 pathology.¹ The first stage involves viral replication and innate immune response and it is usually associated with mild symptoms. In the second stage, the adaptive immune response occurs, increasing circulating cytokines and tissue damage. It has been observed that, in ~ 20% of patients with COVID-19, the infection progresses to acute respiratory distress syndrome inducing an abnormal and aberrant host-immune response, the so-called “cytokine storm.”² As a result, the evolution of COVID-19 disease highly resembles the cytokines release syndrome (CRS). Furthermore, both patients with CRS and COVID-19 present high levels of different chemokines and inflammatory mediators, such as IL-6, INF- γ , and tumor necrosis factor (TNF). CRS syndrome is generally associated with monoclonal antibody treatments³; although, a massive immune system response is also present in autoimmune diseases too.⁴

A fundamental characteristic of the immune system is its ability to distinguish self-structures from the non-self-structures (e.g., foreign pathogens). If the immune system fails to recognize self-components, it produces autoantibodies against the body’s cells, tissues, or organs, causing an inflammation, which in turn leads to autoimmune disease.⁵ Viral

pathogens are known to be one of the most common exogenous factors able to trigger autoimmunity.⁶ Specific types of viruses can cause widespread nonspecific lymphocytes B and T activation, promoting the production of autoantibodies and cytokines production. For instance, the presence of Epstein Barr Virus and Parvovirus B19 correlates with Hashimoto’s thyroiditis,⁷ human T-lymphotropic virus-1, and Human Foamy Virus with Graves’ disease,⁸ and herpes simplex with postinfectious autoimmune encephalitis.⁹

Until a few months ago, there was no evidence to support the hypothesis of a correlation between autoimmunity and COVID-19 disease. As of today, only a couple of studies have shown the presence of autoantibodies in patients with SARS-CoV-2. In April 2020, Zhang and colleagues described that patients with COVID-19, affected by coagulopathy and multiple thrombi, were positive for anti-Cardiolipin IgA antibodies, as well as anti- β 2-Glycoprotein IgA and IgG antibodies.¹⁰ In the paper, the authors firmly sustained that antiphospholipid antibodies might be the reason of the thrombotic events. Two months later, Vlachoyiannopoulos *et al.* analyzed 29 Greek patients and found that almost 70% had developed an autoimmune activation as a consequence of SARS-CoV-2 infection.¹¹

Although these data support the idea that autoimmunity could be triggered by COVID-19 infection, more recently, molecular mimicry has also been proposed as a cause of autoimmune phenomena in patients with COVID-19.¹² Therefore, further investigations are required and necessary to clearly define the possible interaction between autoimmune disease onset and SARS-CoV-2 infection.

In this perspective, our study aims to clarify how autoimmunity can be affected by SARS-CoV-2. Our data sustain the existence of a link between COVID-19 infection and autoimmune activation. Based on this study, we suggest to take into appropriate consideration the autoimmune sphere in patients with COVID-19, also in view of the possible use of transferring plasma as therapy.

METHODS

Ethics committee approval

This study was approved by the ethics committee of “SS Antonio and Biagio and Cesare Arrigo” Hospital, Alessandria, Italy. The research study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Study participants

Between March 17 and April 6, 2020, we enrolled 40 adult patients (28 men and 12 women), age 20–97 years, admitted to Alessandria Hospital in Northern Italy. All the patients were positive for SARS-CoV-2. The presence of infection was confirmed by real-time reverse-transcriptase-polymerase chain reaction (PCR) on nasopharyngeal swab samples (Cobas SARS-CoV-2 kit, Roche; Cobas 6800 Roche). All patients were hospitalized in our center. All the samples analyzed in this study were collected after hospital admission. In addition, we selected as reference controls 40 blood donors aged 24–60 years, declaring the exclusion of any previously established autoimmune disorders.

Laboratory measurements

Blood biochemical indicators

The number of lymphocytes was determined using a flow cytometry system (Advia 2120i; Siemens).

Blood biochemical analyses, such as creatinine (mg/dL), lactate dehydrogenase (LDH, U/L), CRP (mg/dL), complement 3 (C3, mg/dL), and complement 4 (C4, mg/dL) were measured using a fully automated spectrophotometric/immunoturbidimetric and ion selective electrode measurement system (Advia XPT analyzer; Siemens). Ferritin (ng/mL) and the inflammatory marker IL-6 (mg/dL) were detected using Siemens immunoassay systems (Ferritin: Centaur XPT; IL6: Immulite 2000 XPI).

Blood autoimmunity tests

Antiphospholipid antibodies (anti-cardiolipin, anti- β 2-glycoprotein IgA, and IgG) were analyzed using a chemiluminescent assay (ACL AcuStar; Instrumentation Laboratory). The results were positive with a cutoff of 20 U/mL. Anti-Saccharomyces cerevisiae antibodies (ASCA IgA and IgG), proteins myeloperoxidase (MPO) and proteinase 3 (PR3), Connective Tissue Disease (CTD) panel (CTD Screen: human recombinant U1RNP, SS-A/Ro, SS-B/La,

centromere B, Scl-70, Jo-1, fibrillarin, RNA Pol III, Rib-P, PM-Scl, PCNA, Mi-2 proteins, Sm proteins, and native purified DNA) were analyzed by fluorescence enzyme immunoassay Phadia 250-Thermoscientific). The higher-than-normal range was 7 U/mL for ASCA, 3.5 U/mL for MPO, and was 2 U/mL for PR3.

Antineutrophil cytoplasmic antibodies (ANCA) and antinuclear antibodies (ANAs) were detected by indirect immunofluorescence using EUROIMMUN test kits. ANCA was performed at the dilution of 1:20, whereas ANA was analyzed through 3 serial dilutions (1:80; 1:160; and 1:320). The confirmatory tests were performed by line-blot technology using the following kits: EUROLINE Myositis DL-1530-4G and EUROLINE Scleroderma DL-1532 G. EUROLINE Myositis is specific for the following antigens: Mi-2 alpha, Mi-2 beta, TIF1g, MDA5, NXP2, SAE1, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-2, EJ, OJ, and Ro-52. EUROLINE Scleroderma includes these antigens: Scl-70, CENP A, CENP B, RP11, Rp155, fibrillarin, NOR90, Th/To, PM-Scl100, PM-Scl75, Ku, PDGFR, and Ro-52. Only bands having an intensity higher than 11 were considered positive, according to the manufacturer's instructions (EUROIMMUN, Luebeck, Germany).

Statistical analyses

Clinical data were collected using REDCap (version 10.2.3, 2020; Vanderbilt University) electronic data capture tools.^{13,14} Patients were clustered into 2 groups based on the age: < 60 years and \geq 60 years. Values are indicated as mean \pm SD. The Excel asymmetry function was used to check the distribution of the data (Excel version 2009; Microsoft Office Professional 2016, Redmond, WA). Values having a normal distribution were analyzed using independent sample *t*-test (Excel version 2009, Microsoft Office Professional 2016). Values that did not present a normal distribution were evaluated using the Mann–Whitney *U* test (<https://www.socscistatistics.com/tests/mannwhitney/default2.aspx>). Categorical data were compared using the χ^2 test (<https://www.socscistatistics.com/tests/chisquare/default2.aspx>). Two tailed $P < 0.001$ were considered statistically significant.

RESULTS

Demographics, baseline, and clinical characteristics of patients with COVID-19

Demographics, baseline, and clinical characteristics of 40 patients with COVID-19 who were symptomatic and hospitalized are summarized in Table 1 and Table S1.1. The age of the patients considered in this study was between 20 and

TABLE 1 Clinical characteristic of patients with COVID-19

	Characteristics	All patients (40)	< 60 years (13)	≥ 60 years (27)
Demographics	Age, years (+/- SD)	66.8 (+/- 17.46)	46.92 (+/- 12.3)	76.37 (+/- 9.74)
	Age, years (min-max)	20-97	20-59	60-97
	Sex			
	M	28/40 (70%)	09/13 (69.23%)	19/27 (70%)
	F	12/40 (30%)	04/13 (30.77%)	8/27 (29.63%)
	Survival rate	29/40 (72.50%)	12/13 (92.31%)	17/27 (62.96%)
Symptoms	Fever	30/40 (75%)	12/13 (92.31%)	18/27 (66.67%)
	Chills	None	None	None
	Dry cough	23/40 (57.50%)	08/13 (61.54%)	15/27 (55.56%)
	Cough with phlegm	None	None	None
	Conjunctivitis	None	None	None
	Rhinorrhea	None	None	None
	Headache	None	None	None
	Muscle pain	02/40 (5%)	02/13 (15.38%)	None
	Fatigue	02/40 (5%)	None	02/27 (7.41%)
	Nausea	None	None	None
	Vomiting	None	None	None
	Diarrhea	03/40 (7.50%)	01/13 (7.69%)	02/27 (7.41%)
	Dyspnea	24/40 (60%)	06/13 (46.15%)	18/27 (66.67%)
	Hemoptysis	None	None	None
	Hematemesis	None	None	None
	Ageusia	02/40 (5%)	02/13 (15.38%)	None
	Anosmia	01/40 (2.50%)	01/13 (7.69%)	None
	Other symptoms	04/40 (10%)	02/13 (15.38%)	02/27 (7.41%)
	Coexisting disorders	Coexisting disorder on admission	07/40 (17.50%)	05/13 (38.46%)
BPCO		03/40 (7.50%)	None	03/27 (11.11%)
Diabetes		07/40 (17.50%)	None	07/27 (25.93%)
Hypertension		24/40 (60%)	03/13 (23.08%)	21/27 (77.78%)
Coronary disease		04/40 (10%)	None	04/27 (14.81%)
Cerebrovascular disease		1/40 (2.50%)	None	01/27 (3.70%)
Hepatitis B infection		None	None	None
Cancer (in the last 5 years)		04/40 (10%)	01/13 (7.69%)	03/27 (11.11%)
Chronic renal disease		06/40 (15%)	none	06/27 (22.22%)
Immunodeficiency		01/40 (2.50%)	01/13 (7.69%)	None
Ischemic heart disease		07/40 (17.50%)	None	07/27 (22.22%)
Ictus		02/40 (10%)	None	02/27 (7.41%)
Dementia		01/40 (2.50%)	None	01/27 (3.70%)
Chronic liver disease		None	None	None
HIV infection		None	None	None
Atrial fibrillation		05/40 (12.50%)	None	05/27 (18.52%)
DVT		None	None	None
PE		None	None	None
Others disorders		17/40 (42.50%)	03/13 (23.08%)	14/27 (51.85%)

The table summarized the demographic and clinical characteristics of 40 patients with COVID-19. The patients were clustered based on the age of 60 years.

BPCO, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; DVT, deep-vein thrombosis; PE, pulmonary emboli.

97 years with a mean age of 66.8 years (SD: 17.46). Patients included 28 men (70%) and 12 women (30%). Eleven patients (27.5%) died because of SARS-CoV-2 infection. Thirty patients (75%), more specifically all the patients under 60 years of age, presented fever at hospital admission. The most common clinical symptoms were dry cough (57.5%) and dyspnea (60%); none of them presented nausea or vomiting and only 7.5% had diarrhea. Only seven patients (17.5%) had one co-existing disorder, but none had a previous clinical history of autoimmune disease. Based on the clinical picture, patients were treated with hydroxychloroquine, or antiviral therapy (ritonavir or darunavir-cobicistat), anticoagulant therapy, broad-spectrum antibiotics therapy, or a combination of them. Steroid therapy was not considered based on the international guideline recommendations at the time of our study.¹⁵ The average time of hospitalization was 16.5 days. Whereas 7 of 40 patients (17.50%) needed assisted ventilation, and 32 of 40 required oxygen therapy (80%; Table S1.1).

Radiologic characteristics of patients with SARS-CoV-2

COVID-19 highly affects the respiratory system and in particular the lungs. Radiologic investigations were necessary in 36 patients (90%). The specific clinical features observed were: patchy shadowing (5%), interstitial abnormalities (17.5%), and pulmonary thicknesses (10%). None of the patients showed ground-glass opacity. Only four patients (10%) did not require RX analysis (Table 2).

Inflammatory status of patients with COVID-19

As do all viral infections, the presence of COVID-19 disease leads to an effective inflammatory response. The

inflammatory status of the 40 patients with COVID-19 was evaluated considering the common diagnostic inflammatory markers: LDH, ferritin, number of lymphocytes, creatinine, PCR, C3, C4, and IL-6. The laboratory measurements are summarized in Tables 3 and 4. Table 3 highlights the number of patients having a clinical value out of the normal range. We observed that $\sim 51.50\% \pm 20.51$ (average \pm SD) of the patients presented an increase in the general inflammation markers (LDH, ferritin, creatinine, C3, and C4). Table S3.1 provides an overview of the number of patients with inflammatory marker values above the upper reference range limit and their relative percentage. We also evaluated other markers, more specific of an ongoing inflammatory response, such as number of lymphocytes, PCR, and IL-6. The number of lymphocytes was below the normal value in 57.5% of the patients. PCR was 12 times higher compared with the upper normal range value in 92.5% of the cases. To avoid timing measurements problems, our patients were analyzed for IL-6 after checking the presence of COVID-19 by real-time reverse-transcriptase-PCR. IL-6 was 10 times higher in 85% of all patients with COVID-19 considered. To check if the age could affect the inflammatory markers analysis, we clustered patients based on whether they were under or over 60 years of age. Statistical analyses showed that patients that were 60 years of age or older (≥ 60 years), presented higher values of LDH (715.58 ± 284.31 U/L), ferritin ($1,099.61 \pm 1,011.24$ ng/mL), the lymphocyte number (85.23 ± 123.29), creatinine (1.32 ± 0.89 mg/dL), PCR (9.21 ± 6.52 mg/dL), and IL-6 (85.23 ± 123.29 mg/dL) than the younger patients (< 60 years; LDH: 645.36 ± 202.72 ; ferritin: 889.90 ± 591.65 ; creatinine: 0.77 ± 0.15 ; PCR: 7.59 ± 10.87 ; lymphocyte number: 1.00 ± 0.43). On the contrary, younger patients showed a higher C3 titer (158.07 ± 31.86 mg/dl) compared with the older group (138.23 ± 37.75 mg/dL). C4 does not appear to depend on age, because its value was similar in both age groups (> 60 years: 37.35 ± 13.79 mg/dL; or ≥ 60 years: 37.42 ± 13.22 mg/dL).

Clinical investigation	Patients evaluated		
	(36)	< 60 years (11)	≥ 60 years (25)
Rx Thorax	36/40 (90%)	11/36 (84.61)	25/36 (92.59)
• Ground-glass opacity	None	None	None
• Patchy shadowing	2/36 (5%)	None	2/25 (7.69%)
• Interstitial abnormalities	7/36 (17.5%)	None	7/25 (26.92%)
• Pulmonary thicknesses	4/36 (10%)	2/11 (15.38%)	2/25 (7.69%)

TABLE 2 Radiological investigations of the 40 patients with COVID-19

The table summarized the clinical radiologic features of the 36 patients with COVID-19 that had Rx thorax analysis. Only four patients did not require this analysis. The major part of the group of patients showed interstitial abnormalities (17.5%) and were above age 60 years (26.92%). Patchy shadowing (5%) was also observed in the elderly population (7.69%). Pulmonary thicknesses (10%) were present in our cohort of patients independently of age (> 60 years: 15.38%; ≥ 60 years: 7.69%).

COVID-19, coronavirus disease 2019.

TABLE 3 Patients with COVID-19 with the classical inflammatory markers out of range

Inflammatory markers	Reference range	All patients		
		< 60 years	≥ 60 years	
LDH	230–500 U/L	30/40 (75%)	64.28%	80.77%
Ferritin	10–291 ng/mL	32/40 (80%)	7.86%	80.77%
Lymphocytes number	0.9–5.2 × 1,000/ mcl	23/40 (57.50%)	35.71%	73.08%
Creatinine	0.4–1 mg/dL	16/40 (40%)	7.14%	57.69%
CPR	0–0.8 mg/dL	37/40 (92.50%)	8.57%	96.15%
C3	82–160 mg/dL	12/40 (30%)	42.86%	23.07%
C4	12–36 mg/dL	21/40 (52.50%)	50%	53.85%
IL-6	0–5.9 mg/dL	34/40 (85%)	71.43%	92.31%

The table summarizes the common diagnostic inflammatory markers analyzed in all 40 patients with COVID-19 (LDH, ferritin, lymphocytes number, creatinine, CPR, C3, C4, IL-6). The patients were clustered based on the age of 60 years. The analytes were evaluated in patients with COVID-19 under 60 years (< 60 years) and having an age of 60 or above (≥ 60 years). The specific reference range value of each analyte is indicated in the first column. Both in all patients and in the patients clustered by age, we reported how many patients had values out of the reference range and their relative percentage.

C3, complement 3; C4, complement 4; COVID-19, coronavirus disease 2019; LDH, lactate dehydrogenase.

TABLE 4 List of the inflammatory markers analyzed in the 40 patients with COVID-19

Inflammatory markers	Normal range	All patients	< 60 years	≥ 60 years	P value
LDH	230–500 U/L	690.03 ± 259.18	645.36 ± 202.72	715.58 ± 284.31	0.31 (<i>t</i> -test)
Ferritin	10–291 ng/mL	1005.29 ± 892.40	889.90 ± 591.65	1099.61 ± 1011.24	0.26 (<i>t</i> -test)
Lymphocytes number	0.9–5.2 × 1,000/mcl	3.80 ± 13.80	1.00 ± 0.43	5.31 ± 17.17	0.67 (M-W)
Creatinine	0.4–1 mg/dL	1.13 ± 0.76	0.77 ± 0.15	1.32 ± 0.89	0.69 (M-W)
CRP	0–0.8 mg/dL	10.33 ± 12.78	7.59 ± 10.87	9.21 ± 6.52	0.21 (<i>t</i> -test)
C3	82–160 mg/dL	145.38 ± 36.45	158.07 ± 31.86	138.23 ± 37.75	0.02 (<i>t</i>-test)*
C4	12–36 mg/dL	37.35 ± 13.37	37.35 ± 13.79	37.42 ± 13.22	0.40 (<i>t</i> -test)
IL-6	0–5.9 mg/dL	58.59 ± 102.92	11.99 ± 11.01	85.23 ± 123.29	0.02 (M-W)*

Value of common diagnostic inflammatory markers in all the patients with COVID-19 and in the patients clustered by age 60 years are summarized in the table. The specific normal range value of each analyte is indicated in the first column. All the values are expressed as mean ± SD. These two subgroups were compared using statistical analysis. When the population had normal distribution (asymmetry between –2 and +2) Student *t*-test was considered (*t*-test). Mann–Whitney (M-W) test was run in the other cases. C3 and IL-6 showed statistical significance between the two groups considered.

C3, complement 3; C4, complement 4; COVID-19, coronavirus disease 2019; LDH, lactate dehydrogenase.

*Statistically significant.

If age is considered as a critical discriminator value, the only two statistically significant analytes were C3 (*t*-test *P* value: 0.02*) and IL-6 (Mann–Whitney *P* value: 0.02*).

These results further sustain how the immune system promptly reacts against COVID-19 and restate the key role of IL-6 during the inflammation process related to this disease.

Immunological markers

Autoimmunity defines an over-reaction of the immune system against itself. The inflammation can either help the organism to fight the virus, or cause a strong harmful autoimmune response. The 40 patients with COVID-19 considered were analyzed for autoimmune autoantibodies to determine the involvement and correlation of autoimmunity

with COVID-19 disease. Table 5 summarizes the autoantibodies analyzed in both patients with COVID-19 and healthy subjects. We considered the most common autoantibodies associated with inflammation, such as ANA, anti-Cardiolipin, anti-β2-Glycoprotein, anti-extractable nuclear antigens, anti-PR3, anti-MPO, ANCA, and ASCA (IgA and IgG). Although it is common to detect different type of antibodies also in healthy individuals, the patients considered did not have any previous clinical record of antibody presence. Our analysis showed that our patients were positive for ANA (57.50%), ASCA (IgA: 25%; IgG: 17.50%), and anti-Cardiolipin (12.50%) antibodies. Anti β2-Glycoprotein (5%), anti-extractable nuclear antigens (2.50%), and anti-PR3 (2.50%) antibodies were present in < 5% of the patients. Furthermore, 25% of the patients had ANCA antibodies. In particular one patient had c-ANCA (anti-PR3

TABLE 5 List of the autoantibodies detected in patients with COVID-19 and healthy individuals

Autoantibodies	All patients (40)		Healthy subjects (40)		χ^2 (with Yates Correction)
	Pos	Neg	Pos	Neg	<i>P</i> value
ANA	23 (57.50%)	17 (42.50%)	05 (12.50%)	35 (87.50%)	0.0001*
Anti-Cardiolipin	05 (12.50%)	35 (87.50%)	05 (12.50%)	35 (87.50%)	0.7353
Anti β 2-Glycoprotein	02 (5%)	38 (95%)	01 (2.50%)	39 (97.50%)	1.0000
ENA	01 (2.5%)	39 (97.50%)	00 (0%)	40 (100%)	nv
Anti-PR3	01 (2.5%)	39 (97.50%)	00 (0%)	40 (100%)	nv
Anti-MPO	00 (0%)	40 (100%)	00 (0%)	40 (100%)	nv
ANCA	10 (25%)	30 (75%)	01 (2.50%)	39 (97.50%)	0.0094*
ASCA IgA	10 (25%)	30 (75%)	01 (2.50%)	39 (97.50%)	0.0094*
ASCA IgG	07 (17.5%)	33 (82.50%)	01 (2.50%)	39 (97.40%)	0.0624

The table summarizes the number of patients and healthy subjects showing the presence of the most common autoantibodies: anti-ANA, anti-Cardiolipin, anti- β 2-Glycoprotein, anti-ENA, anti-PR3, anti-MPO, ANCA, ASCA, IgA, and IgG. The results are expressed as positivity (pos) or negativity (neg) of a patient for an autoantibody, based on the presence and absence of the autoantibody analyzed. The patients with COVID-19 and healthy subjects were compared using χ^2 statistical analysis with Yates correction. The presence of ANA and ASCA IgA between the two groups considered was statistically significant.

ANCA, antineutrophil cytoplasmic antibodies; anti-ANA, antinuclear antibody; anti-ENA, anti-extractable nuclear antigens; anti-MPO, anti-myeloperoxidase; anti-PR3, anti-proteinase 3; ASCA, anti-Saccharomyces cerevisiae antibodies; nv, not valuable.

*Statistically significant; *P* value < 0.01).

positive, 2.50%) antibodies, whereas the other nine patients had X-ANCA antibodies (22.5%). Based on our results, patients with COVID-19 had a significant prevalence of ANA, ANCA, and ASCA IgA antibodies compared with healthy subjects (ANA: 12.50%; ASCA IgA: 2.50%; and ANCA: 2.50%; *P* value < 0.01). MPO antibodies were not detected in any of the patients and healthy subjects considered in this study.

To check if autoimmunity could affect patient's survival, we compared the patients with positive antibodies who survived versus those who died (Table S5.1). Focusing on the autoantibodies with a significant prevalence, the percentage of deceased patients was 39.13% for ANA, 30% for ASCA, and 40% for ANCA positive patients. All the ANCA positive deceased patients showed an X-ANCA pattern (50%; Table S5.1).

The samples that were positive for autoantibodies were also analyzed using a confirmatory immune line blot test. However, the presence of the specific antibodies involved was determined in just one patient, who also presented anti-Pm-SCL100 and anti-Ro52 antibodies. It was later found that this patient had cancer.

We further evaluated our patients with COVID-19 considering only those with an ANA titer above the 1:160 dilution. The results confirmed a significant prevalence of ANA antibodies in patients with COVID-19 compared with healthy subjects (*P* = 0.0032).

These results clearly highlight that COVID-19 disease is linked to autoimmunity. ANA, ANCA, and ASCA antibodies could be detected in association with COVID-19 viral infection. Their presence could negatively affect the outcome of patients with COVID-19.

DISCUSSION

SARS-CoV-2 infection currently represents the worst global pandemic disease and efforts are being made worldwide to find a possible cure. Different research studies have focused on trying to define the mechanism behind this pathology. It has been known that SARS-CoV-2 can trigger a strong harmful immune response in some patients.¹⁶ However, its specific pathogenic mechanism of action it is not yet completely known.

Different studies have already shown that the SARS-CoV-2 infection determines a higher production of inflammatory cytokines.^{17,18} In particular, it has been observed that this immune response can either be helpful to fight the viruses, or exacerbate the number of inflammatory chemokines leading to a process known as "inflammatory storm," that could worsen the patients' already critical conditions.^{19,20} According to this, the patients with COVID-19 considered in this study showed increased levels of the classical inflammatory markers. Furthermore, patients having high levels of IL-6 had the worst clinical outcome. Our data support the concept that high IL-6 levels could be a useful diagnostic value for identifying subjects with a poor prognosis. This hypothesis has been already supported by a careful meta-analysis performed by Coomes and Haghbayan.^{21,22}

Imbalanced immune response in certain conditions could also lead to the development of autoantibodies. Up to now, no clear investigations have been performed to determine if SARS-CoV-2 infection can activate an autoimmune response.

One recent study has described the autoimmune characteristics of 21 patients with COVID-19. In this work, the authors found that patients with COVID-19 had a prevalence of anti-52kDa SSA/Ro, anti-60 kDa SSA/Ro, and ANA antibodies (20%, 25%, and 50%, respectively); they conclude that the autoimmune mechanism is activated in patients with COVID-19.²³ In June 2020, another cohort of 29 patients was analyzed, defining the presence of autoimmunity after COVID-19 infection.¹¹ In accordance with these studies, our data sustained that not only inflammation, but also autoimmunity is likely triggered by the COVID-19 infection. In our cohort of 40 patients with COVID-19, there was a significant prevalence of ANA, ANCA, and ASCA IgA antibodies compared with healthy individuals. ANA antibodies are important markers in the diagnosis of different autoimmune diseases, mainly ANA-associated rheumatic diseases. However, ANAs must always be evaluated in association with the clinical features, because even healthy subjects can be ANA-positive (25% of the healthy population presents ANA antibodies).^{24–26} In our cohort, 23 patients (57.50%) were positive for ANA antibodies; more than twice of what is described in healthy people. This percentage reached 81.81% (9/11; Table S5.1) when the deceased patients were considered (11/40, 27.50%, results section Demographic, baseline, and clinical characteristics of patients with COVID-19 and Table 1). All the patients with COVID-19 analyzed in our study did not have a clinical record of previous autoimmune disease at the time of their hospital admission for SARS-COV-2 infection. Thus, we could speculate that ANA positivity might correlate with hospitalization in medium-high intensity care department and a fatal outcome.

Patients positive for ANCA were 25% in our cohort. ANCA antibodies are usually considered biomarkers for ANCA-associated vasculitis. Atypical pattern (X-ANCA) can be found in other conditions, such as gastrointestinal tract diseases.²⁷ In our study, of the 9 patients (22.50%) that were X-ANCA positive, 50% died and the other 50% had a long and complicated hospitalization (Table S5.1). In particular, two patients required intubation and recovery in the intensive care unit; another remained in the sub-intensive care unit for a long period under high pressure positive ventilation. Clinical data, together with our analyses, strongly support the hypothesis of an association between X-ANCA detection and a more severe respiratory disease in patients with COVID-19. Therefore, based on our results, patients positive with COVID-19 having ANA and ANCA antibodies, respectively, the 39.13% and 40% of the patients with COVID-19, had the worst outcome and died (Table S5.1).

ASCA antibodies were also detected in our patients: 25% had ASCA IgA antibodies, whereas 17.50% had ASCA IgG. ASCA isotypes IgA or IgG normally correlate with inflammatory bowel disease. Furthermore, they have been detected in the serum samples of 60–70% of patients having Crohn's

disease.²⁷ However, in our study, only one patient that was positive for ASCA antibodies had the typical gastrointestinal symptoms (i.e., diarrhea). Therefore, we could assume that there is no correlation between the presence of these autoantibodies and the clinical phenotype. This result could be explained by the stringent selection of the patients with COVID-19 analyzed, that could have included a bias in the population. Indeed, the patients admitted to hospital were the ones having severe respiratory symptoms. Patients with gastrointestinal symptoms and moderate respiratory distress were treated at home and did not require hospitalization and consequently were not evaluated.

ANA, ANCA, and ASCA are not the only antibodies described in literature to be found in patients with COVID-19. Recently, Zhang and colleagues have reported 3 patients affected by COVID-19 who developed cerebral thrombi. These patients had coagulopathy, thrombocytopenia, anti-Cardiolipin IgA, and anti- β 2-Glycoprotein IgA and IgG antibodies.¹⁰ Usually, the presence of antiphospholipid antibodies is fundamental for the diagnosis of the antiphospholipid syndrome. However, these antibodies can also be detected transiently in patients with critical disease and different infections.²⁸ In another study, other 5 patients with COVID-19 were described to be positive for the same autoantibodies. In this work, the authors did not clearly explain whether the increased rate of arterial thrombotic events in these patients was caused by the presence of the antibodies.²⁹ We also detected antiphospholipid antibodies in our cohort, although at a low rate. In details, we found that only 5 patients (12.50%) were positive for anti-Cardiolipin and 2 (5%) for anti- β 2-Glycoprotein. However, there were no significant differences compared with the healthy subjects. According to literature and our data, we could infer that the increase of thromboembolic events that normally occurs in patients with COVID-19 might not be influenced by the presence of antiphospholipid antibodies, but could be also due to other factors.

The results obtained in this study firmly sustained that COVID-19 is associated with autoimmunity, in particular ANA, ASCA, and ANCA antibodies development. To support our findings, it has to be mentioned that 1 of our 40 patients with COVID-19 had a peculiar clinical outcome. At the beginning of his hospitalization, after autoantibodies investigations, his results were negative. However, his clinical condition was highly severe, he had high critical pulmonary and renal disfunctions, and after 1 month of hospitalization, there were no improvements. Therefore, it was decided to repeat the autoimmune analyses. During this second evaluation, we detected a strong ANA positivity (pattern cytoplasmic 1:160, centriole 1:320, and granular 1:160) and the myositis blot was positive for M2beta and Ku antigens.³⁰ Moreover, another case of onset of autoimmune diseases (Systemic Lupus Erythematosus) following a COVID-19 infection has recently been described in the literature.³¹

This clinical case emphasizes the importance to keep in mind the role of autoimmunity in patients with COVID-19. Autoimmune response could explain, in some cases, the lack of clinical improvement or a long recovery despite the resolution of the viral infection. In this regard, it is already known that systemic rheumatic diseases are often characterized by multiorgan involvement and lung complications are frequent. Pulmonary interstitial disease is the most represented pulmonary complication and its evolution is often poorly foreseeable. Thus, autoimmunity may also negatively affect the patients with COVID-19 respiratory system, which is already in a critical condition.

CONCLUSIONS

This study shows that most of the patients with COVID-19 enrolled have an altered autoimmune profile. Our data sustained that autoimmunity is linked to SARS-CoV-2 infection, because none of the patients had a previous autoimmune disease. This is in accordance with what has recently been described in the literature concerning the onset of autoimmune diseases upon COVID-19 infection.¹¹

Data related to patients with a medium-severe clinical profile highly suggest that there might be a correlation between the response to SARS-CoV-2 and the specific individual autoimmune response. This could be the reason for the wide variability of clinical manifestations related to a single pathogen. Although the considerations made are preliminary and the study is monocentric, we have definitely started to reveal the link between COVID-19 disease and autoimmunity. Further studies using other independent data sets will be necessary to check whether the autoimmune response in patients with COVID-19 changes based on the disease stages, confirming the clinical-laboratory correlation. Furthermore, it will be fundamental to perform follow-up at different time points (e.g., at 3 and 6 months posthospitalization), to be able to prove whether the immunological changes that we observed in our population, were only transitory, or if the alteration might persist longer and lead to chronic autoimmune disease. Follow-up will also allow the evaluation of the possible effects derived from the therapy used. For this reason, we already obtained the ethics committee's approval to conduct a prospective observational study to evaluate these patients.

In conclusion, this research shows, albeit the limitation of the sample size, that the SARS-CoV-2 correlate to an altered autoimmune response. Our results could help clinicians to understand why drugs normally used to treat autoimmune disease may also be useful toward SARS-CoV-2 improving public health. Finally, it is important to consider autoimmunity in patients with COVID-19, to be able to choose the right therapy and decide if plasma transfer could be a good option. Different autoantibodies are not only disease markers but can

also be pathogenic, therefore transferring autoantibodies or performing transfusions with neutralizing antibodies might not be the best choice for these patients.¹¹

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CONFLICT OF INTEREST

All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

M.C.S., S.T., L.A., and P.D.G. wrote the manuscript. M.C.S., P.S., and A.S. designed the research. M.C.S., S.T., A.R., E.L., R.B., and R.G. performed the research. M.C.S., S.T., R.B., L.A., P.D.G., A.S., and P.S. analyzed the data. L.A., P.D.G., and A.S. contributed new reagents/analytical tools.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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