Characteristics of patients with suspected COVID-19 pneumonia and repeatedly negative RT-PCR

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Abstract

Objectives. Challenges remain and there are still a sufficient number of cases with epidemiological, clinical features and radiological data suggestive of COVID-19 pneumonia that persist negative in their RT-PCR results. The aim of the study was to define the distinguishing characteristics between patients developing a serological response to SARS-CoV-2 and those who did not.

Methods. RT-PCR tests used were TaqPath 2019-nCoV Assay Kit v1 (ORF-1ab, N and S genes) from Thermo Fisher Diagnostics and SARS-COV-2 Kit (N and E genes) from Vircell. Serological response was tested using the rapid SARS-CoV2 IgG/IgM Test Cassette from T and D Diagnostics Canada and CMC Medical Devices and Drugs, S.L, CE.

Results. In this cross-sectional study, we included a cohort of 52 patients recruited from 31 March 2020 to 23 April 2020. Patients with positive serology had an older average age (73.29) compared to those who were negative (54.82) ($P<0.05$). Sat0$_2$ in 27 of 34 patients with positive serology were below 94% ($P<0.05$). There was a frequency of 1.5% negative SARS-CoV-2 RT-PCRs during the study period concurring with 36.7% of positivity.

Conclusions. Clinical features and other biomarkers in a context of a positive serology can be considered crucial for diagnosis.

INTRODUCTION

In December 2019, the city of Wuhan, the capital of Hubei province in China, was the epicentre of an outbreak of pneumonia of unknown cause. In January 2020, Chinese scientists isolated a new coronavirus as the cause of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), clinically named coronavirus disease (COVID-19), declaring an international public health emergency and the beginning of a global pandemic (WHO 11 March 2020) [1]. Up until this moment there are 3559222 infected cases and 78726 deaths [2] in Spain after several waves.

RT-PCR continues to remain as the gold standard for SARS-CoV-2 identification due to its high sensitivity and specificity. However, challenges remain and there are still a sufficient number of cases with highly suggestive clinical and radiological signs of COVID-19 pneumonia, as well as epidemiological exposure, who persistently present negative RT-PCRs. Delay in microbiological confirmation of disease in a hospital environment can negatively impact diagnosis, isolation of patients and therapy, as well as access to available clinical trials.

A serological test is not used routinely for the diagnosis of COVID-19 because, in the early phase of the disease, during the first 5–6 days after the onset of symptoms, the immune response is scarce [3]. Nonetheless, that has been under evaluation in different clinical situations. Given the uncertainty of these, our aim was to analyse the clinical features and microbiological characteristics in this cohort of patients and confirm infection in cases of suspicion of a false negative RT-PCR. On the other hand, we tried to determine the results according to time since onset of symptoms and initial day of testing and final RT-PCR.

Table 1. Demographic, clinical, laboratory, imaging features and therapy received of patients in this study

	Total (*n*=52)	Serologic test negative (*n*=18)	Serologic test positive (*n*=34)	*P* value
Age, years	67.1	54.8	73.2	0.003
Sex				
Female	22 (42.3%)	10	12	0.239
Male	30 (57.7%)	8	22	
Days of hospitalization				
0-7 days	14 (26.9%)	6	8	0.763
>8 days	38 (73%)	12	26	
Fever (≥37.5 °C)	18 (34.6%)	4	14	0.227
Peripheral oxygen saturation				
≤94	33 (63.5%)	6	27	0.002
>94	19 (36.5%)	12	7	
Pneumonia				
Mild, low-grade	36 (69.2%)	15	21	0.129
Severe	16 (30.8%)	3	13	
Ferritin, ng ml^{-1}				
<150/300 (fem/male)	15 (32.6%)	10	5	No *P* value
>150/300 (fem/male)	31 (67.4%)	5	26	No *P* value
D-Dimer, ng ml^{-1}				
≤500	10 (20%)	5	5	No *P* value
>500	40 (80%)	12	28	No *P* value
Lymphocyte count (103 ml^{-1})				
<1000	29 (58%)	9	20	0.763
≥1000	21 (42%)	8	13	
Lactate dehydrogenase(U l^{-1})				
<190	5 (10.2%)	5	0	No *P* value
190-390	30 (61.2%)	8	22	No *P* value
>390	14 (28.6%)	4	10	No *P* value
C-reactive protein (mg l^{-1})				
<10	8 (16%)	7	1	No *P* value
>10	42 (84%)	11	31	No *P* value
Time from illness onset to initial RT-PCR, days				
0-7	35 (67.3%)	14	21	No *P* value
8-14	9 (17.3%)	1	8	No *P* value
>14	8 (15.4%)	3	5	No *P* value
Time from illness onset to last RT-PCR, days				
0-7	17 (32.7%)	7	10	0.763
8-14	17 (32.7%)	5	12	
>14	18 (34.6%)	6	12	

Table 1. Continued

	Total (*n*=52)	Serologic test negative (*n*=18)	Serologic test positive (*n*=34)	*P* value
Time from illness onset to testing serology, days				
0-7	8 (15.4%)	2	6	No *P* value
8-14	23 (44.2%)	9	14	No *P* value
>14	21 (40%)	7	14	No *P* value
Number of RT-PCR tested per patient, mean	3.03	3,05	3,11	
Imaging features				
Consolidation	7 (13.4%)	3	4	No *P* value
Ground-glass opacity	19 (36.53%)	4	15	0.142
Bilateral pulmonary infiltration	16 (30.76%)	5	11	1
Non-specific	10 (19.23%)	6	4	No *P* value
Treatment				
Hydroxychloroquine	46 (88.4%)	13	33	No *P* value
Azithromycin	31 (59.6%)	7	24	0.076
Ceftriaxone	26 (50%)	7	19	0.555
Antithrombotic prophylaxis	25 (48.07%)	4	21	0.037

PATIENTS AND METHODS

Design and settings

We conducted a retrospective cross-sectional study including patients admitted in La Paz Hospital, Madrid (Spain) with clinical signs and symptoms compatible with COVID-19, mainly pneumonia. Patients were enrolled between 31 March and 23 April 2020, during the highest incidence of infection. A cohort of 52 patients were recruited, with them having at least two negative determinations to SARS-CoV-2 by real-time RT-PCR in respiratory tract samples and positive (IgG +, IgM +/-) or negative (IgG -, IgM -) serological response.

Tests

RT-PCR tests used were TaqPath 2019-nCoV Assay Kit v1 (ORF-1ab, N and S genes) from Thermo Fisher Scientific and SARS-COV-2 Kit (N and E genes) from Vircell Diagnostic. Serological response was tested using the rapid SARS-CoV2 IgG/IgM Test Cassette from T and D Diagnostics Canada and CMC Medical Devices and Drugs, S.L, CE (sensitivity 81.3%, specificity 90.7% in clinical settings) [4].

Data collection

Participants were classified as having symptoms consistent with COVID-19, mainly pneumonia. Survey data included demographic and epidemiological parameters (variables: age, sex, exposure history, close contacts), clinical parameters (variables: fever, presence of severe or mild pneumonia, mechanical ventilation), clinical outcome (variables: days of hospitalization, recovery or death), microbiological (variables: average number of RT-PCRs tested per patient, days from

illness onset to performing serological test and initial and last RT-PCR from illness onset, in days), analytical (peripheral oxygen saturation (Sat02), D-dimer, C-reactive protein (CRP), lactate dehydrogenase (LDH), ferritin, lymphocyte count), imaging features (consolidation, ground-glass opacity, non-specific or bilateral pulmonary infiltration) and the following pharmacological treatment received: hydroxychloroquine, azithromycin, ceftriaxone and antithrombotic prophylaxis. Further clinical data were extracted from the patient's electronic medical records using a standardized form.

Analysis

We characterized the enrolled cohort using descriptive statistic, stratified by SARS-CoV-2 antibody results. We compared groups using the Chi square test for categorical variables and Kolmogorov-Smirnov test for continuous variables to identify potential factors associated with positive serology. Univariate and multivariate logistic regression analyses were performed. Variables with a *P*-value <0.05 in the univariate analysis were included in the multivariate analysis. OR were calculated with 95% confidence intervals (95% CI). Data were analysed with SPSS Statistics 20 (IBM, Armonk, NY).

RESULTS

We enrolled 52 patients, including 30 (57.7%) males and 22 (42.3%) females. The average number of RT-PCRs tested per patient was 3.03 (some patients with five or six tests), with at least two of them negative. There were 18 negative serologies (34.6%), whereas there were 34 positive serologies (65.4%).
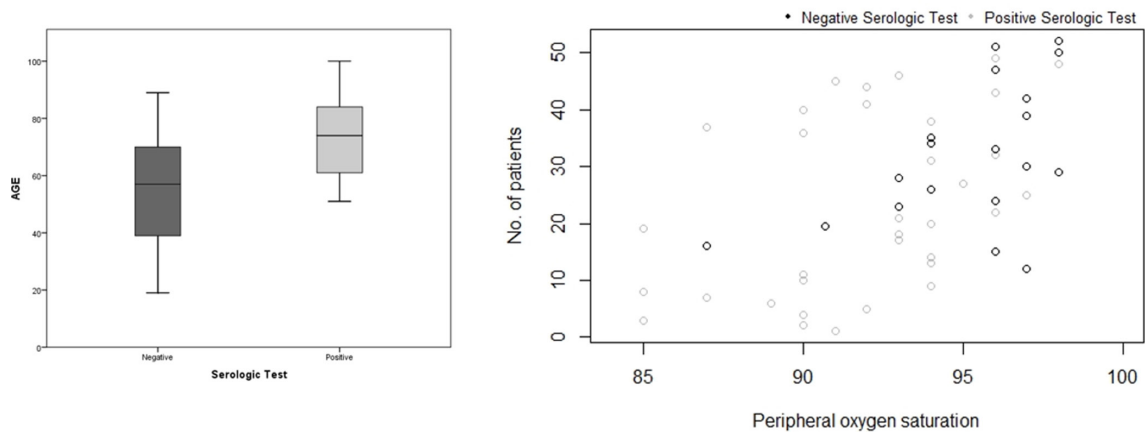
Fig. 1. Age and peripheral oxygen saturation variables in this study.

Demographic, clinical, laboratory, imaging features and treatments received are summarised in Table 1.

By multivariate analyses, a statistically significant association with SARS-CoV-2 seropositivity was found for age and peripheral oxygen saturation in comparison with negative results. Patients with positive serologies had an older average age (73.29) compared to those with negative ones (54.82) (OR: 1.068; 95% CI: 1.023–1.115; P=0.003). Twenty-seven out of 34 patients with positive serology had peripheral oxygen saturation below 94% (SpO2 >94% as protective factor, OR: 0.130; 95% CI: 0.036–0.469; P=0.002). Data is shown in Fig. 1.

Out of the 52 patients, 25 (48.07%) of them received antithrombotic prophylaxis (including three patients that finally switched to antithrombotic therapy). There was found a statistically significant association between SARS-CoV-2 seropositivity and antithrombotic prophylaxis (P=0.037).

The time from illness onset to performing serological tests was 7 days in eight patients (15.4%), 8 to 14 days in 23 (44.2%) and more than 14 days in 21 patients (40.4%). Time after symptom onset for testing initial PCR were between 0–7 days from 67.3% of the cases and 8–14 days (17.3%) or more 14 days from 15.4% of the patients. Days after symptom onset for testing last PCR were 0–7 days from 17 patients (32.7%), 8–14 days from 17 (32.7 %) and >14 days from 18 patients (34.6%). No statistically significant association with SARS-CoV-2 seropositivity was found for these nor any of the variables studied. Data are shown in Fig. 2.

For laboratory tests, it was found that approximately 58% of patients had lymphopenia, 89.8% showed LDH more than 190 U l^{-1}, 84% had C-reactive protein above 10 mg l^{-1} and 80% of patients had elevated D-dimer (>500 ng ml^{-1}). The imaging features were characterized by the ground-glass opacity (36.53% of patients), bilateral pulmonary infiltration (30.76%), non-specific imaging (19.23%) and consolidation (13.4%). Out of the 52 patients, 46 (88.4 %)

received hydroxychloroquine, 31 (59.6 %) azithromycin and 26 patients (50%) received ceftriaxone.

Five patients died, three of them with positive serological response for SARS-CoV-2 and two patients who remained without serological response (patients 24 and 33) were highly immunosuppressed. Mechanical ventilation was used in two patients who died.

Several characteristics of the 18 patients with serological response negative to SARS-CoV-2 and repeatedly negative RT-PCRs were shown in Table 2.

DISCUSSION

The coronavirus disease caused by SARS-CoV-2 has posed a serious threat to public health. Limited pre-existing immunity is assumed to account for the extraordinary rise in cases worldwide. Serological tests are being studied in the different stages of disease due to the uncertainty of its interpretation to current date. Several studies reported clinical sensitivity for SARS-CoV-2 real time PCR assays performed on upper respiratory swab samples to be in the range of 60–70% [5]. Other authors described sensitivity on nasopharyngeal specimen is highest within 5 days of symptom onset at 80–95% and declines below 80% after [6]. Most of the samples in this study were upper respiratory tract samples (nasopharyngeal and oropharyngeal swabs). Lower respiratory samples are invasive and imply high-risk aerosol-generating procedures when performed. In this study, only patient 16's sample was a bronchoalveolar lavage which was negative for RT-PCR SARS-CoV2. Patient was on ocrelizumab for multiple sclerosis and had radiological findings compatible with COVID-19 pneumoniae. Patient 52's sample was obtained by fibro-bronchoscopy aspirate, diagnosed with pulmonary tuberculosis. Nonetheless, the percentages of invasive samples in clinical practice during the study period of highest COVID-19 incidence were scarce, due to the risk of aerosolization.
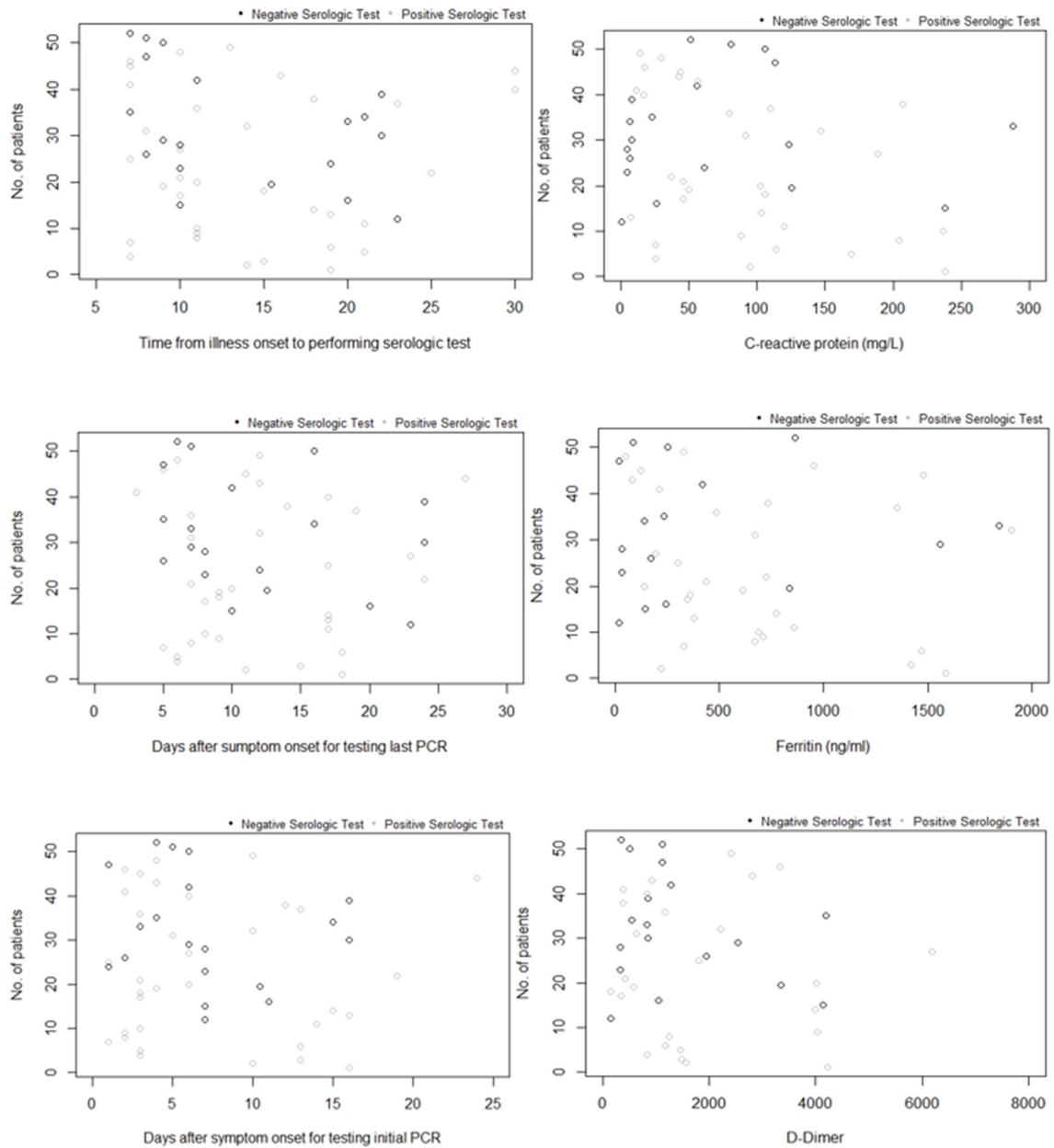
Fig. 2. Graphics of different parameters included in this study.

Viral load varies depending on a sample's nature [7, 8]. It is suggested that lower respiratory tract infections had higher viral loads than upper respiratory tract. In another study [9], bronchoalveolar lavage fluid showed the highest positive rates (93%), followed by sputum (72%), nasal swabs (63%), pharyngeal swabs (32%) and blood (1%). The determination of viral load is not recommended in any clinical setting, although the detection of SARS-CoV-2 RNA in serum might indicate an increased risk of progress to critical disease and death [10].

The likelihood of detecting SARS-CoV-2 RNA is highly dependent on the type of specimen obtained, the timing of its collection and quality of the sample with sufficient cellularity. Highest amounts of SARS-CoV-2 can be found in the upper respiratory tract (i.e. the nasopharynx) during the first several days following symptom onset (typically 5–6 days following exposure) and subsequently declines over the course of the following week [11]. Of the 52 patients, 35 hospitalized patients (67.3% of total) were tested in the first 7 days after symptom onset. None of them had a positive result on successive RT-PCR assays. Only 21 patients seroconverted finally. The rest of the patients (*n*=17) were tested over 8 days after symptom onset, of which 13 seroconverted. Most of patients in this study had a positive

Table 2. Clinical diagnosis of patients with serological response negative to SARS-CoV-2 and RT-PCRs repeatedly negative

Patient	Age	Day of testing serology since time of onset	Sp O2 (%)	Biomarkers					Clinical diagnosis
				Lymphocyte count (×10³ ml⁻¹)	C- reactive protein (mg l⁻¹)	Ferritin (ng ml⁻¹)	LDH (U l⁻¹)	D-Dimer	
12	39	Day +23	97	1850	0.5	19	166	151	Mild disease
15	63	Day +10	96	373.76	2.38	144	144	4130	Community-acquired pneumonia
16	34	Day +20	87	1188	26.1	242	347	1045	COVID-19 pneumonia Ocrelizumab previously
23	53	Day +10	93	1060	4.6	33	295	330	Previous Chest-X-ray is similar
24	56	Day +19	58*	320	61.4	ND	179	ND	HIV-1 immunosuppression
26	82	Day +8	94	1290	6.6	171	170	1941	Community-acquired pneumonia
28	70	Day +10	93	1060	4.6	33	295	330	Acute pulmonary embolism
29	19	Day +9	98	500	123.2	1561	451	2540	Oncologic patient
30	70	Day +22	97	540	8	ND	395	840	Necrotizing pneumonia
33	61	Day +47	96*	530	288	1844	503	829	Cancer.
34	47	Day +21	94	2010	7	137	303	530	COVID-19 pneumonia
35	43	Day +7	94	1957	22.9	233	259	4190	COVID-19 pneumonia
39	70	Day +22	97	540	8	ND	395	840	Lymphoproliferative process
42	89	Day +11	97	2978	56	416	222	1280	Mild disease
47	78	Day +8	96	1240	113.2	16	213	1110	Community-acquired pneumonia
50	26	Day +9	98	760	106	252	ND	500	COVID-19 pneumonia Methotrexate previously
51	31	Day +8	96	1460	81.2	85	166	1120	Metapneumovirus positive
52	57	Day +7	98	1340	51.2	864	251	340	Pulmonary TB

serology test after two or 3 weeks of the onset symptoms. Accordingly, the serological criteria can be located on the revised diagnostic of COVID-19 based on the findings from the second week of symptom onset in hospitalized patients with repeatedly negative RT-PCRs.

Cases with an initial false-negative diagnosis for RT-PCR [12–15] and subsequently found to be positive for SARS-CoV-2 were described in other reports. Their findings suggest that tests were either too early post-symptom onset or too late in the disease. Similarly, it has been reported that the occurrence of a newly positive result within 7 days was uncommon (3–3.5%) [16]. Here, there was a frequency of 1.5% repeatedly negative SARS-CoV-2 RT-PCRs (52 of 3420 patients) during the study period concurring with 36.7% of positivity (3420 of 9312 patients).

In this study, there was not a statistically significant association between SARS-CoV-2 serologic positivity (confirmed COVID-19 pneumonia) or time (days) since onset symptoms to initial RT-PCR or final RT-PCR testing. The false-negative rate of RT-PCR based SARS-CoV-2 test by time since exposure is already described [17]. In this context, serological tests have been incorporated in diagnostic criteria to confirm the diagnosis of COVID-19 [18, 19]. Despite this, as shown in Table 2, there are four patients with serological negative results and compatible with COVID-19 pneumonia. Negative results for RT-PCR and serology do not completely rule out SARS-CoV-2 infection, especially in severity disease and immunocompromised states.

There were 34 patients who presented with a positive serology (65.38%). This is a significantly larger number than the remaining 18 patients whose serology was negative (34.6%). An increased number of patients with positive serology presented with fever, more than 8 days of hospitalization, were male and had elevated ferritin, D-dimer, LDH, C-reactive protein and lower lymphocyte counts than the serologically negative group.

In this study, age (P=0.003) and peripheral oxygen saturation (P=0.002) were found as predictors for developing COVID-19 pneumonia with a serological positive response to SARS-CoV-2. As shown previously, age was also an independent predictor of critical disease and death [20–22]. Laboratory parameters, such as lactate dehydrogenase, C-reactive protein, ferritin, D-dimer and lymphocyte count as well as findings on chest X-ray/CT scan can help define the disease severity. Approximately 96% of patients with COVID-19 presented with chest CT abnormalities, such as multiple bilateral and peripheral ground-glass opacities and consolidation [23]. In this study, only ten of 52 patients (19.2%) had non-specific chest X-ray or CT scan. Imaging features can also be a great help in false-negative RT-PCRs [24].

A limitation of our study is the absence of a comparison control group, which would enable the analysis of the magnitude of association between coronavirus infection with RT-PCR SARS-CoV-2 positivity and serological response. Nonetheless, our study provided relevant information about the COVID-19 infection in hospitalized patients with repeatedly negative RT-PCR and few data have been reported about that.

According to the results, we were not able to identify a clear cause of false negative RT-PCRs because those were not performed on the lower respiratory tract. These sampling techniques are associated with unnecessary risks to health care workers due to close contact with patients [25]. It is possible that defining the date of symptom onset may have been difficult for some patients. Nevertheless, the number of retesting RT-PCRs per patient in upper respiratory tract have been high. On the other hand, other published reports describe important heterogeneity in viral load both between and within individuals [26].

In conclusion, we have not determined if these patients' samples were negative because the virus was not replicating or because patients were presenting with an inflammatory process. There was a frequency of 1.5% negative SARS-CoV-2 RT-PCRs during the study period concurring with 36.7% of positivity. Age (P<0.05) and the level of Sat0$_2$ (P<0.05) were linked to a positive serology for SARS-CoV-2 in patients with negative RT-PCR results. Clinical features and other biomarkers in a context of a positive serology can be considered crucial for diagnosis.

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