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SHORT COMMUNICATION

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Medium-term serostatus in Spanish case series recovered from SARS-CoV-2 infection

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Abstract

The medium-term serologic response of SARS-CoV-2 infection recovered individuals is not well known. The aims were to quantify the incidence of seropositive failure in the medium term in a cohort of patients with different COVID-19 severity and to analyze its associated factors. Patients who had recovered from mild and severe forms of SARS-CoV-2 infection in an Academic Spanish hospital (March 12–May 2, 2020), were tested for total anti-SARS-CoV-2 antibodies by electrochemiluminescence immunoassay (Elecsys Anti-SARS-CoV-2 test; Roche Diagnostics GmbH). The non-seropositive status (seropositive failure) incidence (95% CI) was determined. Associations were tested by multiple logistic regression in a global cohort and severe pneumonia subpopulation. Of 435 patients with PCR-confirmed SARS-CoV-2, a serological test was carried out in 325: 210 (64.6%) had severe pneumonia (hospitalized patients), 51 (15.7%) non-severe pneumonia (managed as outpatients), and 64 (19.7%) mild cases without pneumonia. After a median (IQR) of

Oscar Moreno-Pérez and Jose Manuel Ramos, contributed to the manuscript equally and share first authorship.

On behalf COVID19-ALC research group.

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76 days (70–83) from symptom onset, antibody responses may not consistently develop or reach levels sufficient to be detectable by antibody tests (non-seropositive incidence) in 6.9% (95% CI, 4.4–10.6) and 20.3% (95% CI, 12.2–31.7) of patients with and without pneumonia, respectively. Baseline independent predictors of seropositive failure were higher leukocytes and fewer days of symptoms before admission, while low glomerular filtrate and fever seem associated with serologic response. Age, comorbidity or immunosuppressive therapies (corticosteroids, tocilizumab) did not influence antibody response. In the medium-term, SARS-CoV-2 seropositive failure is not infrequent in COVID-19 recovered patients. Age, comorbidity or immunosuppressive therapies did not influence antibody response.

KEYWORDS

antibodies, case series, chemiluminescence immunoassay, COVID-19, risk factors, SARS-CoV-2, seroconversion

1 | INTRODUCTION

More than 90% of individuals infected with SARS-CoV-2 develop antibodies about 1 week after symptom onset, persisting for at least 3 months.[1] The duration of antibody rises is currently unknown, and there are scant data on the presence of antibodies in the medium or long term.[2] However, titers of neutralizing antibodies against the SARS-CoV-2 spike protein were detectable for at least 5 months after primary infection.[3]

Numerous immunoassays for the detection of antibodies to SARS-CoV-2 are emerging rapidly and have the potential to improve the diagnosis and monitoring of infection in different scenarios. Challenges arise in terms of sample collection, clinical translation, population studied, and sampling biases.[4,5]

Knowing the seroconversion rate is essential when interpreting seroprevalence studies, due to the implications for understanding the spread of infection at the population level and decision-making in health policy.[6]

The objectives of the study were to quantify the incidence of SARS-CoV-2 infection non-seropositive status in the medium term and to analyze the factors associated with the non-production of anti-SARS-CoV-2 antibodies in a cohort of patients with COVID-19.

2 | METHODS

Retrospective cohort study, of patients who had recovered from symptomatic SARS-CoV-2 infection diagnosed in the emergency department by RT-PCR between March 3 to May 2, 2020.

Of 435 patients with PCR-confirmed SARS-CoV-2, a serological study was not carried out or was invalid (carried out less than 14 days after symptoms onset) in 110 patients, so finally, 325 were included in the analysis. Patients were classified into pneumonia (includes severe—hospitalized patients and non-severe pneumonia (managed on an outpatient basis, hospital follow-up at home)), and mild cases without pneumonia (managed by primary care physician). The diagnosis of pneumonia required the demonstration of opacity on chest imaging (chest x-ray) in a patient with a clinically compatible syndrome; if lung involvement was suspected based on clinical features despite a negative chest radiograph, we obtained a computed tomography. The criteria for non-severe pneumonia included mild unilobar or multilobar alveolar pneumonia (radiological opacities < 50% pulmonary area) without dyspnea, sat02 ≥ 95% (Fi02 0.21), PaO_2:FiO_2 > 300 and a respiratory rate <20 rpm, normal glutamic oxaloacetic transaminase (GOT)/glutamic pyruvic transaminase (GPT) and lactate dehydrogenase (LDH), D-dimer < 1000 ng/ml, lymphocyte count > 1200 mm^3, and a normal 50 meters walking test (pulse oximetry saturation: desaturation < 5 points, and > 93%). Patients without serology, excluded from the analysis, did not differ in severity from the study population.

The main outcome was non-seropositive status at the time of evaluation: anti-SARS-CoV-2 antibody responses may not consistently develop or reach levels sufficient to be detectable by antibody tests. Blood samples were analyzed by electrochemiluminescence immunoassay (ECLIA) (Elecsys Anti-SARS-CoV-2 test, Roche Diagnostics GmbH) to detect total antibodies anti– SARS-CoV-2 including IgG, using a recombinant protein which represents the nucleocapsid antigen (N), the most sensitive target for serological diagnosis of infection with SARS-CoV-2.[7–9] The test was high-throughput and had a short turnaround time, being suitable for routine care settings. The sensitivity of this test was 96.8% ≥14 days after PCR-positivity and specificity of 99%.[10]

The non-seropositive status incidence (95% CI) at the time of evaluation was determined. Multiple logistic regression models were built to explore which risk factors present at diagnosis were

TABLE 1 Univariate analysis of factors related with negative serostatus to SARS-Cov-2

	Negative serostatus (n = 31)	OR (95% CI)	p
Type of patient			**.002**
Non-pneumonia[a]	13/64 (20.3)	1	
Pneumonia[b]	18/261 (6.9)	0.29 (0.13–0.63)	
Demographics			
Age			**.040**
<65	16/221 (7.2)	1	
≥65	15/104 (14.4)	2.15 (1.02–4.55)	
Gender			.546
Males	15/174 (8.6)	1	
Females	16/151 (10.6	1.25 (0.59–2.63)	
Nosocomial case			1
No	31/319 (89.7)	NC	
Yes	0/6 (0.0)		
Long-term care resident			.607
No	31/315 (9.8)	NC	
Yes	0/10 (0.0)		
Health professional			.855
Yes	6/67 (9.0)	1	
No	25/258 (9.7)	1.09 (0.48–2.77)	
Comorbidities			
Charlson index			**.008**
<3	14/216 (6.5)	1	
≥3	17/109 (15.6)	2.65 (1.26–5.69)	
Hypertension			.299
No	17/206 (8.3)	1	
Yes	14/119 (11.8)	1.49 (0.70–3.12)	
Diabetes			.185
No	25/286 (8.7)	1	
Yes	6/39 (15.4)	1.92 (0.72–4.76)	
Obesity			.667
No	20/220 (9.1)	1	
Yes	7/97 (7.2)	0.77 (0.31–1.90)	
Cardiovascular disease			.516
No	25/279 (9.0)	1	
Yes	3/23 (13.0)	1.78 (0.42–3.57)	
Chronic respiratory disease			.193
No	23/265 (8.6)	1	
Yes	8/56 (14.3)	1.34 (0.74–4.16)	
Immunosuppression			.683
No	29/208 (9.4)	1	

(Continues)

TABLE 1 (Continued)

	Negative serostatus (*n* = 31)	OR (95% CI)	*p*
Yes	2/16 (12.5)	1.38 (0.42–6.66)	
Clinical presentation			
Clinical duration[c]			**<.001**
>3 days	12/231 (5.1)	1	
≤3 days	18/84 (21.4)	5.07 (2.32–11.06)	
Fever			**.004**
No	15/86 (17.4)	1	
Yes	16/239 (6.7)	0.35 (0.16–0.72)	
Dry cough			.290
No	13/108 (12.0)	1	
Yes	18/215 (8.4)	0.67 (0.32–1.48)	
Wet cough			.977
No	26/272 (9.6)	1	
Yes	5/53 (9.4)	0.99 80.36–2.72)	
Dyspnea			.905
No	16/170 (9.4)	1	
Yes	15/153 (9.8)	1.04 (0.49–2.19)	
Diarrhoea			.132
No	26/242 (10.7)	1	
Yes	4/79 (5.1)	0.65 (0.21–1.31)	
Confusion			.148
No	27/305 (8.9)	1	
Yes	3/15 (20.0)	1.46 (0.68–10.09)	
Fatigue			.144
No	21/187 (11.2)	1	
Yes	8/126 (6.3)	0.53 (0.22–1.26)	
Myalgias-arthralgias			.056
No	23/199 (11.6)	1	
Yes	6/117 (5.1)	0.41 (0.16–1.05)	
Anosmia-dysgeusia			**.012**
No	29/266 (10.9)	NC	
Yes	0/47 (0.0)		
Initial assessment			
PaO2:FiO2			.639
≥300	11/161 (6.8)	1	
<300	4/45 (8.9)	1.92 (0.40–4.40)	
Systolic BP (mmHg)			.323
≥100	30/305 (9.8)	NC	
<100	0/9 (0.0)		
Diastolic BP (mmHg)			.253

	Negative serostatus (*n* = 31)	OR (95% CI)	*p*
≥60	26/289 (9.0)	1	
<60	4721 (16)	1.92 (0.62–6.02)	
Heart rate			.121
≤100	2/61 (3.3)	1	
>100	14/146 (9.6)	3.12 (0.68–14.28)	
Analytical assessment			
Leukocytes (/μl)			**<.001**
≤7900	8/221 (3.6)	1	
>7900	16/74 (21.6)	7.34 (2.99–18.0)	
Lymphocytes (/μl)			.321
>880	20/221 (9.0)	1	
≤880	4/74 (5.4)	0.57 (0.19–1.77)	
eGFR (45 ml/min/1.73m^2)			**.009**
≥60	17/253 (6.7)	1	
<60	8/41 (19.5)	3.36 (1.35–8.41)	
C-reactive protein (g/L)			.383
≤9	21/225 (9.3)	1	
>9	4/71 (5.6)	0.58 (0.19–1.76)	
Procalcitonin (ng/ml)			.344
≤0.13	17/196 (8.7)	1	
>0.13	4/76 (5.3)	0.58 (0.19–1.78)	
Ferritin (/μg/L)			.128
≤1202	15/167 (9.0)	1	
>1202	1/55 (1.8)	0.18 (0.24–1.45)	
Lactate dehydrogenase (U/L)			.774
≤319	17/201 (8.5)	1	
>319	5/68 (7.4)	0.86 (0.30–2.42)	
D-dimers (μg/ml)			.666
≤0.9	19/207 (9.2)	1	
>0.9	5/67 (7.5)	0.78 (0.28–2.23)	
Troponin T (ng/L)			**<.001**
≤14	9/201 (4.5)	1	
>14	11/63 (17.5)	4.53 (1.71–11.4)	
Brain natriuretic peptide (pg/ml)			**<.001**
≤211	9/199 (4.5)	1	
>211	11/60 (18.3)	4.73 (1.89–12.07)	
Creatine phosphokinase (U/L)			.530
≤128	16/210 (7.6)	1	
>128	7/70 (10.0)	1.34 (0.53–3.42)	
Aspartate aminotransferase (U/L)			.225

(Continues)

TABLE 1 (Continued)

	Negative serostatus (n = 31)	OR (95% CI)	p
≤44	21/214 (9.8)	1	
>44	4/76 (5.3)	0.95 (0.169–1.53)	
Alanine aminotransferase (U/L)			.783
≤41	19/214 (8.9)	1	
>41	6/76 (7.9)	0.80 (0.33–2.29)	
Interleukin 6 (pg/ml)			.436
≤54	10/124 (8.1)	1	
>54	2/44 (4.5)	0.54 (0.11–2.58)	
X-Ray			
Opacities of lung surface on X-rays			**.009**
≤50%	13/206 (6.3)	1	
>50%	18/119 (15.1)	2.46 (1.26–5.26)	
Treatment			
Hydroxicloroquine			**.001**
No	11/44 (25.0)	1	
Yes	17/262 (6.5)	0.22 (0.09–0.24)	
Azitromycine			**.002**
No	16/98 (16.3)	1	
Yes	11/207 (5.3)	0.28 (0.64–0.12)	
Ceftriaxone			.334
No	7/69 (10.1)	1	
Yes	9/141 (6.4)	0.60 (0.21–1.69)	
Antiretrovirals			.916
No	12/159 (7.5)	1	
Yes	4/50(8.0)	1.02 (0.32–3.44)	
Tocilizumab			**.007**
No	31/263 (10.8)	NC	
Yes	0/62 (0.0)		
Steroid			.069
No	29/261 (11.1)	1	
Yes	2/64 (3.1)	3.84 (0.90–16.7)	
Noninvasive mechanical ventilation			.677
No	14/176 (8.0)	1	
Yes	2/34 (5.9)	0.72 (0.15–3.33)	

Note: Data shown as *n* (%) unless specified otherwise. In bold, statistically significant differences.

Abbreviations: BP, blood pressure; CI, confidence interval; eGFR, estimated glomerular filtration rate; IOT, intubation orotracheal; NC, not calculable; OR, odds ratio; PaO2:FiO2, pressure arterial of oxygen: fraction of inspired oxygen.

[a]Non-pneumonia: nonhospitalized [mild case].

[b]Pneumonia: hospitalized [severe] and nonhospitalized [mild]).

[c]Days of symptoms before admission.

associated with a higher non-seropositive status incidence; odds ratios (OR) with (95% CI) were estimated. IBM SPSS Statistics v25 (Armonk) was used for analyses. $p < .050$ defined statistical significance.

3 | RESULTS

Of the 325 patients in the global cohort, 210 (64.6%) had severe pneumonia (hospitalized patients), 51 (15.7%) non-severe pneumonia (managed as outpatients, hospital at home follow-up), and 64 (19.7%) mild cases without pneumonia.

3.1 | Non-seropositive incidence

Anti-SARS-CoV-2 antibodies were measured at a median (range) of 76 days (IQR, 70–83; range 14–119) after disease onset. Out of 325 in the global cohort, 31 patients (9.5%; 95% CI, 6.8–13.2) were classified as non-seropositive at that time period. The non-seropositive status incidence was 7.6% (95% CI, 4.7–12.0), 3.9% (95% CI, 1.1-13-2), and 20.3% (95% CI, 12.2–31.7) in patients with severe pneumonia, non-severe pneumonia and without pneumonia, respectively.

3.2 | Seropositive failure associated factors—global cohort

Table 1 shows the associations between negative serostatus and explanatory variables. In the univariate analysis, age, comorbidity, and the absence of pneumonia were associated with a lower frequency of antibody detection, this association was lost after adjusting for confounding factors.

After adjustment (Figure 1), in the global cohort baseline, independent predictors of seropositive failure were higher leukocytes and fewer days of symptoms (<3 days) before clinical evaluation.

3.3 | Seropositive failure *associated factors*—severe pneumonia subpopulation

In the severe pneumonia subpopulation, higher leukocytes and fewer days of symptoms (<4) before admission, remained as a risk factor for seropositive failure.

4 | DISCUSSION

The present study showed that the non-seropositive incidence eleven weeks after disease onset varies according to the clinical severity, being threefold higher in mild cases. Neither age, comorbidity, nor the use of immunosuppressive drugs had an impact on the seropositive rate. However, the impact on the immune response of higher leukocytes and fewer days of symptoms before admission will need to be confirmed in future studies as at this time the relationship between seropositivity and leukocyte counts or with a lower number of days with symptoms before admission has not been described in other studies.[11]

Small sample sizes and short follow-up post-symptom onset (limited to 60-65 days follow-up), constitute the main limitations of the available evidence, about the immune response to SARS-CoV-2 infections.[11]

In hospitalized patients, published seroconversion rates range from 85% to 100%.[12] Liu et al.[13] stratifies hospitalized patients by severity, with a global seroconversion failure of 15%, all severe patients seroconverted (day 43-48 after disease onset).

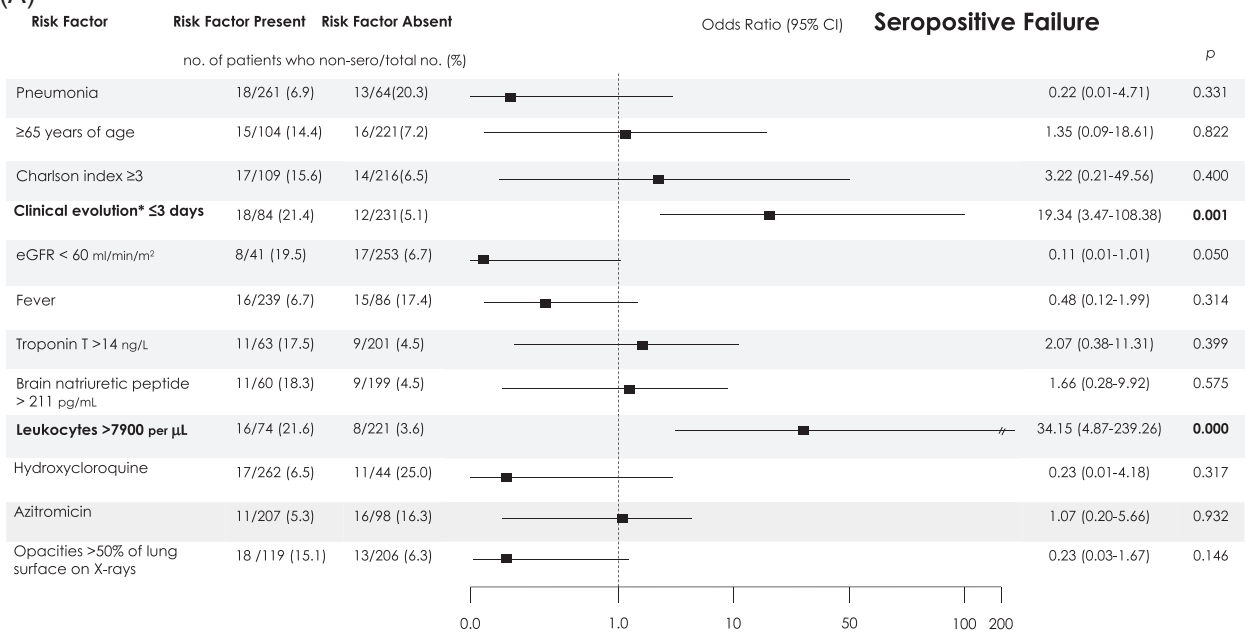
In the mild outpatient population, non-seroconversion rates range from 4.2% to 10%.[5,14–16] Fafi-Kremer et al.,[5] published the largest series of 160 hospital staff who had recovered from mild outpatients forms of COVID-19 and reported seroconversion of 95.6% by rapid immunodiagnostic tests (median 24 days after symptom onset). Shirin et al.[14] reported that mildly symptomatic individuals (n = 108) developed an IgG response by day 14 in 95% of individuals and rose to 100% by day 30; in contrast, asymptomatic individuals (n = 63) developed antibody responses significantly less frequently, with 45% positive for IgG by day 30 after infection.

In our case series, as expected, more severe SARS-CoV-2 infections appear to lead to a robust immune response.[12] Antibodies to the N protein are the most sensitive target for serological diagnosis of infection with SARS-CoV-2,[7] the N proteins being essential for viral survival and expansion,[8,9] whereas other antigens can react against antibodies to other coronaviruses.[7] This specificity is determined by Complementary Determining Regions (CDRs), localized on the N-terminus of the antibody.[17] The different characteristics of the patients and the commercial SARS CoV-2 antibody tests employed, could be contributing to discrepancies in the literature. Given the robustness of the method used in our series,[15] and the concordance of seroconversion failure in the subpopulation with severe disease, bias due to the test used is unlikely.

Finally, elevated WBC count and short duration of clinical evolution, as independent predictors of seropositive failure, could be related to a more robust innate response early in the course of infection that mitigates against a vigorous adaptive response and generates a limitation of viral expression.[18]

Our study has some limitations. This is an observational, retrospective, single-center study, and the collection of data was not standardized in advance. Also, we did not include asymptomatic individuals. As it takes place in a clinical setting, a complementary independent confirmatory test addressing different surface antigens was not performed. The sensitivity of the Elecsys Anti-SARS-CoV-2 in clinical practice differs from manufacturer data, keeping high overall diagnostic sensitivity and specificity, which range from 68.8% to 97.2% and from 99.05% to 100%, respectively.[19] As we lack a sequence of repeat serologic studies, we cannot determine for sure

(A)



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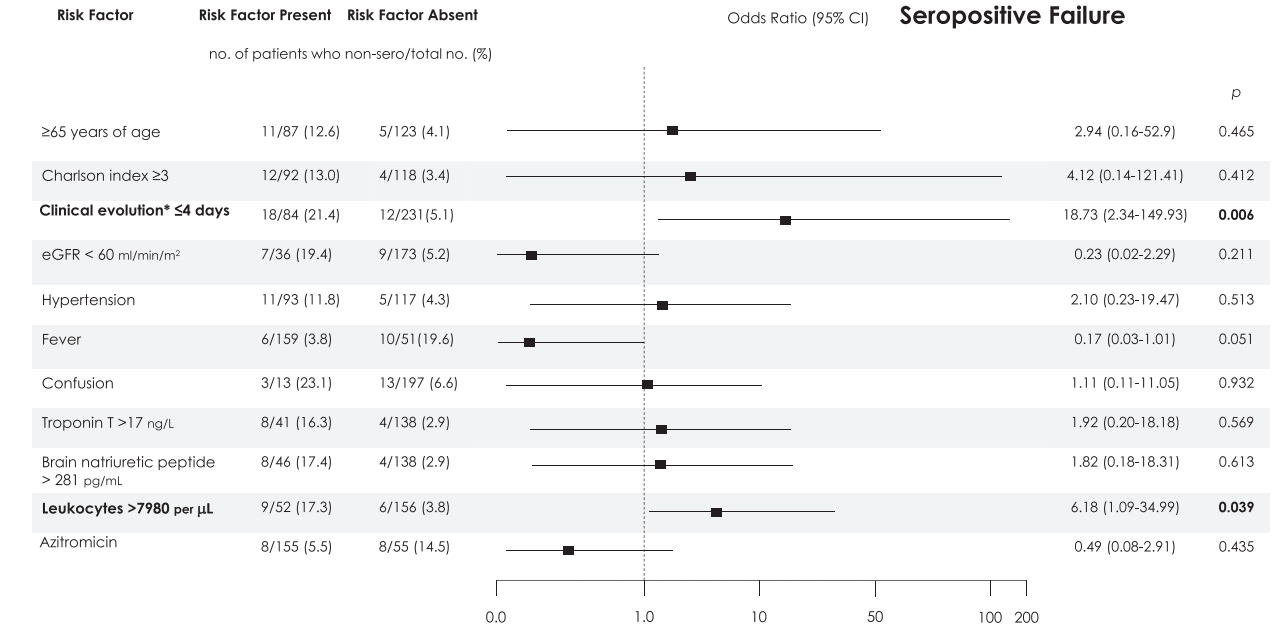

FIGURE 1 Independent Predictors of Seropositive failure at the time of evaluation from multivariable logistic-regression analysis. (A) Global cohort. (B) Severe pneumonia subpopulation numbers and percentages of patients with each risk factor who non-seroconverted (risk factor present) and of patients without each risk factor who seroconverted (risk factor absent) are shown. Variables were included as covariates if they showed significant associations in simple models. The 95% CIs of the odds ratios have been adjusted for multiple testing. R2 models for non-seroconversion: 0.50, global cohort; 0.47, severe pneumonia subpopulation. In bold, independent predictors associated with the outcomes. eGFR by CKD-EPI formula; * on admission. For the purpose of logistic regression models in the global cohort and severe pneumonia subpopulation, variables were categorized regarding their 75-percentiles within each subpopulation, to show the impact of severe extreme values in the outcomes—except for those in which severity is defined by lowest levels, such as clinical evolution and lymphocyte counts, where 25-percentiles were used. For the following variables, standard categorizations were followed: age ≥65 years, Charlson comorbidity index ≥3, eGFR <60 ml/min/1.73 m^2, PaO2:FiO2 < 300. The inclusion of tocilizumab use and anosmia as admission symptom in the logistic regression models (not included in the initial models due to 100% seroconverted), led to renal failure in global cohort and fever in the severe pneumonia subpopulation, reaching statistical significance as protective factors. CI, confidence interval; eGFR, estimated glomerular filtration rate

whether the non-seroconversion rates in patients with infections of varying severity were due to lack of antibody activation, with low-level N protein expression and N antibodies production, or to their rapid decline.

In summary, this experience provides a large case series at a community level that evaluates the incidence of seropositive failure, according to the clinical spectrum produced by the SARS-CoV-2 infection. After 2.5 months, antibody responses may not consistently develop or reach levels sufficient to be detectable by antibody tests in 20% of patients without pneumonia and 6.9% in the COVID-19 pneumonia subpopulation. Our findings, if confirmed, would have direct implications on the interpretation of seroprevalence studies (underestimating rates of infection). Future work is needed to comprehensively characterize the antibody response and associated factors in asymptomatic individuals and minor to mild forms of COVID-19.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Writing—original draft: Jose Manuel Ramos, Esperanza Merino and Oscar Moreno-Pérez; *writing—review and editing*: Jose Manuel Ramos, Esperanza Merino, Oscar Moreno-Pérez, Vicente Boix, Beatriz Valero, Mariano Andres, Jose-Manuel Leon-Ramirez, Joan Gil, Juan Carlos Rodríguez, Pere Llorens and Adelina Gimeno; *conceptualization*: Jose Manuel Ramos, Esperanza Merino, and Oscar Moreno-Pérez; *investigation*: Jose Manuel Ramos, Esperanza Merino, Oscar Moreno-Pérez, Vicente Boix, Beatriz Valero, Mariano Andres, Jose-Manuel Leon-Ramirez, Joan Gil, Juan Carlos Rodríguez, Pere Llorens, and Adelina Gimeno; *methodology*: Jose Manuel Ramos, Esperanza Merino and Oscar Moreno-Pérez; *formal analysis*: Oscar Moreno-Pérez; *project administration*: Esperanza Merino; *funding acquisition*: not applicable.

DATA AVAILABILITY STATEMENT

The data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL

Written informed consent was obtained from all the participants, with approval by the institutional review board (EXP. 200145).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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