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MAJOR ARTICLE

SARS-CoV-2 Seroconversion and Viral Clearance in Patients Hospitalized With COVID-19: Viral Load Predicts Antibody Response

Mar Masiá,[1,a] Guillermo Telenti,[2,a] Marta Fernández,[2] José A. García,[3] Vanesa Agulló,[2] Sergio Padilla,[2] Javier García-Abellán,[2] Lucía Guillén,[2] Paula Mascarell,[2] José C. Asenjo,[2] and Félix Gutiérrez[1]

[1]Hospital General Universitario de Elche and Universidad Miguel Hernández, Elche, Alicante, Spain, [2]Hospital General Universitario de Elche, Elche, Alicante, Spain, and [3]Operational Research Center, Universidad Miguel Hernández, Elche, Alicante, Spain

Background. The interdependencies of viral replication and the host immune response in patients with coronavirus disease 2019 (COVID-19) remain to be defined. We investigated the viral determinants of antibody response, the predictors of nonseroconversion, and the role of antibodies on viral dynamics.

Methods. This was a prospective study in patients hospitalized with COVID-19 that was microbiologically confirmed by real-time polymerase chain reaction (RT-PCR). Serial nasopharyngeal and oropharyngeal swabs and plasma samples were obtained for measuring severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA and antibodies (total and S-IgG/N-IgG), respectively.

Results. Of 132 patients included, 99 (75%) showed positive antibody titers after a median (Q1–Q3) of 11 (8–14) days. The median (Q1–Q3) follow-up was 74.5 (63.0–87.0) days. In an adjusted linear regression model, time to seropositivity was inversely associated with peak log SARS-CoV-2 viral load ($P = .009$) and positively with time to viral clearance ($P = .004$). Adjusted predictors of S-IgG levels were time to viral clearance ($P < .001$), bilateral lung infiltrates on admission ($P = .011$), and the time-dependent SARS-CoV-2 RNA ($P < .001$) and SARS-CoV-2 RNA area under the curve ($P = .001$). Thirty-three (25%) patients showed undetectable antibody titers. Patients who did not seroconvert had higher cycle threshold values of RT-PCR (38.0 vs 28.0; $P < .001$), had shorter time to viral clearance (3.0 vs 41.0; $P < .001$), and were more likely to have SARS-CoV-2 only detected on fecal samples ($P < .001$). Nonseroconvertors had also lower levels of blood inflammatory biomarkers on admission and lower disease severity.

Conclusions. Viral replication determines the magnitude of antibody response to SARS-CoV-2, which, in turn, contributes to viral clearance. COVID-19 patients who do not seroconvert exhibit a differential virological and clinical profile.

Key words. coronavirus; COVID-19; SARS-CoV-2; viral load; seroconversion; antibody responses; viral clearance.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease (COVID-19) has emerged as a rapidly escalating pandemic that has spread to the 5 continents. While an unprecedented amount of knowledge has been generated about this novel coronavirus during the few months that have elapsed since its emergence, several aspects of the natural history of SARS-CoV-2 infection remain to be well characterized. Particularly, little information is available about the interdependencies of viral replication and the immune response. Humoral immune response is crucial for viral clearance by production of virus-specific antibodies that neutralize the entry of free virions into uninfected cells, opsonize virus for inactivation by complement proteins or for elimination by phagocytic immune cells such as macrophages and neutrophils, and inactivate virions or initiate killing of infected cells by triggering the complement cascade and through antibody-mediated cytotoxicity processes [1]. Besides viral elimination, antibody response is necessary for protective immunity against reinfection [2]. On the other hand, the intensity of viral replication might be a determining factor in the magnitude of the host immune response to infection. High viral loads are capable of inducing an immediate activation of extrafollicular B cells that leads to early and intense antibody production [3]. In infections caused by respiratory syncytial virus, the viral load was found to correlate with the strength of the innate immune response [4].

The host immune response differs among the SARS-CoV-2-infected population. A proportion of patients exhibit a dysregulated innate immune response with excessive secretion of proinflammatory cytokines that lead to severe organ damage [5], while others remain asymptomatic. Likewise, the humoral immune response to SARS-CoV-2 varies from

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[a]Equal contribution

Correspondence: Félix Gutiérrez, MD, Infectious Diseases, Hospital General de Elche & Universidad Miguel Hernández, Camí de la Almazara S/N, 03203 Elche, Alicante, Spain (gutierrez_fel@gva.es).

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early and strong antibody release to weaker or absent antibody production [6–8]. In fact, a percentage ranging between 10% and 20% of patients with polymerase chain reaction (PCR)–confirmed SARS-CoV-2 [6, 9] infection show negative immunoglobulin G (IgG) titers. While antibody response has been associated with disease severity in patents with COVID-19 [6, 7, 10], similar to what was described in SARS [11], the additional factors contributing to explain such differences, especially the reciprocal effects of immune response and viral dynamics, have not yet been defined. We aimed to identify the virological and clinical predictors of antibody responses in patients with COVID-19, the predictors of nonseroconversion, and the role of antibodies in viral dynamics.

METHODS

This prospective observational study was carried out at the University Hospital of Elche, Spain. Patients enrolled in the study were all those admitted for COVID-19 between March 10 and May 19, 2020, who were confirmed to be infected with SARS-CoV-2 upon hospital admission through real-time PCR (RT-PCR), mostly from nasopharyngeal smear samples and rarely from fecal samples.

Patients were managed according to a predefined protocol that included diagnostic and therapeutic procedures during the hospital stay. Blood samples for routine lab tests and biomarkers of cytokine release syndrome, serologic tests, and nasopharyngeal samples for SARS-CoV-2 were serially obtained at different time points during the hospital stay. Serum samples for the measurement of levels of antibodies to SARS-CoV-2 were collected and frozen at −80°C. The protocol was approved by the Ethical Committee of the Hospital General Universitario de Elche (Spain) as part of the COVID-19@Spain study.

Therapy for COVID-19 was given following institutional guidelines. Patients received antimicrobial and/or immunomodulatory therapy containing lopinavir/ritonavir (LPV/r), hydroxychloroquine, azithromycin, interferon-β-1b or remdesivir ± methylprednisolone. According to guidelines, tocilizumab and intravenous methylprednisolone were added to initial therapy on admission if any of the pre-established severity criteria were met.

SARS-CoV-2 RNA Measurements

For RNA extraction and RT-PCR analysis for SARS-CoV-2, nasopharyngeal and oropharyngeal flock swabs were placed together into 3 mL of transport medium (VICUM, Deltalab, Rubí, Spain). Viral RNA was extracted from 350 µL of the medium using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and eluted in a final 50-µL nucleic acid elution sample. Eight microliters of RNA was used for detection of SARS-CoV-2 by RT-PCR with a commercially available kit (AllplexTM 2019-nCoV Assay, Seegene, Seoul, Korea), which targeted the E, RdRP, and N genes. Viral load measurements of nasal/throat samples (log10 copies/sample) were performed with a standard curve of 10-fold serial dilutions from an in vitro RNA transcript (Macrogen, Seoul, Korea). The lower limit of detection was 64 copies/sample. The assay procedure was carried out in accordance with the manufacturer's protocol in a CFX96 real-time thermocycler (Bio-Rad, Hercules, CA, USA). The success of RNA extraction and PCR were assessed by the internal control included in the kit, and negative and positive controls were used in each assay.

SARS-CoV-2 Antibody Testing

Both total antibodies (including IgG, IgA, and IgM) against the SARS-CoV-2 surface S1 domain of the spike (S) protein and IgG antibodies against the internal nucleocapsid (N) protein (N-IgG) and surface S1 domain of the S protein (S-IgG) were measured in EDTA plasma samples using commercial techniques. Total antibodies were detected by an immunometric technique (VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total Reagent pack used in combination with the VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total Calibrator, Ortho-Clinical Diagnostics, Rochester, NY, USA) in an automated instrument (VITROS XT 7600 Integrated System) following the manufacturer's instructions. N-IgG (Anti-SARS-CoV-2-NCP IgG ELISA, Euroimmun, Lubeck, Germany) and S-IgG (Anti-SARS-CoV-2 IgG ELISA, Euroimmun, Lubeck, Germany) were detected using commercial semiquantitative enzyme immunosassay kits in an automated instrument (Dynex DS2 ELISA system) following the manufacturer's instructions. Antibody levels were evaluated by calculating the ratio of the optical density (OD) of the patient sample over the OD of the calibrator (sample OD/calibrator OD = S/CO [absorbance/cutoff]). Results were interpreted according to the following criteria: ratio ≤1.1 was defined as negative and ratio >1.1 as positive.

Statistical Analyses

Continuous variables are expressed as median ± 25th and 75th percentiles (Q1, Q3), and categorical variables as percentages. The Wilcoxon test and Student *t* test were used to compare continuous variables, and the chi-square test or Fisher exact test for the comparison of categorical variables. The Pearson and Spearman tests, where appropriate, were used to analyze the correlations between time to seropositivity, viral load, and time to viral clearance. Predictors of time to seropositivity were investigated through multivariate linear regression. Generalized linear mixed-model analysis was performed to analyze factors associated with the levels of S-IgG as a time-dependent variable, and logistic regression was used to analyze variables associated with the categorized IgG variable, and with nonseroconversion. Covariates with a

P value <.05 in the univariate analyses were included in the models. Statistical analysis was performed using R, version 3.6.2.

RESULTS

Of 210 adult patients admitted with COVID-19 during the pandemic, 132 patients with serial available serological samples taken on >14 days since the initiation of symptoms and with confirmed PCR on nasopharyngeal (94%) and/or fecal (6%) samples were included in the study. A flowchart of the patients is shown in Supplementary Figure 1. The median (Q1–Q3) age was 63.5 (54.0–76.0) years, 61.4% were male, 59.5% were active smokers, and the most frequent comorbidities were diabetes (22%), respiratory disease (13.6%), and renal disease (9.8%), with median (Q1–Q3) Charlson comorbidity index of 3 (1–5) (Table 1). The median (Q1–Q3) time from symptom onset to admission was 6.0 (2.0–11.0) days, the median (Q1–Q3) SOFA score was 2.0 (2.0–3.0), and 73 (67%) patients had bilateral lung infiltrates on admission. The median cycle threshold (Ct) value for the E gene of SARS-CoV-2 RNA (Q1–Q3) was 32.7 (27.6–39.0), and the peak viral load was 3.3 (0.0–4.4) log10 copies/sample. The median time to viral clearance (Q1–Q3) was 34.0 (12.0–56.5) days. There was a positive correlation of SARS-CoV-2 viral load with ferritin (P = .005), LDH (lactate dehydrogenase; P = .004), and fibrinogen (P = .001), a trend to a positive relationship with the levels of C-reactive protein (CRP; P = .087), and an inverse association with the blood lymphocyte (P = .011) and neutrophil (P = .037) counts and with the neutrophil-to-lymphocyte ratio (P = .037).

The majority of patients were treated with hydroxichloroquine, azithromycin, or LPV/r, 75 (56.8%) patients received tocilizumab, and 25 (18.9%) received methylprednisolone. The median (Q1–Q3) length of hospital stay was 12.0 (9.0–17.0) days, 14 (10.6%) patients were admitted to the ICU, and 4 (3%) died. The median (Q1–Q3) follow-up duration of the patients was 74.5 (63.0–87.0) days.

Predictors of Time to Seropositivity

The number of patients with positive serological tests during follow-up was 99 (75%). The median (Q1–Q3) time to seropositivity was 11 (8–14) days. Time to seropositivity was inversely associated with peak viral load (P = .007) (Figure 1) and positively associated with time to viral clearance (P = .029) (Figure 2). Additional factors associated with time to seropositivity included age (P = .037), the Charlson comorbidity index (P = .011), neutrophil count (P = .019), and neutrophil-to-lymphocyte ratio (P = .043), all of which had a negative correlation (Table 2).

In a multivariable model adjusted for the significant (P < .05) variables of the univariate analysis, time to antibody production was inversely associated with peak log SARS-CoV-2 viral load

Table 1. Clinical Data of Patients Admitted With COVID-19 Confirmed With Real-time Polymerase Chain Reaction

Variable	No. (%) or Median (Q1–Q3)
Sex, male	81 (61.4)
Age, y	63.5 (54–76)
Active smoking	72 (59.5)
Charlson comorbidity index	3 (1–5)
Comorbidities	
Diabetes	29 (22)
Congestive heart failure	7 (5.3)
Previous AMI	11 (8.3)
Previous stroke	10 (7.6)
Respiratory disease	18 (13.6)
Renal disease	13 (9.8)
Peripheral arterial disease	4 (3)
Clinical status	
Days from symptom onset to admission	6.0 (2.0–11.0)
SOFA score on admission	2 (2–3)
Median follow-up, d	74.5 (63–87)
SpO2/FIO2 on admission	350 (339.3–452.4)
Bilateral lung infiltrates on x-ray	73 (67)
Microbiological data	
SARS-CoV-2 in nasopharyngeal and oropharyngeal samples	124 (94)
SARS-CoV-2 detected only in fecal samples	8 (6)
Cycle threshold (E gen)	32.7 (27.6–39)
Peak SARS-CoV-2 RNA, copies/sample	3.3 (0.0–4.4)
Cycle threshold (E gen) <35	72 (54.5)
Cycle threshold (E gen) 35–38	9 (6.8)
Cycle threshold (E gen) >38	42 (31.8)
Time to viral clearance, d	34 (12–56.5)
Time to seroconversion, d	11 (8–14)
Peak S-IgG, S/CO	26 (18–39.5)
Peak N-IgG, S/CO	19 (14–27)
Biomarkers	12 (9–17)
Interleukin-6, pg/mL	24.2 (12.6–80.6)
Ferritin, ng/mL	302.8 (143.2–497.2)
C-reactive protein, mg/L	48.5 (18.1–87.2)
Fibrinogen, mg/dL	551 (357.9–750.8)
LDH	231 (190–297.5)
Lymphocytes, ×103/µL	1.2 (0.9–1.5)
Neutrophil	4 (2.9–6.5)
Neutrophil/lymphocytes	4.7 (3.5–6.6)
D-dimer, µg/mL	0.7 (0.4–1.4)
NT-proBNP, pg/mL	66 (27–199.7)
Outcomes	
Death	4 (3)
ICU admission	14 (10.6)
Hospital stay, d	12 (9–17)
Concomitant antimicrobial/immunomodulatory drugs, No. (%)	
Hydroxychloroquine	161 (98.8)
Azithromycin	151 (92.6)
Lopinavir/ritonavir	146 (89.6)
Remdesivir	1 (0.6)
Interferon-β-1b	28 (17.2)
Tocilizumab	75 (56.8)
Methylprednisolone	25 (18.9)

Categorical variables are expressed as No. (%) and continuous variables as median (Q1–Q3).

Abbreviations: AMI, acute myocardial infarction; COVID-19, coronavirus disease 2019; ICU, intensive care unit; LDH, lactate dehydrogenase; NT-proBNP, N-terminal pro-brain natriuretic peptide; S/CO, absorbance/cutoff; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SOFA, Sequential Organ Failure Assessment; SpO2/FIO2, peripheral blood oxygen saturation/fraction of inspired oxygen rate.

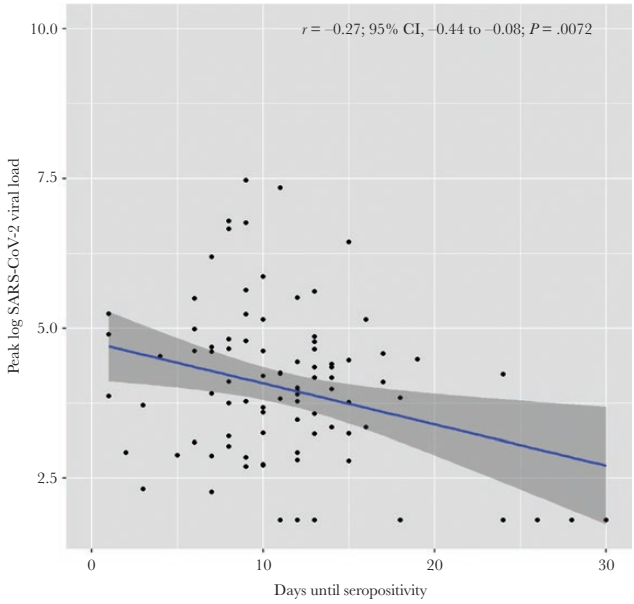

Figure 1. Correlation between peak viral load and time to seropositivity.

(P = .009) and positively associated with time to viral clearance (P = .004) (Table 2).

Predictors of the Intensity of Antibody Response

Factors associated with peak antibody titers are shown in Table 2. Peak levels of S-IgG were associated with male sex (P = .003), bilateral lung infiltrates (P = .004), higher levels of CRP (P = .011) and LDH (P = .005), and lower lymphocyte count (P = .005); there was a mild association with longer time to viral clearance (P = .070). When analyzed as a time-dependent variable, the titer of the antibodies was associated with the closest SARS-CoV-2 RNA (P < .001) and with the area under the curve for the viral load (P < .001).

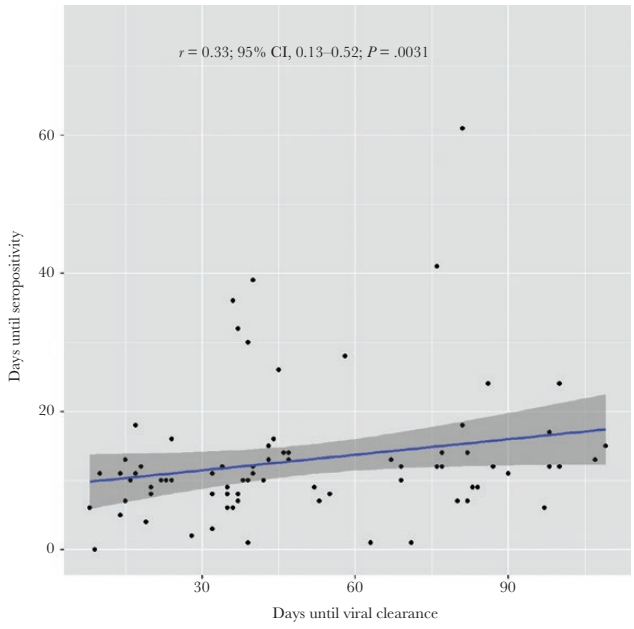

Figure 2. Correlation between time to seropositivity and time to viral clearance.

In a generalized linear mixed-model analysis adjusted for the significant variables of the univariate analysis, levels of S-IgG were associated with bilateral lung infiltrates on admission (P = .011), time-dependent SARS-CoV-2 RNA (P < .001), the area under the curve for the viral load for each antibody titer (P = .001), and time to viral clearance (P < .001).

Predictors of Nonseroconversion

There were 33 (25%) patients who did not seroconvert. Patients' characteristics according to serostatus are shown in Table 3. Patients who did not seroconvert were older (P = .023) and had more comorbidities, with a significantly higher Charlson comorbidity index score (P < .001). The frequency of bilateral lung infiltrates was lower (P = .006), and they had a shorter hospital stay (P = .010). They were more likely to have SARS-CoV-2 RNA only detected in fecal samples (P < .001). The median time to viral clearance (Q1–Q3) was 3.0 (1.0–12.0) days in seronegative patients and 41.0 (32.0–76.0) days in patients who seroconverted (P < .001). The median Ct value was 38.0 (37.0–38.7) in patients who did not seroconvert and 28.04 (25.5–30.5) in those who seroconverted (P < .001). Regarding COVID-19 therapy, patients who did not seroconvert were less frequently treated with LPV/r (P < .001), interferon beta 1 b (P < .001), tocilizumab (P < .001), and steroids (P = .038). The levels of several inflammatory biomarkers on admission were significantly lower in seronegative patients, including ferritin (P < .001), fibrinogen (P = .001), CRP (P = .023), and LDH (P < .001), and they had higher blood lymphocyte (P = .032) and neutrophil (P = .001) counts.

In the adjusted multivariate logistic regression including the significant variables of interest, with the exception of age because of multicollinearity with the Charlson index and the significant comorbidities that were also contained in the Charlson index, the predictors of nonseroconversion in patients with COVID-19 were a higher Ct of RT-PCR (odds ratio [OR], 1.87; 95% CI, 1.09–3.21; P = .023), higher Charlson comorbidity index (OR, 1.35; 95% CI, 1.04–1.76; P = .027), higher peripheral blood oxygen saturation/fraction of inspired oxygen rate (SpO2/FIO2; OR, 1.014; 95% CI, 1.00–1.02; P = .036), higher neutrophil count (OR, 1.38; 95% CI, 0.96–1.97; P = .081), and lower fibrinogen levels (OR, 0.99; 95% CI, 0.99–1; P = .032).

DISCUSSION

The reciprocal interactions between viral replication and the host immune response in patients with COVID-19 have not yet been characterized. Our study suggests a central role for SARS-CoV-2 viral load in the humoral immune response. Higher viral loads were associated with earlier antibody response, and, at the

Table 2. Univariate and Multivariate Models for Predictors of Time to Seropositivity and Peak Antibody Titer

	Time to Seropositivity			Peak S-IgG titer		
Variable	Coefficient	*P*	Adjusted *P*[a]	Coefficient	*P*	Adjusted *P*[a]
Sex, male	0.082	.226		2.262	.003	.8472
Age, y	–0.005	.037	.408	–0.018	.507	
Active smoking	0.085	.255		–0.270	.746	
Charlson comorbidity index	–0.040	.011	.149	–0.243	.182	
SOFA score on admission	–0.016	.555		–0.223	.497	
SpO2/FIO2 on admission	0.000	.776		0.005	.383	
Bilateral lung infiltrates on x-ray	0.06952	.423		2.450	.004	.011
Microbiological data						
Peak log SARS-CoV-2 RNA, copies/sample	–0.048	.009	.009	–0.075	.727	
Log SARS-CoV-2 RNA				0.054	<.001	<.001
Cumulative RNA AUC	0.000	.891		0.003	.548	
Time to viral clearance	0.003	.042	.007	0.031	.070	<.001
Days to seroconversion	–			0.062	.157	
Biomarkers						
Interleukin-6, pg/mL	0.000	.581		0.004	.298	
Ferritin, ng/mL	0.000	.858		0.001	.616	
C-reactive protein, mg/L	0.000	.893		0.013	.011	.213
LDH	0.000	.630		0.013	.005	.150
Fibrinogen, mg/dL	0.000	.093		0.001	.573	
Lymphocytes, ×103/μL	0.079	.228		–2.069	.005	.290
Neutrophil	–0.030	.043	.2674	0.083	.634	
Neutrophil/lymphocytes	0.007	.595		0.081	.262	
D-dimer, μg/mL	–0.045	.126		–0.097	.779	
NT-proBNP, pg/mL	0.000	.213		–0.004	.129	
Outcomes						
Death	–0.007	.976		–2.016	.460	
ICU admission	0.323	.001		0.734	.519	

[a]Adjustment was performed by logistic regression.

Abbreviations: AMI, acute myocardial infarction; AUC, area under the curve; COVID-19, coronavirus disease 2019; ICU, intensive care unit; LDH, lactic dehydrogenase; NT-proBNP, N-terminal pro-brain natriuretic peptide; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SOFA, Sequential Organ Failure Assessment; SpO2/FIO2, peripheral blood oxygen saturation/fraction of inspired oxygen rate.

other end of the spectrum, patients who did not seroconvert showed the lowest viral loads. On the other hand, the kinetics of the humoral immune response predicted the speed of viral elimination, so that earlier antibody response was associated with faster viral clearance.

The dynamics of the antibody response against SARS-CoV-2 are currently under investigation. In order to characterize the viral determinants of the antibody response, we analyzed a cohort of consecutive, unselected patients hospitalized for COVID-19 during the pandemic, with a wide spectrum of severity. Patients were thoroughly evaluated with serial nasopharyngeal and blood samples to assess virological and serological responses. Among the immunoglobulins, we opted for the analysis of IgG levels in order to assess the degree of activation of the humoral immune response [12] and focused on S-IgG, as it is potentially capable of eliminating the virus and might protect against reinfection [13]. We found that the amount of SARS-CoV-2 RNA was an important factor implicated in the humoral immune response against the virus. There was a correlation between viral load and time to seropositivity, and higher peak viral loads were associated with earlier antibody release, whereas very low initial SARS-CoV-2 RNA levels were observed in patients who tested negative for S-IgG, suggesting that the induction of the adaptive humoral immune response might be dependent on the intensity of viral replication. In addition, viral load positively correlated with several other biomarkers of host response, as shown by the association with macrophage-induced molecules like ferritin or CRP, with lymphopenia as a likely reflection of the cytokine release [14], or the acute phase inflammation molecule fibrinogen or LDH, the latter reflecting tissue damage and associated with mortality [15].

Duration of viral shedding varies among COVID-19 patients, and the factors determining such differences need to be further disclosed. Older age, comorbidities, immunomodulatory therapy for COVID-19, and severity of disease have been linked with prolonged viral shedding [16–18]. Our study shows a positive correlation between time to antibody response and time to viral clearance, which would support the implication of the humoral immune response in viral elimination. Therefore, a delay in mounting the adaptive antibody response might contribute

Table 3. Factors Associated With Nonseroconversion in Patients With COVID-19

Variable	Nonseroconverter	Seroconverter	*P*	Adjusted[a] OR (95% CI)	*P*[a]
Sex, male	22 (66.7)	59 (59.6)	.539		
Age, y	71.0 (62.0–77.0)	60.0 (53.0–74.0)	.023	-	
Active smoking	14 (45.2)	58 (64.4)	.089		
Charlson comorbidity index	5.0 (3.0–7.0)	2.0 (1.0–4.0)	<.001	1.35 (1.04–1.76)	.027
Clinical status					
Days from symptom onset to admission	3.0 (1.0–8.0)	7.0 (4.5–10.0)	.004	-	
Days from symptom onset to treatment initiation	3.0 (2.0–10.0)	10.0 (6.5–14.0)	<.001	-	
SOFA score on admission	3.0 (1.0–3.2)	2.0 (2.0–3.0)	.705		
Median follow-up	63.0 (40.0–72.0)	80.0 (71.0–89.0)	<.001	-	
SpO2/FIO2 on admission	380.8 (343–462)	350.0 (339–380)	.051	1.01 (1.00–1.02)	.036
Bilateral lung infiltrates on x-ray	9 (27.3)	54 (56.2)	.006	1.20 (0.28–5.23)	.809
Microbiological data					
SARS-CoV-2 detected only in fecal samples	8 (24)	0	.001		
Days from onset to first serological sample	6 (2–17)	10.0 (8.0–13.0)	.055		
Cycle threshold (E gen)	38 (37–38.7)	28.0 (25.5–30.5)	<.001	1.87 (1.09–3.21)	.023
Time to viral clearance	3 (1–12)	41.0 (32.0–76.0)	<.001		
Biomarkers					
Interleukin-6, pg/mL	20.7 (10–41.8)	25.6 (12.9–88.6)	.189		
Ferritin, ng/mL	132.0 (68.1–369)	377.5 (235–584)	<.002	0.99 (0.99–1)	.173
C-reactive protein, mg/L	35.3 (6.1–66.2)	50.3 (24.4–99.1)	.023	1.10 (0.73–1.66)	.002
LDH	188.2 (159–228)	249.5 (211–301)	<.001	1.00 (0.99–1)	.769
Fibrinogen, mg/dL	420.8 (263–554)	613.0 (440–782)	.001	0.99 (0.99–1)	.032
Lymphocytes, ×103/μL	1.4 (1.1–2.1)	1.2 (0.9–1.4)	.032	0.99 (0.88–1.12)	.951
Neutrophil	5.9 (4.9–8.6)	4.2 (3.3–6.2)	.001	1.38 (0.96–1.97)	.081
Neutrophil/lymphocytes	4.2 (3.3–6.2)	4.7 (3.5–6.6)	.001		
D-dimer, μg/mL	1.0 (0.4–2.1)	0.6 (0.4–1.2)	.121		
NT-proBNP, pg/mL	42.0 (7.4–161)	75 (41.2–200)	.068		
Outcomes					
Death	2 (6.1)	2 (2.0)	.260		
ICU admission	1 (3)	13 (13.1)	.188		
Hospital stay, d	10 (6.5–14)	13.0 (10.0–19.0)	.010		
Anti-COVID-19 therapy					
HCQ-based combinations	33 (100.0)	97 (98.0)	1.00		
Azithromycin	31 (93.9)	92 (92.9)	1.00		
Lopinavir/ritonavir	22 (66.7)	98 (99.0)	<.001		
Remdesivir		1 (1)	1.00		
Interferon-β-1b		28 (28.3)	<.001		
Tocilizumab	5 (15.2)	70 (70.7)	<.001		
Methylprednisolone[b]	2 (6.1)	23 (23.2)	.038		

[a]Adjustment was performed by logistic regression. Categorical variables are expressed as No. (%), and continuous variables as median (Q1–Q3).

[b]1 to 3 bolus of 125-250 mg of methylprednisolone.

Abbreviations: AMI, acute myocardial infarction; COVID-19, coronavirus disease 2019; ICU, intensive care unit; LDH, lactic dehydrogenase; NT-proBNP, N-terminal pro-brain natriuretic peptide; OR, odds ratio; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SOFA, Sequential Organ Failure Assessment; SpO2/FIO2, peripheral blood oxygen saturation/fraction of inspired oxygen rate.

to explaining the elongated viral shedding observed in a proportion of patients. Prolonged viral shedding was otherwise associated with higher levels of IgG, probably reflecting a higher release of antibodies due to prolonged exposure to the virus [16]. In addition to the virological, other factors were associated with the intensity of the antibody response. We found that greater severity of disease, as reflected by more extensive lung involvement, longer duration of hospital stay, and higher levels of inflammatory biomarkers, was positively associated with higher levels of S-IgG.

We identified a proportion of patients with COVID-19 who did not develop antibodies against the virus using 2 different assays to detect both total antibodies (including IgG, IgA, and IgM) against the SARS-CoV-2 surface S1 domain of the S protein and IgG antibodies against the internal nucleocapsid protein and surface S1 domain of the S protein. Serological analyses were obtained a median of 6 days after the onset of symptoms, and up to 63 days afterwards, which entails a wide enough interval as to detect seroconversion. To date, no studies have characterized patients with COVID-19 failing to generate the antibody response.

We found that these patients exhibited a differential clinical, biological, and virological profile. An interesting finding was that all but 1 of the nonseroconverters showed high cycle threshold values of RT-PCR. This would suggest that low SARS-CoV-2 viral load might be insufficient to stimulate adaptive humoral immunity and generate the antibody response. The reasons why some COVID-19 patients show such low viral loads remain unknown and might hypothetically include genetic factors related to susceptibility and/or immune response to the virus. Genetic factors could potentially lead to downregulation of ACE-2 receptors, which would hamper the entry of the virus into the cells [19]. In a proportion of these patients, SARS-CoV-2 RNA could only be detected in the feces, which could denote a barrier for viral entry in the respiratory tract cells. Nonseroconverters were older and had a higher frequency of comorbidities. Although not probably the most relevant, both factors might have partially contributed to the undetectable antibody titers, as a decline in the humoral response has been described with age and comorbidities [20, 21]. An alternative explanation for the seronegative status in COVID-19 patients might be the earlier intervention of the innate immune cells like neutrophils, interferon, or natural killer cells for viral clearance [1], before the adaptive immune response reached a significant level. Patients who did not generate antibodies showed higher levels of neutrophils. An early and efficient innate immune response might hypothetically have resulted in the low levels of viral shedding and low severity of disease observed in seronegative patients.

Limitations of the study include that SARS-CoV-2 RNA measurements were performed in upper respiratory tract samples, which may exhibit in some cases lower sensitivity than lower tract specimens. All patients received antiviral agents with potential effects on viral dynamics, and a majority of them also received immunomodulators, which might have a detrimental effect in the immune response; however, seronegative patients were less frequently treated with these drugs than those who seroconverted.

In conclusion, in patients with COVID-19, viral replication determines the magnitude of the humoral immune response. High viral load predicts an earlier antibody response, while nonseroconversion is linked with very low replication. In addition, the kinetics of the humoral immune response predicts the speed of viral elimination. Further investigation is warranted to deepen our understanding of host–virus interactions in patients with COVID-19 and to assess the outcomes of nonseroconverters after re-exposure to SARS-CoV-2.

Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Patient consent. The patients' written consent was obtained. The design of the work was approved by the Ethical Committee of the Hospital General Universitario de Elche (Spain).

References

1. Newton AH, Cardani A, Braciale TJ. The host immune response in respiratory virus infection: balancing virus clearance and immunopathology. Semin Immunopathol **2016**; 38:471–82.
2. Channappanavar R, Zhao J, Perlman S. T cell-mediated immune response to respiratory coronaviruses. Immunol Res **2014**; 59:118–28.
3. Lee SK, Rigby RJ, Zotos D, et al. B cell priming for extrafollicular antibody responses requires Bcl-6 expression by T cells. J Exp Med **2011**; 208:1377–88.
4. Piedra FA, Mei M, Avadhanula V, et al. The interdependencies of viral load, the innate immune response, and clinical outcome in children presenting to the emergency department with respiratory syncytial virus-associated bronchiolitis. PLoS One **2017**; 12:e0172953.
5. McKechnie JL, Blish CA. The innate immune system: fighting on the front lines or fanning the flames of COVID-19? Cell Host Microbe **2020**; 27:863–9.
6. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis. **2020**; 71:2027–34.
7. Wang Y, Zhang L, Sang L, et al. Kinetics of viral load and antibody response in relation to COVID-19 severity. J Clin Invest. **2020**; 130:5235–44.
8. Long Q, Tang X, Shi Q, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med. **2020**; 26:1200–04.
9. Xiang F, Wang X, He X, et al. Antibody detection and dynamic characteristics in patients with COVID-19. Clin Infect Dis. **2020**; 71:1930–4.
10. Liu X, Wang J, Xu X, Liao G, Chen Y, Hu CH. Patterns of IgG and IgM antibody response in COVID-19 patients. Emerg Microbes Infect **2020**; 9:1269–74.
11. Lee N, Chan PK, Ip M, et al. Anti-SARS-CoV IgG response in relation to disease severity of severe acute respiratory syndrome. J Clin Virol **2006**; 35:179–84.
12. Xiao DAT, Gao DC, Zhang DS. Profile of specific antibodies to SARS-CoV-2: the first report. J Infect **2020**; 81:147–78.
13. Buchholz UJ, Bukreyev A, Yang L, et al. Contributions of the structural proteins of severe acute respiratory syndrome coronavirus to protective immunity. Proc Natl Acad Sci U S A **2004**; 101:9804–9.

14. Tavakolpour S, Rakhshandehroo T, Wei EX, Rashidian M. Lymphopenia during the COVID-19 infection: what it shows and what can be learned. Immunol Lett **2020**; 225:31–32.
15. Yan L, Zhang H, Goncalves J, et al. An interpretable mortality prediction model for COVID-19 patients. Nat Mach Intell **2020**; 2:283–8.
16. Jin C, Zhu L, Gao C, Zhang S. Correlation between viral RNA shedding and serum antibodies in individuals with coronavirus disease 2019. Clin Microbiol Infect. **In press**.
17. Qi L, Yang Y, Jiang D, et al. Factors associated with the duration of viral shedding in adults with COVID-19 outside of Wuhan, China: a retrospective cohort study. Int J Infect Dis **2020**; 96:531–7.
18. Li TZ, Cao ZH, Chen Y, et al. Duration of SARS-CoV-2 RNA shedding and factors associated with prolonged viral shedding in patients with COVID-19 [published online ahead of print July 9, 2020]. J Med Virol. **2020**; doi:10.1002/jmv.26280
19. Gheblawi M, Wang K, Viveiros A, et al. Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. Circ Res **2020**; 126:1456–74.
20. Fulton RB, Varga SM. Effects of aging on the adaptive immune response to respiratory virus infections. Aging Health **2009**; 5:775.
21. Kwetkat A, Heppner HJ. Comorbidities in the elderly and their possible influence on vaccine response. Interdiscip Top Gerontol Geriatr **2020**; 43:73–85.