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The impact of early anti-SARS-CoV-2 antibody production on the length of hospitalization stay among COVID-19 patients

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ABSTRACT COVID-19 has challenged the scientific community in the search for biological markers and information that can contribute to the early management of the severe disease. Given the global scale of COVID-19, including reports of reinfection even in the presence of effective vaccines; consequently, the eradication of SARS-CoV-2 is far from happening. This study aimed to characterize the neutralizing antibody (Nab) geometric mean titers (GMTs) in hospitalized patients with COVID-19 and to evaluate the association with length of stay, comorbidities, and patient outcome. Among the 103 participants, 84 (81.5%) had some previous conditions associated with worsening health and 34 (33%) died. We found that neutralization potency varied greatly across individuals and was significantly higher in patients discharged before 14 days than in patients who stayed longer in the hospital. During the study period, 15 people living with HIV (PLWH) were hospitalized, and no significant difference in clinical characteristics or anti-SARS-CoV-2 Nabs was observed. However, PLWH with severe COVID-19 were younger [42, interquartile range (IQR) = 17.5] than other hospitalized COVID-19 patients (59, IQR = 22, $P < 0.01$). A high anti-HIV-1 antibody GMT of 583.9 (95% confidence interval: 344–990) was detected, demonstrating maintenance of anti-HIV-1 Nab production among PLWH coinfected with SARS-CoV-2. Therefore, these results indicate that neutralizing antibodies are not the only immunological response capable of controlling disease progression, but a high neutralizing response was associated with a shorter length of stay, suggesting the benefit of early-stage immunotherapy treatment of COVID-19. Nevertheless, these data highlight the importance of more Nab screening studies to predict faster recovery.

IMPORTANCE The study provides valuable insights into the sociodemographic characteristics, clinical outcomes, and humoral immune response of those affected by the virus that has devastated every field of human life since 2019; the COVID-19 patients. Firstly, the association among clinical manifestations, comorbidities, and the production of neutralizing antibodies (Nabs) against SARS-CoV-2 is explored. Secondly, varying levels of Nabs among patients are revealed, and a significant correlation between the presence of Nabs and a shorter duration of hospitalization is identified, which highlights the potential role of Nabs in predicting clinical outcomes. Lastly, a follow-up conducted 7 months later demonstrates the progression and persistence of Nabs production in recovered unvaccinated individuals. The study contributes essential knowledge regarding the characteristics of the study population, the early humoral immune response, and the dynamics of Nabs production over time. These findings have significant implications for understanding the immune response to COVID-19 and informing clinical management approaches.

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The major portal of SARS-CoV-2 entry into host cells is through the respiratory tract, with the virus binding to the angiotensin-converting enzyme 2 (ACE-2) receptor on alveolar type 2 (AT2) cells in the epithelium. When attacked by the virus, AT2 cells produce inflammatory mediators, stimulating proinflammatory chemokine production and initiating immunoglobulin production (1). Generally, immunoglobulin production occurs 4 days after the onset of symptoms, enabling the diagnosis of COVID-19 through specific SARS-CoV-2 antibodies (2). The detection of neutralizing antibodies (Nabs) against SARS-CoV-2 helps to evaluate the status of the immune response of asymptomatic/symptomatic COVID-19 patients and severe COVID-19, in addition to indicating protection correlates (3).

Recent studies have demonstrated that the magnitude of Nab responses appears to correlate with viral load, with higher responses reported among patients with more severe disease and older adults (4, 5). Several comorbidities, including respiratory disease, hypertension, diabetes, kidney disease, and HIV-1 infection, have been identified as risk factors associated with severe COVID-19 manifestation (6). Nonetheless, few studies describing the anti-SARS-CoV-2 Nab response in HIV/COVID-19 coinfections have been performed thus far (7). The identification and characterization of Nabs can help prevent new infections in exposed individuals, and passive immunization can be used as a treatment against disease progression to severe forms. The aim of this study was to provide preliminary data on the Nab immune response to SARS-CoV-2 in a group of hospitalized subjects with COVID-19 in Rio de Janeiro, Brazil, including those with COVID-19 associated with HIV-1 infection. For this, we calculated the seroconversion rate of SARS-CoV-2 Nabs using the pseudovirus (psV) neutralization assay for a cohort of 103 hospitalized COVID-19 patients with or without HIV-1-associated infection and evaluated its association with clinical features of COVID-19.

MATERIALS AND METHODS

Human samples

Plasma samples analyzed in this study were obtained from a clinical COVID-19 follow-up study that included moderate/severe hospitalized patients at the COVID-19 Pandemic Hospital Center INI/FIOCRUZ, Rio de Janeiro, Brazil, between June 2020 and May 2021 (RECOVER-SUS study-NCT04807699). A total of 103 hospitalized participants who tested positive for SARS-CoV-2 by nasopharyngeal sampling using RT-qPCR were selected. The variant circulating at the beginning of the pandemic was B.1.1.33 in Rio de Janeiro. Clinical presentation was defined according to the World Health Organization (WHO) COVID-19 severity classification within the first 24 h of hospitalization (8). Plasma samples were collected on the first day of hospitalization (D1), after 14 days (D14), and between 7 and 11 months (D300) after hospital admission. None of the patients were vaccinated for COVID-19 during the blood collection period and we lack information regarding reinfections between the time of hospital discharge and the blood sample collection at D300. The local ethics committee approved this study, and all participants signed an informed consent form; all the samples were deidentified.

Cells

HEK293T-ACE2 and HEK293T/17 cells were cultured in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS), 2 mM of L-glutamine, and 200 µg/mL of hygromycin B (Thermo Fisher Scientific, USA) at 37°C with 5% CO_2. HEK293T-ACE2 cells were a donation from Biodefense and Emerging Infections Research Resources Repository -BEI resources (BEI catalog number: NR-52511), and the TZM-bl cell line was obtained through the NIH AIDS Research and Reference Reagent Program.

Pseudovirus production and titration

All transfections were performed in T75 culture flasks, and the psV was produced in HEK293T/17 cells cultured 24 h before reaching 50%–70% confluence on the day of the experiment. The production of the HIV envelope-based psV and the HIV psV neutralization assay was performed as described by de Almeida et al. (9). SARS-CoV-2 particles were produced using the Luciferase-IRES-ZsGreen backbone obtained from the BEI resource, which was established by Crawford et al. (10). Briefly, 1 µg of HDM-IDT Spike-fixK (BEI catalog number: NR-52514), 6 µg of pHAGE-CMV-Luc2-IRES-ZsGreen-W backbone (BEI catalog number: NR-52516), 1.4 µg of HDM-Hgpm2 (BEI catalog number: NR-52517), 1.4 µg of HDM-tat1b (NR-52518), and 1.4 µg of pRC-CMV-Rev1b (NR-52519) were used and cotransfected with Lipofectamine 3000 (Thermo Fisher Scientific, USA), and Opti-MEM I Reduced Serum Medium (Gibco) was used, as well. For the positive control, VSV G-pseudotyped (psV VSV-G) lentiviral particles (HDM-Hgpm2, HDM-tat1b, and pRC-CMV-Rev1b) with the ZsGreen backbone were produced using the plasmid pHEF expressing vesicular stomatitis virus glycoprotein (envelope) (VSV-G), obtained from the NIH-HIV Reagent Program (catalog number: RP-4693). Pseudovirus supernatants were collected approximately 72 h post-transfection, and the FBS concentration in the virus-containing culture medium was adjusted to 20% (i.e., for each 1 mL of virus harvested, 0.125 mL of FBS was added), filtered through a 0.45-µm filter, and stored at −80°C.

For median tissue culture infectious dose ($TCID_{50}$) assay measurements, 293T-ACE-2 cells (4×10^4 cells in 100 µL of growth medium/well) were added to each well, followed by 20 mg/mL of DEAE-dextran with 11 pseudovirus dilutions, added after 24 h. Serial fivefold dilutions of pseudovirus were quadruplicated in 96-well culture plates in a volume of 100 µL of growth medium/well. After 48 h of incubation (37°C in 5% CO_2), 100 µL of culture medium was removed from each well, and 100 µL of Britelite Plus Reagent (PerkinElmer) was added to the cells. After 2 min of incubation at room temperature to allow cell lysis, 150 µL of the cell lysate was transferred to black, solid 96-well plates for luminescence measurements using a GloMax Navigator Microplate Luminometer (Promega, USA). Wells with pseudovirus SARS-CoV-2-containing supernatant that was not toxic to the cells based on light microscopy inspection, and that produced at least relative luminescence units (RLUs) 10 times greater than the background were scored as positive. The $TCID_{50}$ was calculated using the Reed–Muench method and using the macro described on the $TCID_{50}$; the cutoff macro is available on the Los Alamos HIV Immunology Database (https://www.hiv.lanl.gov/content/nab-reference-strains/html/home.htm).

Pseudovirus neutralization assay

Neutralization activity was expressed as Nab titers, defined as the interpolated plasma dilution that produced a 50% reduction in virus infectivity or a 50% (ID_{50}) and 90% inhibitory dose (ID_{90}), observed as reductions in Luciferase reporter gene expression after a single round of virus infection of 293T-ACE-2 cells, given that some components essential for viral replication or persistent infection were deleted from the genome. For each sample, residual infection was estimated across eight serum dilutions ranging from 1:20 to 1:43,740; threefold serial dilutions of samples were performed in duplicate (96-well flat bottom plate) in DMEM growth medium (100 µL/well). An amount of virus to achieve a $TCID_{50}$ of 100,000 RLU equivalents was added to each well in a volume of 50 µL. The plates were then incubated for 90 min at 37°C in a total volume of 150 µL of growth medium. 293T-ACE-2 cells (4×10^4 cells in 100 µL of growth medium) were added to each well, and after 24 h, 20 mg/mL of DEAE-dextran was added. The normalized percent (%) inhibition was calculated using uninfected cells (100% inhibition), and as a negative control, we used prepandemic plasma (0% inhibition) as a reference. For positive control, we used anti-SARS coronavirus recombinant human IgG1 (BEI catalog number: NR-52392). After 48 h of incubation, 150 µL of culture medium was removed from each well, and 100 µL of Britelite reagent (PerkinElmer, USA) was added to the

cells. After 2 min of incubation at room temperature to allow cell lysis, 150 µL of the cell lysate was transferred to black, solid 96-well plates for luminescence measurements using a GloMax Navigator Microplate Luminometer (Promega, USA). The assays were evaluated as having passed when the following parameters were met: the mean RLU of the virus control was 10× higher than the background of the cell control; and the standard deviation of RLU in the virus control well was 30%. All data were analyzed using Excel Macros Nab analysis provided by the Global HIV Vaccine Enterprise, available on the Los Alamos HIV Immunology Database (https://www.hiv.lanl.gov/content/nab-reference-strains/html/home.htm).

Statistical analysis

P values were calculated by parametric (*t*-test), nonparametric Mann-Whitney (Wilcoxon rank-sum) test, or Fisher's exact test for nominal and continuous numeric variables, respectively (Tables 1 and 2; Fig. 1). Multiple linear fixed-effects models were used to evaluate patient differences related to neutralization antibodies titers. The model's fixed systematic component was adjusted by confounding variables (age, gender, self-declared skin color, number of comorbidities, and days since first symptoms of COVID-19 at hospital admission). The mean and 95% confidence intervals (95% CIs) were used, and the results were also presented graphically for the estimated mean marginal effects, where all other variables included in the multiple linear fixed models remained in equal proportions or their average values, and contrasts were constructed from these estimated mean marginal effects. R software (https://www.r-project.org/about.html) version 4.1.1 packages 'lme4', 'emmeans', and their dependencies were used to perform the statistical analyses. For each parameter, a nonparametric analysis of variance (ANOVA) was performed, and a paired *t*-test was performed to compare Nab titers. To evaluate the continuous ID_{50} and ID_{90} outcomes, we used a simple linear regression model assuming normal distribution for these outcomes on the log scale. Statistical significance was declared at a *P* value <0.05. Analysis was performed with the statistical computing software GraphPad Prism 9.

TABLE 1 Sociodemographic characteristics of study participants[a]

Characteristics	Overall	Hospitalization stay days		*P* value
		1–14	>14	
Total	103 (100%)	50 (49%)	53 (51%)	
Gender				
Male	57 (55.3%)	28 (56%)	29 (54.7%)	1
Female	46 (44.7%)	22 (44%)	24 (45.3%)	
Skin color				
Brown	71 (68.9%)	30 (60%)	41 (77.4%)	**0.03**
White	14 (13.6%)	6 (12%)	8 (15.1%)	
Black	8 (7.8%)	5 (10%)	3 (5.7%)	
Unknown	10 (9.7%)	9 (18%)	1 (1.9%)	
State of origin				
RJ	101 (98.1%)	49 (98%)	52 (98.1%)	1
AM	2 (1.9%)	1 (2%)	1 (1.9%)	
Schooling				
High school	42 (40.8%)	23 (46%)	19 (35.8%)	0.58
Elementary school	20 (19.4%)	8 (16%)	12 (22.6%)	
University education	8 (7.8%)	4 (8%)	4 (7.5%)	
First grade	22 (21.4%)	10 (20%)	12 (22.6%)	
Illiterate	6 (5.8%)	4 (8%)	2 (3.8%)	
Age	57 (IQR = 23.5)	56 (IQR = 23.6)	58 (IQR = 25.4)	0.84

[a]Values are presented as number (%) or median (IQR). Data are presented as absolute (relative) frequencies or median (IQR). *P* values were calculated either by nonparametric Mann-Whitney (Wilcoxon rank-sum) test or by Fisher's exact test for nominal and continuous numeric variables, respectively. Statistical significance is indicated with the following notations: $P < 0.05$.

TABLE 2 Clinical characteristics and symptoms of study participants[a]

Comorbidities	Levels	Overall	Hospitalization days		*P* value
			1–14	>14	
Systemic arterial hypertension	No	54 (52.4%)	24 (48%)	30 (56.6%)	0.49
	Yes	49 (47.6%)	26 (52%)	23 (43.4%)	
Diabetes mellitus	No	70 (68%)	31 (62%)	39 (73.6%)	0.29
	Yes	33 (32%)	19 (38%)	14 (26.4%)	
Chronic obstructive pulmonary disease	No	93 (90.3%)	48 (96%)	45 (84.9%)	0.12
	Yes	10 (9.7%)	2 (4%)	8 (15.1%)	
Cerebrovascular accident	No	101 (98.1%)	50 (100%)	51 (96.2%)	0.50
	Yes	2 (1.9%)	0 (0%)	2 (3.8%)	
Cardiovascular failure	No	99 (96.1%)	48 (96%)	51 (96.2%)	1
	Yes	4 (3.9%)	2 (4%)	2 (3.8%)	
Rheumatic diseases	No	102 (99%)	49 (98%)	53 (100%)	0.98
	Yes	1 (1%)	1 (2%)	0 (0%)	
Current smoking	No	96 (93.2%)	49 (98%)	47 (88.7%)	0.14
	Yes	7 (6.8%)	1 (2%)	6 (11.3%)	
Active tuberculosis	No	99 (96.1%)	48 (96%)	51 (96.2%)	1
	Yes	4 (3.9%)	2 (4%)	2 (3.8%)	
HIV serological status	Negative	88 (85.4%)	45 (90%)	43 (81.1%)	0.10
	Positive	15 (14.6%)	5 (10%)	10 (18.9%)	
Clinical data during COVID-19					
Fever	No	51 (49.5%)	26 (52%)	25 (47.2%)	0.77
	Yes	52 (50.5%)	24 (48%)	28 (52.8%)	
Oxygen saturation	No	63 (61.2%)	33 (66%)	30 (56.6%)	0.44
(<95%)	Yes	40 (38.8%)	17 (34%)	23 (43.4%)	
Cough	No	40 (38.8%)	19 (38%)	21 (39.6%)	1
	Yes	63 (61.2%)	31 (62%)	32 (60.4%)	
Coryza	No	97 (94.2%)	47 (94%)	50 (94.3%)	1
	Yes	6 (5.8%)	3 (6%)	3 (5.7%)	
Dyspnea	No	26 (25.2%)	14 (28%)	12 (22.6%)	0.69
	Yes	77 (74.8%)	36 (72%)	41 (77.4%)	
Anosmia	No	95 (92.2%)	45 (90%)	50 (94.3%)	0.65
	Yes	8 (7.8%)	5 (10%)	3 (5.7%)	
Ageusia	No	96 (93.2%)	47 (94%)	49 (92.5%)	1
	Yes	7 (6.8%)	3 (6%)	4 (7.5%)	
Diarrhea	No	95 (92.2%)	46 (92%)	49 (92.5%)	1
	Yes	8 (7.8%)	4 (8%)	4 (7.5%)	
Nausea	No	99 (96.1%)	49 (98%)	50 (94.3%)	0.65
	Yes	4 (3.9%)	1 (2%)	3 (5.7%)	
Headache	No	92 (89.3%)	45 (90%)	47 (88.7%)	1
	Yes	11 (10.7%)	5 (10%)	6 (11.3%)	
Myalgia	No	82 (79.6%)	39 (78%)	43 (81.1%)	0.88
	Yes	21 (20.4%)	11 (22%)	10 (18.9%)	
Oxygen supplementation[b]	No	20 (19.4%)	16 (32%)	4 (7.5%)	**0.004**
	Yes	83 (80.6%)	34 (68%)	49 (92.5%)	
WHO Clinical Progression Scale	WHO Scale	8 (IQR = 0)	8 (IQR = 0)	8 (IQR = 0)	0.51
Outcome	Discharge	69 (67%)	37 (74%)	32 (60.4%)	0.21
	Death	34 (33%)	13 (26%)	21 (39.6%)	
Symptoms	Days until hospitalization	8.6 (SD = 3.9)	7.5 (SD = 5)	9.7 (SD = 9)	0.05

[a]Values are presented as number (%), median (IQR) or mean (SD: standard deviation). *P* values were calculated by parametric (*t*-test), nonparametric Mann-Whitney (Wilcoxon rank-sum) test, or Fisher's exact test for nominal and continuous numeric variables, respectively.
[b]Ventilatory support.
[c]Statistical significance is indicated with the following notations: $P < 0.05$.

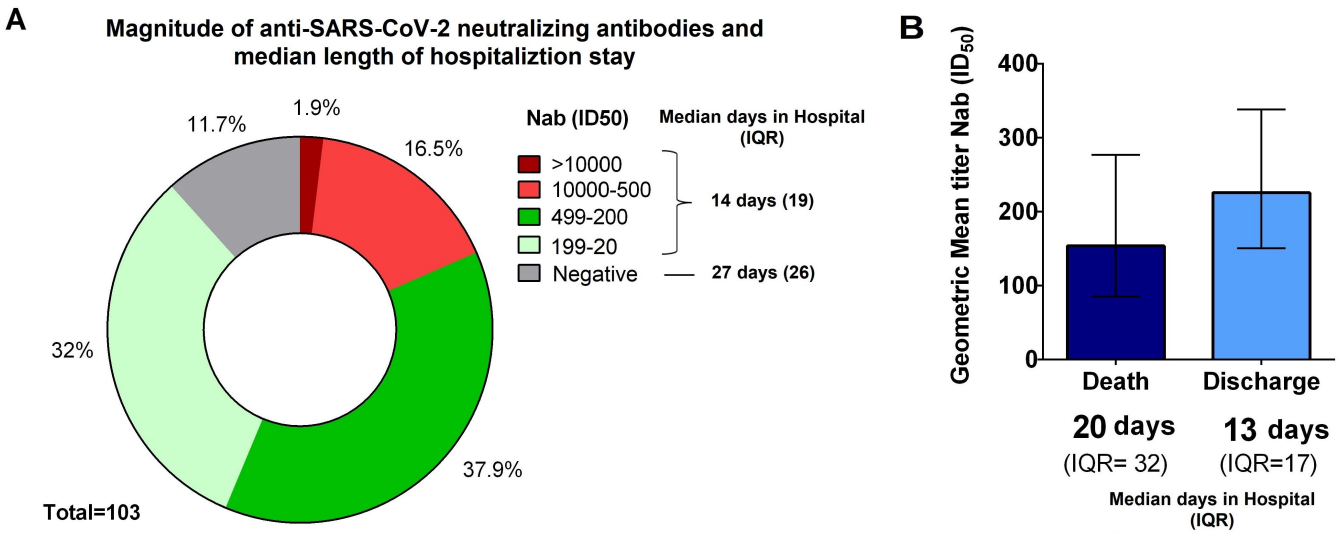

FIG 1 Relationship between length of hospitalization (median in days) and, magnitude of humoral response and clinical outcome. (A) The parts of the whole graph indicate the four ranges of neutralizing antibody titers by response magnitude and the range with titers below 20 (negative), indicating the frequency of participants in each range (percentage). The legend indicates the time of hospital stay of patients with Nab and those without Nab. (B) The bar graph shows the geometric mean anti-SARS-CoV-2 titers (GMT) for the group of patients who died and those who were discharged from the hospital and the median length of hospital stay for each group. The bars indicate the 95% confidence interval of GMT. Mann-Whitney test was performed to compare median days between the groups (IQR: interquartile range).

RESULTS

Characteristics of the study population

Plasma samples from 103 hospitalized participants were analyzed for Nab response to SARS-CoV-2. Ten (10.3%) patients were classified with moderate COVID-19 and 93 (90.3%) as severe. Sociodemographic and clinical data were collected for the present study on the first day of hospital admission. Participants were categorized according to median [15 days, interquartile range (IQR) = 21] length of hospitalization, into short hospitalization (less than 14 days) and long hospitalization (over 14 days) groups, to correlate sociodemographic characteristics (Table 1), clinical data (Table 2), and Nab response. Age and gender were not significantly distinct between the short and long hospitalization groups, but 71 (68.9%) participants self-declared that they had brown skin, and one significant difference in this frequency was observed (P = 0.032). Most patients were male [57 (55.3%)], and the median age was 57 (IQR = 23.51) years (Table 1).

The most common symptoms observed were fever, cough, chest pain, coryza, dyspnea, anosmia, ageusia, diarrhea, abdominal pain, nausea, headache, myalgia, and oxygen saturation below 95%. The presence of these symptoms varied among the participants, with dyspnea symptoms being the most frequent among the participants (74%), followed by oxygen saturation below 95% (37.5%) and myalgia (21%). We observed that most individuals required oxygen (80.6%) and remained hospitalized for longer periods, $P < 0.01$ (Table 2). Major comorbidities included hypertension [49 (47.1%)], diabetes mellitus [33 (31.7%)], and myalgia [22 (21.2%)]. Other comorbidities were present in less than 20% of the participants.

Among the individuals included in this study, 15 (14.4%) tested positive for HIV-1 infection. Most people living with HIV (PLWH) were diagnosed with HIV-1 infection during hospitalization for COVID-19 treatment and were not on antiretroviral treatment, at least at baseline blood sample. Of them, 9 (60%) were male, 14 (93.3%) self-declared having brown skin, and none had obesity, anosmia, loss of taste, nausea, previous stroke, heart failure, or coronary or rheumatic disease. Only 3 (20%) PLWH had systemic arterial hypertension, which was different from the group without HIV infection, where

the majority, 46 (54.1%), had this comorbidity (P = 0.02). The median age of patients hospitalized with COVID-19 was 57 years. However, among PLWH/people with COVID-19, the median age was 38 years (IQR = 18.22), and among those with only COVID-19, the median age was 60 years (IQR = 22; P < 0.01) (Table S1).

Early humoral immune responses among hospitalized patients with moderate and severe COVID-19

Humoral anti-SARS-CoV-2 immune responses were assessed at study entry for COVID-19 patients with moderate and severe disease. When we compared these two groups in relation to the magnitude of the response, we did not observe statistical differences (Fig. S1). We noted variable levels of Nab anti-SARS-CoV-2 ID_{50} in their plasma samples collected on the first day of hospitalization, corresponding to approximately 9 days after the onset of the first symptoms (mean of 8.6 days, standard deviation = ±3.9). Two patients (1.9%) had very high titers of Nabs (ID_{50}) of 1:11,532 and 1:14,962, respectively, 17 (16.5%) had high neutralization titers, between 1:500 and 1:10,000 Nab ID_{50}, whereas 39 (37.9%) patients developed Nab titers between 1:499 and 1:200, and 33 (32%) between 1:199 and 1:20. Twelve patients (11.6%) had Nab titers under the detectable limit of the assay (<1:20). Therefore, approximately 88% of COVID-19 patients exhibited robust neutralization of SARS-CoV-2 pseudovirus neutralization assay at hospitalization. Patients who had detectable production of Nabs were hospitalized for a shorter median time (14 days, IQR = 19) than those who did not have detected Nab (27 days, IQR = 27), P = 0.0363 (Fig. 1).

Association between anti-SARS-CoV-2 neutralizing antibodies and the clinical condition of COVID-19 patients

Considering the COVID-19 discharge (n = 69) or death (n = 34) outcomes in our study group, we observed that the geometric mean titer (GMT) of anti-SARS-CoV-2 Nab among patients with a death outcome was 140 (95% CI: 74–263), whereas a Nab GMT of 254 (95% CI: 170–380) was observed for those with a discharge outcome. Hospitalization time was longer for patients who died (20, IQR = 32) than for those who were discharged (13, IQR = 17), P = 0.0114 (Fig. 1). Of the 69 COVID-19 patients who were discharged, 7 (10%) had no Nab response on D1 and were hospitalized for a median of 21 (IQR = 22) days. Of the 34 patients who died, 5 (14%) had no Nab on D1 and were hospitalized for a median of 38 (IQR = 50) days.

We further explored the clinical manifestations associated with the Nab (ID_{50} and ID_{90}) levels of the 103 hospitalized patients with COVID-19, and we did not identify any association between the production of Nab titers and the main comorbidities such as arterial hypertension, diabetes, or obesity. However, Sidak's multiple comparisons analysis showed that anosmia and ageusia (P < 0.01) were associated with higher Nab titers. All patients with anosmia or ageusia had detectable Nab titers (Fig. 2). The Nab GMT of patients who did not have anosmia [32.2 (95% CI: 25–42)] or ageusia [31.7 (95% CI: 24–41)] was lower than that of patients who had anosmia [123 (95% CI: 28–536)] or ageusia [187 (95% CI: 40–869)].

To analyze other relevant clinical factors associated with Nabs, we investigated the hospitalization time in relation to initial Nab production on the first hospital day. We stratified the samples into two groups: 50 (48.6%) patients who stayed for up to 14 days and 53 (51.4%) patients who stayed for more than 14 days.

Of the 50 patients who stayed less than 14 days, 10 (20%) died, and the 53 who stayed longer than 14 days, 21 (40%) died. We found significantly lower psV SARS-CoV-2 Nab ID_{50} reciprocal plasma dilutions among patients hospitalized for more than 14 days (logFC = 0.34; P = 0.024) than among those with shorter hospitalization stays (Fig. 3). The anti-SARS-CoV-2 Nab titer of patients with less than 14 days to hospital discharge [GMT = 422 (95% CI: 341–2,014)] was significantly higher than that of patients with more than 14 days to hospital discharge [GMT = 149 (95% CI: 32–1,687)] (P < 0.05). To better understand the role of humoral immunity against SARS-CoV-2 infection, we analyzed the

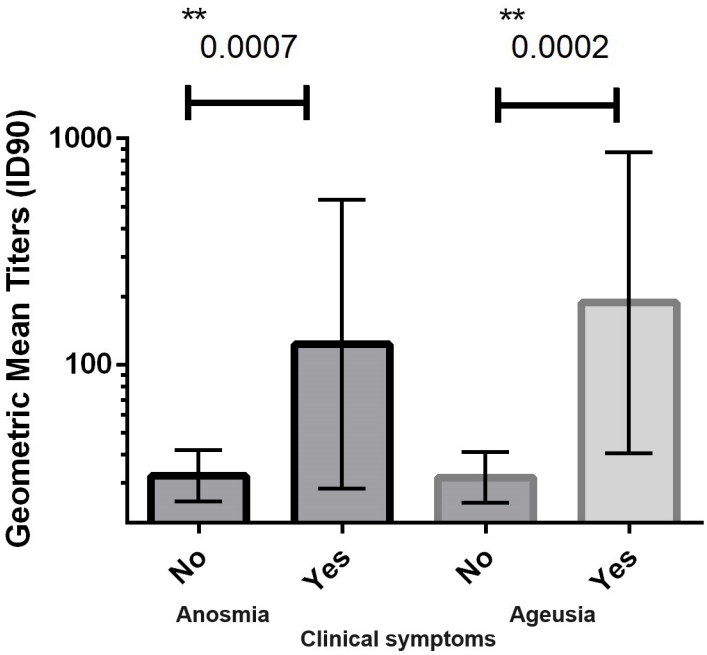

FIG 2 Clinical symptoms of anomia and ageusia of SARS-CoV-2 infection are influenced by neutralizing antibody titers. The COVID-19 patients (n = 103) were divided into groups according to the presence or absence of these symptoms and analyzed for their Nab titers with 90% inhibition (ID_{90}). The bar line represents the geometric mean with a 95% confidence interval. For each parameter, a nonparametric ANOVA (Sidak's multiple comparisons test) was performed, and statistical significance is indicated with the following notation: $^{**}P < 0.001$.

dynamics of changes in the Nab response initially at 14 days post-hospitalization (D14) compared to those obtained at hospital admission (D1). As shown in Fig. 4A, plasma samples from D14 were available for 39 (73.5%) of the 53 patients who had longer

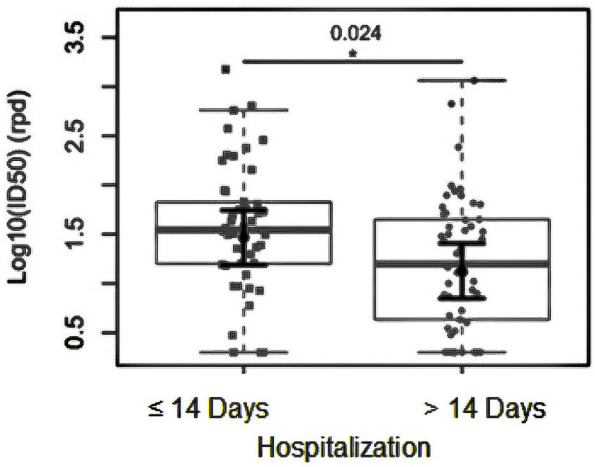

FIG 3 PsV SARS-CoV-2 neutralizing antibody ID_{50} reciprocal plasma dilutions and hospitalization stay (in days). The sample distributions of data are represented in box plots and strip plots in gray. In black, the center circle represents the expected mean marginal effect for each group estimated from linear multiple fixed-effects models. The fixed effects were adjusted by age, gender, self-declared skin color, number of comorbidities, and days since the first symptoms of COVID-19 at hospital admission. Black horizontal bars represent the 95% confidence intervals of the expected mean marginal effects by group. Statistical significance is indicated with the following notation: $^{*}P < 0.05$.

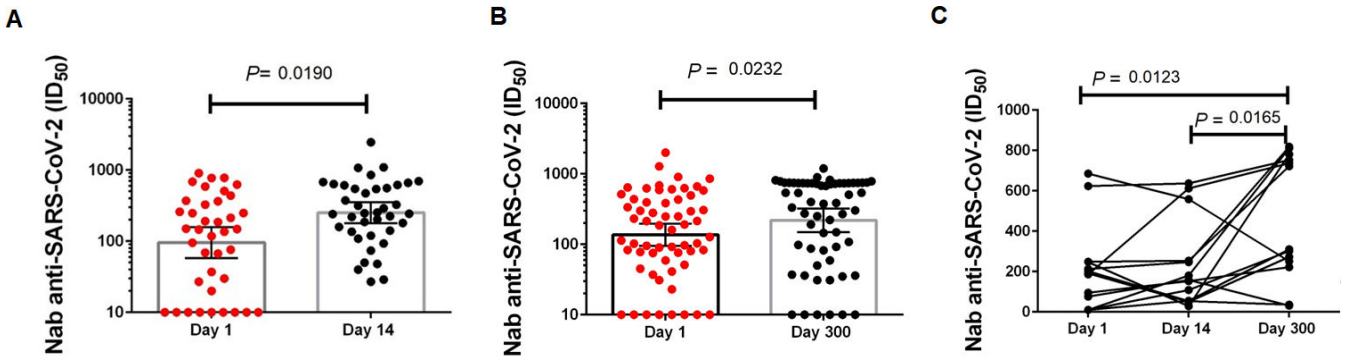

FIG 4 Longitudinal humoral immune response of inpatients with COVID-19. (A) Nabs (ID_{50}) of COVID-19 patients at admission D1 compared to the Nab (ID_{50}) assessment 14 days after hospital admission. (B) Nabs (ID_{50}) of COVID-19 patients at admission D1 compared to Nab (ID_{50}) assessment 300 days after hospital admission. (C) Changes in Nab titers (ID_{50}) for each patient are shown over time. Data from patients for the first sample D1, after 14 days, and the last collected sample D300 are shown (nonparametric ANOVA and a paired *t*-test were performed).

hospital stays (>14 days). There was a significant increase ($P = 0.0190$) in Nab titers (ID_{50}) between D1 [GMT = 95 (95% CI: 57–158)] and D14 [GMT = 251 (95% CI: 178–354)].

Patients who were discharged from the hospital were invited to return to the INI/FIOCRUZ after 7 months for a new blood collection and clinical follow-up. We performed the Nab assay at this point, hereby called D300, days median was 297 (IQR = 57) and, the minimal and maximum points obtained to D300 were 215 and 339 days after hospital admission. In this analysis, we compared the Nab titers (ID_{50}) of D1 and D300 for the 60 patients who returned for the study, and we observed an increased progression and persistence in the production of Nabs among these SARS-CoV-2-infected and unvaccinated individuals. The GMT for D1 was 136 (95% CI: 94–197), and the GMT for D300 was 219 (95% CI: 149–321) ($P = 0.0232$) (Fig. 4B).

It was possible to compare D1, D14, and D300 for only 13 patients, and we observed the following values of Nab D1 (ID_{50}) GMT = 108 (95% CI: 44–266); Nab D14 (ID_{50}) GMT = 219 (95% CI: 120–400); and Nab D300 (ID_{50}) GMT = 317 (95% CI: 161–624) ($P = 0.0123$ for D1 versus D300 and $P = 0.0165$ for D14 versus D300) (Fig. 4C).

Additionally, we evaluated the geometric mean titers of the anti-SARS-CoV-2 Nab ID_{50} of the 15 COVID-19 patients living with HIV-1 (PLWH/COVID-19) included in our study group and compared it with HIV-negative COVID-19 patients. The PLWH/COVID-19 participants [GMT = 148 (95% CI: 54–407)] showed no differences in anti-SARS-CoV-2 Nab production when compared to individuals without HIV-1 infection [GMT = 222 (95% CI: 153–321)], $P = 0.950$. As expected, the VSV-G pseudotyped virus control did not exhibit neutralization for any of the samples at a 1:20 dilution (<20 Nab) (Fig. 5). As a control, we also performed an HIV-1 pseudovirus neutralization assay to determine the anti-HIV Nab titers of the PLWH/COVID-19 participants on antiretroviral therapy. A high anti-HIV-1 antibody GMT of 583.9 (95% CI: 344–990) was detected, demonstrating maintenance of anti-HIV-1 antibody production in individuals with HIV-1 hospitalized for COVID-19.

DISCUSSION

Age is one of the most important prognostic factors associated with lethality in SARS-CoV-2 infection (6). However, in our study, which included hospitalized patients with moderate to severe COVID-19, age was not shown to be a determining factor for the production or magnitude of anti-SARS-CoV-2 Nabs in adults. In the population evaluated, we observed that 55% of those hospitalized patients were men, the mean age was 57 years, and the mortality rate was 33%. The same was observed in the SIVEP-Gripe study, where 678,235 patients were admitted to Brazilian hospitals and showed a mean age of 59 years and 35% mortality (11). Perazzo et al. (12) in a prospective multicenter study (RECOVER-SUS) that assessed hospital mortality, analyzed a cohort that included 1,589 participants and found that 54.5% of those admitted were men aged 62 and that 27.0%

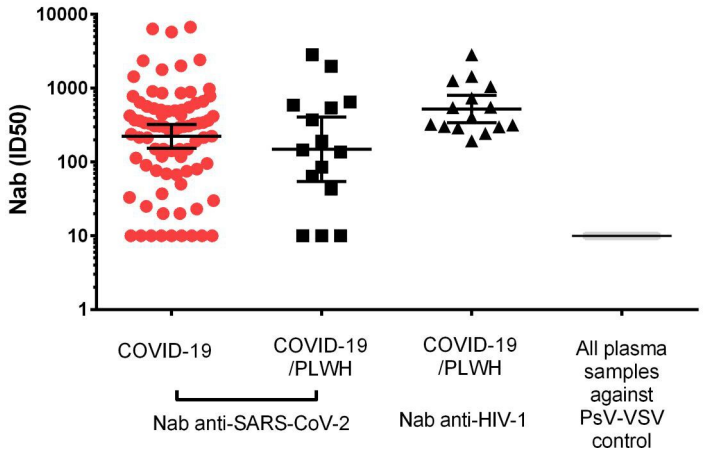

FIG 5 Neutralizing antibody responses from SARS-CoV-2-infected and HIV-coinfected patients. Nabs during the first day of hospitalization of 85 COVID-19 patients not infected with HIV and 15 PLWH coinfected with SARS-CoV-2 (COVID-19/PLWH) are shown. Fifteen patients (COVID-19/PLWH) showed Nab titers against SARS-CoV-2 and HIV-1 pseudovirus. The psV VSV-G was tested together as a negative control, and the 50% inhibition in virus infectivity was undetected. Negative values (titers below 20) were considered 10 for calculating the GMT. The horizontal bars indicate the GMT, and the bars indicate 95% confidence intervals. Nonparametric ANOVA and a paired *t*-test were performed.

died during hospitalization (12). The sociodemographic characteristics of the population of our study reflect the data presented by Zeiser et al. and Perazzo et al., indicating a consensus on the profile of hospitalized patients with COVID-19 in Brazil. Most men are aged between 50 and 60 years and have approximately 30% mortality.

The most frequent comorbidities observed in our study were hypertension, diabetes, and obesity. Hypertension is more frequent among elderly individuals and subjects affected by other comorbidities (13). In a multivariate analysis after adjustment for age and other cardiovascular risk factors, hypertension did not play an independent role in COVID-19 development (14). Our study observed similar anti-SARS-CoV-2 Nab titers between groups with and without these main COVID-19 comorbidities. However, the survey by Karuna et al. noted that hypertension was independently associated with low Nab titers (15). According to some researchers, more than half of COVID-19 patients experienced hyperglycemia, and approximately 33% developed diabetic ketoacidosis (16). Marchand et al. were the first to observe an increased risk of type 1 diabetes development among patients with COVID-19 (17). In our study, the proportion of diabetes patients was 31%, but the presence of diabetes was not related to the ability to produce Nabs, similar to the results observed by Dispinseri et al. (18).

We observed a correlation between anosmia and ageusia with high Nab titers. The American Academy of Otolaryngology—Head and Neck Surgery released the COVID-19 Anosmia Reporting Tool for clinicians, which revealed that anosmia was noted in 73% of COVID-19 cases and was the presenting symptom in 26.6% of cases (19). The incidence of anosmia among COVID-19 patients, however, varies in different studies. According to a meta-analysis by Tong et al., (20) the prevalence of olfactory dysfunction among COVID-19 patients was estimated to be 52% genetic factors, different SARS-CoV-2 strains, viral load (cases of viral damage), specificities of the different evaluated populations, or antibody complexes (21). However, this last concept must be explored since we found higher Nab titers among patients with anosmia. For example, anosmia is one of the first clinical signs of some neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases. IgG deposits are found in dopaminergic neurons in these patients, suggesting that neurons may be targets for these immunoglobulins (22). In addition, changes in the blood−brain barrier due to inflammation may facilitate the entry of IgG (23). Ko et al. (24), evaluated 15 asymptomatic patients who presented only anosmia, and the production

of Nabs was observed in 80.0% of patients (25). However, the underlying biological basis of anosmia remains obscure, and our study has some limitations. First, our cohort was limited to hospitalized patients, and the results could be different among people with SARS-CoV-2 infection with mild symptoms.

Most hospitalized patients had positive Nab titers (88%) with, on average, 9 days of symptoms. Other studies also observed the presence of antibodies in acute infection, with a negativity range in 10%–25% of patients (26, 27). Notably, 7 out of 12 (58%) recovered patients had Nab titers that were under the detectable level, suggesting that other immune responses may have contributed to the recovery of these patients. Previous studies have suggested that cellular immunity can mitigate severe infection, and T cells display strong cross-reactivity to SARS-CoV-2 (28). Whether these patients with COVID-19 without Nabs have a high risk of rebound or reinfection or a poor response to vaccination should be explored in further studies. Our data revealed that individuals who recovered from COVID-19 experienced relatively robust antibody responses to SARS-CoV-2 pseudovirus neutralization assay in the acute infection phase. However, patients who arrived at the COVID-19 hospital center with a moderate to critical condition and had low titers remained hospitalized for more than 14 days, and 92.5% needed ventilatory support. Therefore, contrary to what we expected, these patients who initially showed a low neutralizing response did not show milder clinical characteristics, such as the asymptomatic individuals with lower antibodies, as seen in other studies (29, 30).

Analysis of the humoral response across multiple cohorts of COVID-19 patients showed that a natural SARS-CoV-2 infection can elicit Nabs in most cases, but accumulating evidence indicates that the magnitude of the response varies greatly among individuals (31, 32). Kaneko et al. demonstrated that SARS-COV-2 infection has mechanisms capable of influencing cell differentiation and dysregulation of the specific humoral immune response, which may limit the memory response (33). It was also demonstrated that plasmablast expansion without somatic hypermutation during the acute phase of COVID-19 plays an important role in the early production of Nabs, which may reflect pre-existing B repertoire memory (34).

We observed that after 14 days of hospitalization, Nab GMTs increased in relation to D1, as observed in other cohorts, where the kinetics of Nab induction were reported to be similar for immunoglobulin seroconversion (32). Patients who died of COVID-19 were also able to increase antibody production at D14; however, this delay in the initial immune response may have facilitated the expansion of the viral load, leading to prolonged hospital stays and/or death. Some clinical studies that have adopted the use of convalescent plasma or monoclonal antibodies have observed patient improvement if administered at the beginning of infection (35), and when the serum is administered late (28 days), there was no improvement in the patient (36). The testing of Nabs in the acute phase of COVID-19 can be a great ally for decision-making in clinical trials of convalescent plasma administration as therapy.

The most extensive study on HIV/COVID-19 coinfection is from a Western Cape, South Africa cohort. PLWH who contracted SARS-CoV-2 died at 2.39 times the rate of patients without HIV with COVID-19. However, HIV was a minor risk factor for severe COVID-19 compared with comorbidities such as cardiopulmonary diseases (37). In our analysis of the production ability of anti-HIV and anti-SARS-CoV-2 antibodies among coinfected patients, we did not observe differences in relation to the mono-infected group (SARS-CoV-2), indicating that HIV-1 is not an aggravating factor for COVID-19 in terms of humoral responsiveness, but we also observed that a low titer of antibodies resulted in a longer hospitalization. Mishra et al. observed that some HIV-1 Nabs recognize viral glycan shields of SARS-CoV-2, showing cross-reactivity with SARS-CoV-2 (38). How much the polyclonal cross-neutralizing antibody response can contribute to the improvement of the patient has not yet been elucidated; however, in this study, we verified that a potent neutralizing humoral response at the beginning of the infection is associated with a faster recovery. It is crucial to emphasize that the presence of antiretroviral drugs in

the sera might have an impact on our lentiviral-based assays, both for SARS-CoV-2 and HIV pseudovirus neutralization. However, the fact that neutralization was not observed in the VSV-G assays indicates that there may not be substantial interference from the antiretroviral therapy administered to the patients.

All protection mechanisms of COVID-19 are not yet clear; however, Nabs are considered a significant correlate of protective immunity and vaccine success, and it has been shown that the rate of SARS-CoV-2 infections among vaccinated individuals is lower among individuals with high Nab levels (39). Extensive efforts to understand the relationship between SARS-CoV-2 and the immune system have highlighted Nab detection assays as essential for screening for potent antibodies for future immunotherapeutic designs. Here, we showed an important relationship between the rapid production of Nabs and shorter hospital stays among patients with moderate and severe COVID-19.

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ETHICS APPROVAL

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Instituto Nacional de Infectologia Evandro Chagas (INI)/FIOCRUZ, Rio de Janeiro, Brazil, under the approval number CAAE 32449420.4.1001.5262. All participants or their legal representatives signed an informed consent form prior to enrollment in the study.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental file 1 (Spectrum00959-23-s0001.docx). Table S1.
Supplemental file 2 (Spectrum00959-23-s0002.tif). Fig. S1.
Supplemental file 3 (Spectrum00959-23-s0003.docx). Legend to Fig. S1.

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