Reduced neutralization against Delta, Gamma, Mu, and Omicron BA.1 variants of SARS-CoV-2 from previous non-Omicron infection

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Abstract
The understanding of the host immune response to SARS-CoV-2 variants of concern is critical for improving diagnostics, therapy development, and vaccines. Here, we analyzed the level of neutralizing antibodies against SARS-CoV-2 D614G, Delta, Gamma, Mu, and Omicron variants in D614G infected healthcare workers during a follow-up up to 6 months after recovery. We followed up 76 patients: 60.5% were women and 39.5% men. The 96.1% and 3.9% were symptomatic and asymptomatic, respectively. The most frequent symptoms were headache, myalgia, and cough. The 65.8%, 65.8%, and 92.1% of the infected individuals were positive for neutralizing antibodies against D614G variant at 2, 4, and 6 months of follow-up, respectively. The 26.3%, 48.7% and 65.8% of patients neutralized Delta variant, 19.7%, 32.9% and 52.6% of patients neutralized Gamma, 7.9%, 19.7% and 44.7% of patients neutralized Mu, and 4.0%, 9.2% and 15.8% of patients neutralized Omicron. Low neutralization against Gamma and Mu variants was observed during the follow-up, and very low against the Omicron variant was detected during the same period. The median of neutralizing antibody titers against D614G and Delta variants increased significantly during the follow-up. An association was observed between the levels of neutralizing antibodies against D614G and Delta variants and the severity of the disease. Our results suggest an immune escape from neutralizing antibodies with the Omicron variant because of the many mutations localized in the S protein.

Keywords Delta variant · Gamma variant · Mu variant · Omicron SARS-CoV-2 variant · Neutralizing antibodies · Follow-up immune response

Introduction
A novel human coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causal agent of coronavirus disease 2019 (COVID-19) has rapidly spread...
A fundamental biomarker of the protective immune response in patients infected with SARS-CoV-2 is the presence of neutralizing antibodies (NAb), which are responsible for blocking the recognition and entry of the virus into cells. Antibody responses to spike glycoprotein (S) are thought to be the main target of neutralizing activity during viral infection [3]. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection [4]. Neutralizing antibodies in COVID-19 patients can be detected around day 7 after symptom onset at variable levels [5, 6]. However, the duration of NAb and their temporal dynamics is variable in convalescent patients. Until 40% of asymptomatic individuals became seronegative for IgG from 8 weeks after onset of symptoms, and until 33% of SARS-CoV-2 infected patients display no neutralizing activity 39 days after symptom onset [7, 8]. In addition, NAb decreased between 82 and 148 days after onset of symptom in mild-to-moderate COVID-19 patients [9].

In late 2020, the Delta variant of concern (VOC, B.1.617.2) was first detected in India and caused an devastating epidemic before spreading globally [10]. The delta variant contains 7 mutations and one deletion in the spike (S) gene [11, 12]. Most of these mutations localize to the receptor binding domain (RBD) and the N-terminal domain (NTD) of the spike glycoprotein, which are the major targets of neutralizing Abs in convalescent and vaccinated individuals [13, 14]. Although the emergence of different SARS-CoV-2 variants have spread throughout the world, until the appearance of the Omicron variant (B.1.1.529), a great genetic variability of the virus and an important immune escape had not been described. This variant was recently first identified in South Africa on November 2, 2021 and was designated a new VOC on November 26 [15]. Since its appearance, Omicron has spread worldwide at a faster rate than previous variants [16, 17]. The Omicron variant contains 21 mutations in the spike (S) gene, many of which are known to evade antibody neutralization or to enhance spike/hACE2 receptor binding [18–22].

On the other hand, the Gamma variant (p.1) was identified in November 2020 in Brazil and designated as a VOC in January 2021 [23]. The Gamma variant contains 12 mutations in the spike (S) gene [24]. This variant spread worldwide, it has been detected in at least 88 countries, with a higher prevalence in American countries, including Haiti, Brazil, and Trinidad and Tobago [24]. In addition, the Gamma variant has reported a large circulation in other South American countries, such as Uruguay, Argentina, Venezuela, and Chile. Similarly, the Mu variant of interest (VOI, B.1.621) was first reported in early January 2021 in Colombia [25]. This variant contains 12 mutations in the S gene [26]. Since its detection, the Mu variant has spread to 57 countries, with a higher presence in the British Virgin Islands, Colombia, Venezuela, Panama, Ecuador, Dominican Republic, Bolivia, and Chile [26].

A significant decrease in neutralization against the Omicron variant was observed between 7–25 and 120–210 days after the onset of symptoms in patients infected with the Alpha variant [27–30]. In addition, a decrease in neutralization against Omicron variant was suggested between 2 and 24 days after the onset of symptoms in patients infected with Delta variant [31]. Sera from COVID-19-convalescent patients collected 6 or 12 months after symptoms displayed low or no neutralizing activity against Omicron [32]. However, the studies were carried out on a few patients and had no follow-up, which did not allow the temporary evaluation of the dynamics of neutralizing antibodies. The aim of this study was the examine the temporal dynamic of the cross-neutralization of Delta, Gamma, Mu, and Omicron variants by antibodies produced from previous infection with D614G variant during the early pandemic in Chile.

Materials and methods

Study design and participants

This study was conducted at Clinica Indisa, one of the biggest private hospitals in Santiago de Chile to manage patients with COVID-19. We recruited between August 14 and September 20, 76 HCW with SARS-CoV-2 positive RT-PCR for follow-up. Participants in this study were infected with SARS-CoV-2 during the first half of 2020, a time when the D614G virus was the dominant circulating SARS-CoV-2 strain in Chile. All hospital staff was invited to participate in the investigation. Those volunteers with more than 2 months after the date of onset of symptoms were excluded. The study was originally designed with a 12-month follow-up, but it was suspended due to the start of vaccination against SARS-CoV-2 in Chile.

Data collection, follow-up and sampling

Sociodemographic information of the participants and details related to COVID-19 were accessed, which included: age in years, gender, symptom onset date, RT-PCR result date, COVID-19 symptoms (fever, cough, sore throat, myalgias, headache, dyspnea, abdominal pain, chest pain, anosmia, ageusia, diarrhea, asthenia, other), history of hospitalization for COVID-19, and required ICU management.
To evaluate neutralizing antibody responses against SARS-CoV-2, we obtained for each participant three blood samples at 2 (M2), 4 (M4), and 6 (M6) months after symptoms onset or date of positive RT-PCR (in asymptomatic patients). The samples were obtained in CI and analyzed at the Virology Reference Laboratory of de Public Health Institute of Chile, within the first 72 h since their obtaining; they were kept and transported refrigerated at 5–8 °C.

**Serum neutralization test**

Samples from 76 individuals were tested for neutralization against D614G (GISAID Accession Number EPI_ISL_3509539), Delta (GISAID Accession Number EPI_ISL_2894958), Gamma (GISAID Accession Number EPI_ISL_1321471), Mu (GISAID Accession Number EPI_ISL_4652657), and Omicron (GISAID Accession Number EPI_ISL_8187478) variants. SARS-CoV-2 strains obtained from our Biosafety level-3 laboratory (BSL-3) by viral isolation in tissue cultures. Neutralization assays were carried out by the reduction of cytopathic effect (CPE) in Vero E6 cells (ATCC CRL-1586). The titer of NAb was defined as the highest serum dilution that can neutralize virus infection, at which the CPE was absent compared with the virus control wells (cells with CPE). Briefly, the Vero E6 cells (4 × 10⁴ cells/well) were seeded in 96-well plates. A fresh aliquot of a viral stock previously titrated and frozen at −80 °C was used in each neutralization assay. For the neutralization assay, 100 μl of each SARS-CoV-2 variant (at a dose of 100 TCID₅₀) were incubated with serial dilutions of heat-inactivated sera samples (dilutions of 1:10, 20, 40, 80, 160, 320, 640, and 1280) from patients and human normal serum as a negative control for 1 h at 37 °C. Then, a mixture of samples and virus were added to the 96-well Vero E6 cells). Cytopathic effect on Vero E6 cells was analyzed 7 days after infection. Neutralization was defined as the absence of CPE compared to virus controls. All samples were tested duplicated. For each test, human normal and a COVID-19 patient serum were used as a negative and positive control, respectively. The negative control was serum from a blood donor negative for SARS-CoV-2 IgM and IgG by the OnSite™ COVID-19 IgG/IgM Rapid Test (CTK Biotech, Ref. R0180C). The positive control was a serum from a recovered COVID-19 patient, who agreed to donate blood for plasmapheresis.

**Statistical analysis**

Traditional methods were used for descriptive results, the median and interquartile range for non-normally distributed numeric data, frequency distributions for categorical data, and geometric means and logs for titers expressed as dilutions or its reciprocal. Statistical analyses were carried out using Prism, version 9.0 (GraphPad), google sheets, and SPSS v23. Exploratory analysis was done with a correlation test or bivariate analysis. Nonparametric tests were preferred. Mann–Whitney t-test, Kruskal–Wallis, Wilcoxon matched-pairs signed-rank, and Friedmann test were used to compare data. All the tests were 2-tailed, and p < 0.05 was considered statistically significant. For statistical analysis, a value of 5 was assigned for NAb titer < 10 (ND).

**Ethical aspect**

The protocol was approved by Scientific-Ethics Committee Andres Bello University, of Metropolitan Region, Chile, through the document No. 021/2020 dated August 10, 2020. Participants consented to follow-up with sampling and provide access to epidemiological and clinical information related to COVID-19 available in the Hospital's epidemiology unit.

**Results**

**Patient characteristics and serum collection**

Seventy-six healthcare workers SARS-CoV-2 RT-PCR confirmed were enrolled. Their characteristics are presented in Table 1. The median [Interquartile (IQR)] age of the participants was 37.0 (30.0–47.8) years, 46 (60.5%) were women and most (61.8%) worked in direct contact with COVID-19 patients. Most of the participants 45 (59.2%) presented a symptomatic infection without fever, 3 (3.9%) had an asymptomatic infection, and only 5 (6.6%) were hospitalized, but not in ICU. The most frequent symptoms related to COVID-19 were headache (63.2%), myalgia (61.8%), cough (55.3%), and fever (32.9%). The median [Interquartile (IQR)] time from the onset of symptoms and to blood sampling was 62 (59–65) days for the M2 time point, 121 (117–124) days for M4, and 180.5 (177–184.8) days for M6.

**Changes in NAb titers over time**

We analyzed the neutralizing activity of serum samples of 76 HCWs at the first (M2), second (M4), and third (M6) sampling. The level of neutralizing antibodies against the D614G, Delta, Gamma, Mu, and Omicron strains of SARS-CoV-2 was evaluated in each of the samples. The 65.8% (n = 50) harbored NAb against G614G with a neutralizing titer ≥ 10 (1:10 dilution) at M2 and M4 sampling. At M6, serum samples from 92.1% (n = 70) of the HCW harbored NAb with a neutralizing titer ≥ 10. Six cases showed an undetectable level of neutralizing antibodies at M6 sampling (three of them from the beginning and three whose titers became negative during follow-up) (Table 2).
Table 1 Characterization of the study population and frequency of symptoms and signs related to COVID-19 in 76 non-Omicron SARS-CoV-2 infected healthcare workers

| Characteristic                        | n  | %        |
|---------------------------------------|----|----------|
| Total no. of patients                 | 76 | 100.0%   |
| Age, median (IQR)                    | 37.0 | (30.0–47.8) |
| Gender                                |    |          |
| Male                                  | 30 | 39.5%    |
| Female                                | 46 | 60.5%    |
| Occupation                            |    |          |
| Medical Doctors                       | 13 | 17.1%    |
| Nurses                                | 16 | 21.0%    |
| Health technicians                    | 22 | 28.9%    |
| Others                                | 25 | 32.9%    |
| Direct contact with COVID-19 patients | 47 | 61.8%    |
| Severity of the disease               |    |          |
| Asymptomatic                          | 3  | 3.9%     |
| Symptomatic without fever             | 45 | 59.2%    |
| Symptomatic with fever                | 23 | 30.3%    |
| Hospitalization                       | 5  | 6.6%     |
| Most common symptoms                  |    |          |
| Headache                              | 48 | 63.2%    |
| Myalgia                               | 47 | 61.8%    |
| Cough                                 | 42 | 55.3%    |
| Fever                                 | 25 | 32.9%    |
| Sore throat                           | 23 | 30.3%    |
| Anosmia                               | 23 | 30.3%    |
| Time from onset of symptoms (days) to blood sample collection, median (IQR)*   |     |          |
| First sample (2 months)               | 62.0 | (59.0–65.0) |
| Second sample (4 months)              | 121.0 | (117.0–124.0) |
| Third sample (6 months)               | 180.5 | (177.0–184.8) |

*In asymptomatic HCW the date of PCR assay was used

The geometric mean titer (GMT) of neutralizing antibodies was 16.5 (12.6–21.7), 14.9 (11.4–19.5) and 48.0 (34.0–67.8) in M2, M4 and M6 sampling. NAb titers increased significantly between M2 and M6 (p < 0.0001) and between M2 and M4 (p < 0.0001) (Fig. 1a). When months 2 and 6 are compared, 39.5% (n = 30) of participants showed similar levels during all periods, 52.6% (n = 40) exhibited raising titers in at least two dilutions, and only 7.9% (n = 6) had diminishing results. No significant correlation was found between age (Rho Spearman: 0.086; p = 0.47), sex (p = 0.859; U of Mann–Whitney) or Ct value (Rho Spearman: −0.18; p = 0.12) and titers. An association between NAb levels and the severity of disease was observed in month 6 (Fig. 2a). Differences were statistically significant between asymptomatic with symptomatic cases without fever (p = 0.02; U of Mann–Whitney), asymptomatic and symptomatic with fever (p = 0.02), and asymptomatic with hospitalized cases (p = 0.01). Table 3 shows that the highest rise in titers between M2 and M6 was observed in those samples that showed NAb in M2 less than 10 (or ND) and 1:10–1:40.

On the other hand, the 26.3% (n = 20), 48.7% (n = 37) and 65.8% (n = 50) harbored NAb against Delta VOC with a neutralizing titer ≥ 10 (1:10) at M2, M4 and M6 sampling, respectively. Twenty-six (34.2%) showed always undetectable level of neutralizing antibodies during the follow-up (Table 2). The GMT of neutralizing antibodies was 6.4 (5.7–7.2), 8.2 (7.1–9.5) and 14.1 (10.9–10.3) in M2, M4 and M6 sampling. NAb titers increased significantly between M2 and M4 (p = 0.003), M2 and M6 (p < 0.0001) and between M4 and M6 (p = 0.002) (Fig. 1b). When months 2 and 6 were compared, the 55.3% (n = 42) of participants showed similar levels during all periods, 43.4% (n = 33) exhibited raising titers in at least two dilutions, and only 1.3% (n = 1) had diminishing results. An association between NAb levels and the severity of disease was observed at month 6 (Fig. 2b). Differences were statistically significant between asymptomatic with hospitalized (p = 0.03; U of Mann–Whitney), symptomatic without fever with hospitalized cases (p = 0.03). No significant correlation was found between age, sex or Ct value and titers. Table 3 shows that the highest rise in titers between M2 and M6 was observed in those samples that showed NAb in M2 less than 10 (or ND) and 40.

The 19.7% (n = 15), 32.9% (n = 25) and 52.6% (n = 40) showed NAb against Gamma VOC with a neutralizing titer ≥ 10 (1:10) at M2, M4 and M6 sampling, respectively. Thirty-six (47.4%) showed always an undetectable level of neutralizing antibodies during the follow-up (Table 2). The GMT of neutralizing antibodies was 5.9 (5.4–6.4), 6.5 (5.9–7.2), and 8.3 (7.3–9.5) in M2, M4, and M6 sampling. NAb titers raised significantly between M2 and M6 (p < 0.0001); and between M4 and M6 (p = 0.006) (Fig. 1c). When months 2 and 6 are compared, 88.2% (n = 67) of participants showed similar levels during all periods, and 11.8% (n = 9) exhibited raising titers in at least two dilutions. A significant difference in Nab levels was detected between symptomatic cases with and without fever (p = 0.0238) (Fig. 2c). No significant correlation was found between age, sex, or Ct value and titers. Table 3 shows that the highest rise in titers between M2 and M6 was observed in those samples that showed NAb in M2 less than 10.

The 7.9% (n = 6), 19.7% (n = 15) and 44.7% (n = 34) showed NAb against Mu VOI with a neutralizing titer ≥ 10 (1:10) at M2, M4 and M6 sampling, respectively. Forty-two (55.3%) showed always an undetectable level of neutralizing antibodies during the follow-up (Table 2). The GMT of neutralizing antibodies was 5.3 (5.1–5.5), 5.7 (5.4–6.1), and 7.5 (6.6–8.4) in M2, M4, and M6 sampling. NAb titers increased significantly between M2 and M6 (p < 0.0001) and between M4 and M6 (p = 0.004) (Fig. 1d). When months 2 and 6 are compared, 90.8% (n = 69) of participants showed
similar levels during all periods, and 9.2\% (n = 7) exhibited raising titers in at least two dilutions. Four, six, and one of these samples simultaneously showed increased NAb titers against Delta, Gamma, and Omicron variants in the same period, respectively. A significant difference in Nab levels was detected between symptomatic cases with and without fever (p = 0.0243), and between symptomatic with fever and hospitalized cases (p = 0.0422) (Fig. 2d). No significant correlation was found between age, sex, or Ct value and titers. Table 3 shows that the highest rise in titers between M2 and M6 was observed in those samples that showed NAb in M2 less than 10.

Finally, 4.0\% (n = 3), 9.2\% (n = 7) and 15.8\% (n = 12) harbored NAb against Omicron VOC with a neutralizing titer ≥ 10 at M2, M4 and M6 sampling, respectively. Sixty-four (84.2\%) always showed an undetectable level of neutralizing antibodies during the follow-up (Table 2). The GMT of neutralizing antibodies was 5.1 (5.0–5.3), 5.4 (5.1–5.7), and 5.8 (5.3–6.5) in M2, M4, and M6 sampling. NAb titers increase significantly between M2 and M6 (p = 0.01) (Fig. 1e). No significant differences were detected between M2 and M4 or M4 and M6 samples. Also, no association was observed between NAb levels and disease severity (Fig. 2e).

Discussion

The generation of neutralizing antibodies and their maintenance over time frequently correlates with protection against viral infections [33]. The duration of the immune response to SARS-CoV-2 variants is not yet resolved. Some studies with the wild-type variant suggest that antibody levels decline rapidly, while others indicate that they persist for at least 8 months after infection [34–38]. It has been shown that most SARS-CoV-2 infected develop neutralizing antibody responses within 2 months post-infection, and these antibodies are maintained for at least 3 months [9, 37–39]. However, the levels of neutralizing antibodies appear to be variable among infected patients. Although knowledge is available on the immune response against SARS-CoV-2 infection during the acute and convalescence stages up to 3 months, there is little information about the neutralizing antibody response in more extended periods after infection.

Here we report that in HCW, mostly infected with mild-to-moderate disease, neutralizing antibody titers were stable over time in 94\% of the patients for at least 6 months post-SARS-CoV-2 infection. This result is similar to those reported in other populations of SARS-CoV-2 infected during 5–7 months of follow-up [9, 40, 41]. Some studies have
reported a decrease of neutralizing antibodies between 82 and 148 days after COVID-19 symptom onset [9]. Unexpectedly, but in agreement with earlier observations, seroconversion in mild COVID-19 cases might take a longer time to mount [34]. Most patients with mild COVID-19 seroconvert 4 weeks after illness [34]. The initial serum antibody titer was likely produced by plasmablasts, and plasmablast-derived antibodies peak 2–3 weeks after symptom onset. Given an immunoglobulin G half-life of ~21 days, the sustained antibody titers observed here over time are likely produced by long-lived plasma cells in the bone marrow. However, our results contrast with those found in asymptomatic patients, in which only 71.6% of infected patients developed neutralizing antibodies [8]. The titers of NAb rapidly waned at 8 weeks after infection in this population until disappeared in 40% of them [8]. Recently, a rapid decrease in titers and disappearance of neutralizing antibodies was reported 2 months after infection in HCW with mild disease [42]. Also, the decline of NAb 2 months after infection was reported in 35 infected patients, although it did not disappear the NAb response [35]. Likewise, a higher neutralizing antibody titer associated with increased severity was reported 2 months after infection [35]. In our study, we found significant differences of NAb against D614G variant at month 6 among asymptomatic with symptomatic and hospitalized patients. We detected differences of NAb against Delta variant at month 6 among asymptomatic with symptomatic without fever and hospitalized patients. We found differences of NAb against Gamma and Mu variants at month 6 among symptomatic with and without fever patients. In addition, the Mu variant showed differences in levels of NAb among symptomatic with fever and hospitalized patients at month 6. No association was observed between NAb levels against the Omicron variant and disease severity. However, this no association could be due to the general low neutralization against Omicron or the low proportion of samples with detectable NAb against Omicron. It is unknown why the neutralizing antibody response correlates with disease severity in some viral variants and not in others. A higher viral load could lead to more serious illness and generate a stronger antibody response through higher levels of viral antigen. Alternatively, antibodies could have an etiological

Fig. 2 Neutralization antibody titers among severity groups in 76 non-Omicron SARS-CoV-2 infected healthcare workers. Plasma neutralization titers against D614G (lineage B.1.1.7) and variants of SARS-CoV-2 in 76 HCW participants from Clinica Indisa infected with non-Omicron SARS-CoV-2. A Neutralization titers against D614G strain (A), Delta VOC (B), Gamma VOC (C), Mu VOI (D), and Omicron VOC (C) in samples of volunteers drawn at 6 months after the date of infection. A, Asymptomatic; S, Symptomatic without fever; SWF, Symptomatic with fever; H, Hospitalized. Geometric mean titer (GMT) and 95% CIs are indicated. The threshold/limit of detection was the 1:10 dilution. Non detectable results were assigned a value of 5.0. Neutralization titers were determined by the reduction of cytopathic effect (CPE) in Vero E6 cells. 1Friedman test, 2Wilcoxon signed-rank test
Infection with SARS-CoV-2 D614G variant produced a sustained neutralizing response against this variant, relatively low against Delta, very low against Gamma and
Mu, and almost absent against Omicron between 2 and 6 months post-infection. The neutralization titers against Delta decreased between 2.6 and 3.4 times compared to the WT strain. Neutralizing antibodies against Gamma and Mu decreased between 2.8–5.8 and 3.1–6.4 times concerning the D614G strain, respectively. In contrast, the neutralization against Omicron decreased between 3.2 and 8.1 times in relation to the D614G strain. Similar results were previously reported [28, 44]. However, we did not detect a decrease in antibodies at 6 months from the onset of symptoms as previously reported [44]. Also, a significant decrease in neutralization against Omicron was detected between 2 and 25 days after the onset of symptoms in some patients infected with Alpha [27, 28] and Delta [31] variant, at 1 and 3 months in 15 patients infected with the Alpha variant [45], and at 7 months in 23 patients infected with the Alpha variant [30]. Here we find that after 6 months of SARS-CoV-2 infection, neutralizing antibody levels between Delta, Gamma, Mu, and Omicron variants were significantly different from each other, with the only exception between Mu and Omicron. Similar results were recently reported in Colombia [46]. Compared with other variants, neutralizing antibody titers from serum samples of 17 SARS-CoV-2-infected persons were lower against Mu than against Delta and Gamma variants except for Omicron. Unfortunately, none of the previous studies followed up on patients. Thus, our research is more robust and reproducible because samples at 2, 4, and 6 months were obtained from the same patients.

Previously, it was reported in 16 patients that the neutralization titers were stable or slightly decreased over time with the D614G and Delta variants [32]. Only 33% of the samples displayed neutralizing activity against Omicron at M6, whereas 94% were active against Delta [32]. Our results at 6 months show a higher decrease in neutralization than previously reported. We detected at 6 M that 15.8% and 65.8% of the convalescent sera neutralized Omicron and Delta, respectively. This difference could explain by the different severity of patients in both studies. Planas D et al. analyzed 50% of severe COVID-19 cases; in contrast, we studied 6.6% of severe COVID-19 patients. Consequently, it is expected that the GMT of antibody titers will be higher in populations with severe cases compared to mild diseases [35, 47].

Our study has several limitations. First, here we do not evaluate the response of CD8+ and CD4+ T cells or other types of antibodies that could mediate the antibody-dependent cytotoxicity that protects patients from severe disease. Recently, cross-reactivity mediated by spike-specific CD8+ and CD4+ T cell responses against Delta and Omicron VOC was described in people previously infected or immunized with the Ad26.COV2.S or BNT162b2 vaccines, despite having low neutralizing antibody titers against both variants [48, 49]. Second, the follow-up time in the patients was 6 months. Unfortunately, it was necessary to suspend the follow-up at 8 months due to the start of SARS-CoV-2 vaccination in Chile, which generated a high booster effect in the neutralizing response against the D614G variant. Third, 89.5% of the cases studied were mild disease, which could suggest the presence of lower titers on average compared to severe cases. Successive studies should include a higher percentage of severe and asymptomatic cases to understand the dynamics of the NAb response in these patients.

Our results show in previously non-Omicron SARS-CoV-2 infected people, with follow-up up to 6 M from the onset of symptoms, a regular and very low cross-neutralization against the Delta and Omicron variants, respectively. In addition, low neutralization against Gamma and Mu variants was observed. These results suggest an immune escape from neutralizing antibodies with the Gamma, Mu, and Omicron variants as a consequence of the many mutations localize in RBD and NTD domains in the S protein [28, 50]. Furthermore, the decrease in cross-neutralization with Delta, Gamma, and Mu variants, and especially Omicron, suggests the need for vaccination in people previously infected with non-Omicron SARS-CoV-2 to avoid reinfections with new variants.

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**Author contributions**
PP participated in the study design, selection and clinical follow-up of patients, writing, critical review of the content and approved the final version of the manuscript. JF contributed to the study design, experimental assays, critical review of the content and approved the final version of the manuscript. MA contributed to the analysis of results, writing, critical review of the content and approved the final version of the manuscript. CA and AMM participated in the selection and clinical follow-up of patients. NB contributed to the analysis of results, and approved the final version of the manuscript. HSM, CE, and MB participated in the experimental assays, and approved the final version of the manuscript. RF, MTV, and AO participated in the study design, critical review of the content and approved the final version of the manuscript. ER contributed to the study design, experimental assays, writing, critical review of the content and approved the final version of the manuscript.

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**Availability of data and materials**
The data and materials that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

**Code availability**
Not applicable.

**Declarations**

**Conflict of interest**
The authors declare that there is no conflict of interest.
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