Persistence of Neutralizing Antibodies and Clinical Protection up to 12 Months After Severe Acute Respiratory Syndrome Coronavirus 2 Infection in the Elderly

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Background. Coronavirus disease 2019 (COVID-19) has severely affected the elderly, who are expected to display decreased immune responses due to immunosenescence.

Methods. This study retrospectively assesses neutralizing antibody (NAb) production up to 12 months after infection in long-term care patients. We used Roche Diagnostics immunoassay to quantify anti-spike (S) antibodies and a competitive immunoassay from YHLO as a surrogate test for NAb.

Results. We included 91 patients (mean age, 86 years). There was no significant variation in anti-S titers over time. There was a significant decrease of NAb titers between month 3 and month 6 but no further significant change up to month 12. Overall, 75 of 91 (82%) and 52 of 91 (57%) patients had, at least once, anti-S titers >75 U/mL and NAb titers >50 AU/mL, respectively, corresponding to a significant neutralizing activity in vitro. All 68 patients studied at M12 had detectable anti-S antibodies and 60 (88%) had detectable NAb; 60 of 68 (88%) and 29 of 68 (42.6%) still had anti-S titers corresponding to a significant neutralizing activity in vitro. All 68 patients studied at M12 had detectable anti-S antibodies and 52 of 91 (57%) patients had, at least once, anti-S titers >75 U/mL and NAb titers >50 AU/mL. Higher NAb titers were correlated with severe infection, higher levels of C-reactive protein, and lower lymphocyte counts. No patient developed reinfection.

Conclusions. Elderly people can display robust and persistent humoral response after severe acute respiratory syndrome coronavirus 2 infection, with NAb lasting up to 12 months.

Keywords. COVID-19; elderly; neutralizing antibodies; YHLO immunoassay; long-term care.

Since December 2019, a new pandemic has emerged, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has affected the elderly in particular [1]. Symptomatology in elderly persons may include classic symptoms of coronavirus disease 2019 (COVID-19) such as fever, upper respiratory tract symptoms, myalgia, and diarrhea [2], but also atypical manifestations such as falls, asthenia, delirium, and altered consciousness [3]. By contrast, approximately 30% of people have an asymptomatic infection [4]. Overall mortality of hospitalized COVID-19 patients was approximately 15%–20%, with a great disparity between age groups. Mortality rates were around 5% in patients <40 years old, increasing to 35% in those 70–79 years old, and up to 60% in those >80 years old [5, 6, 7]. A French study in elderly hospitalized patients with a mean age of 86 years reported an overall mortality rate of 31% [8]. Male sex, age >65 years, and comorbidities such as hypertension, diabetes, cardiovascular diseases, respiratory diseases, and cancer were associated with higher risk of mortality [9–11].

The transmission dynamics of SARS-CoV-2 combined with the initial low availability of testing fueled a rapid spread within and between facilities, leading to high morbidity and mortality among residents in long-term care facilities (LTCFs). Prevention and control are a major challenge in these settings and rely on repeated testing for SARS-CoV-2 by reverse-transcription polymerase chain reaction (RT-PCR). Repeated serological tests can also be useful to identify asymptomatic or pauci-symptomatic infections and allow monitoring of the persistence of antibody response over time.
In most cases, individuals with a confirmed SARS-CoV-2 infection develop immunoglobulin M, immunoglobulin A, and immunoglobulin G antibody responses against the virally encoded spike glycoprotein (S) and nucleoprotein (N) within 1–2 weeks after onset of symptoms [12, 13]. S-glycoprotein is the main target for neutralizing antibodies (NAbS), and a number of highly potent monoclonal antibodies have been isolated that predominantly target its receptor-binding domain (RBD). Nevertheless, the immune response against the N-protein, involved in viral replication and in the production of an immune response to SARS-CoV-2, seems to have a protective role against COVID-19 and could be a future therapeutic target [14, 15]. High NAbS produced after natural infection or vaccination generally correlate with a decreased severity of COVID-19 disease and may be associated with protective immunity against reinfection [16, 17]. Administration of convalescent plasma or monoclonal NAb as a therapeutic intervention has had limited effects on the clinical course of COVID-19, particularly if administered late in the infection, consistent with a substantial role for T-cell response in control and clearance of an ongoing SARS-CoV-2 infection [18, 19].

Thus, studying antibody, memory B-cell, CD4+ T-cell, and CD8+ T-cell memory to SARS-CoV-2 in an integrated manner is likely important for understanding the durability of protective immunity against COVID-19 generated by primary SARS-CoV-2 infection [12, 20].

NAbS play important roles in virus clearance and are considered as key immune factors for protection or treatment against viral diseases. However, both innate and adaptive immunity are altered in the elderly. This phenomenon, called immunosenescence, increases susceptibility to infections, autoimmune disorders, and malignancies. Some individuals experience a sustained innate immune system activation, inducing proinflammatory cytokine secretion and innate immune cells’ recruitment, defined as “inflammaging” [21, 22]. Immunosenescence and inflamming in frail elderly people may impact the cellular and humoral immune response against SARS-CoV-2 [23].

Elderly subjects do develop high SARS-CoV-2 antibody levels, including NAbS, after infection [24, 25]. In a study carried out in 6 London care homes, including 144 hospitalized patients aged >80 years, the seroconversion rate was 100% and 97% in symptomatic and asymptomatic patients, respectively, with NAb lasting up to 5–6 weeks in >80% of cases [26]. Also Figueiredo-Campos et al observed a robust neutralization activity up to 7 months after infection in >90% of subjects in a large cohort of patients and caregivers, but there were very few elderly people included [27].

Regarding the relation between humoral response and clinical outcome, several studies have shown lower antibody titers in young and asymptomatic people with a rapid decrease within 2 or 3 months after the onset of symptoms [28, 29]. Disease severity, age, and male sex were factors identified as positively associated with seroconversion rates and antibody levels [9, 30], with NAb titers increasing with age [24].

While the persistence of humoral response, and particularly NAb levels, for at least 9 months has been reported in the general population, as well as a short-lasting and relatively lower antibody responses in people with asymptomatic or mild infection [31, 32], only a few studies have addressed the nature and the kinetics of antibody response in the elderly [33].

Variants of concern (VOCs), resulting from mutations in the RBD, have emerged at different places and times as a result of viral evolution compared to wild-type virus (https://www.who.int/activities/tracking-SARS-CoV-2-variants). They are more transmissible or virulent and can decrease the effectiveness of vaccines and treatments. In the elderly, some studies have shown a decrease in neutralizing antibody production in the first few months after SARS-CoV-2 infection or primary vaccination, which may determine an escape to new VOCs, especially Omicron [34, 35]. However, no absolute threshold of antibody titer has been established as a correlate of protection against SARS-CoV-2. On the other hand, it has been shown that despite a loss of NAb, a functional memory B-cell response may persist even in the elderly [36].

Thus, the aims of this study were to evaluate the production and persistence of NAb against SARS-CoV-2 in very old and frail unvaccinated patients hospitalized in LTCFs in order to determine their putative protection against reinfection. We also describe the clinical characteristics of patients included in the study and assess association between persistence of NAb and clinical characteristics.

**METHODS**

**Population Study and Sampling**

The Paul Brousse Hospital is a university hospital in the region of Paris (Assistance Publique-Hôpitaux de Paris [AP-HP]) with 415 geriatric beds (34 for acute care, 193 for rehabilitation, and 187 in 5 LTCFs) that house people aged >70 years with severe cognitive impairment and a heavy dependence in activities of daily living. The first wave of the SARS-CoV-2 outbreak between March and April 2020, the second outbreak between October and December 2020, and to a lesser extent, the third wave between March and April 2021 severely affected these wards.

In our LTCF, 91 surviving patients with SARS-CoV-2 infection diagnosed during the first outbreak (Figure 1) had a clinical follow-up and nasopharyngeal and serum sampling for SARS-CoV-2 RNA and serology testing, each time a new case of infection was detected in their unit, and to guide orientation toward single or double rooms, according to SARS-CoV-2 status. Surviving patients up to 9–12 months after the first outbreak were included in this retrospective noninterventional study.
This study, called CovImOld (COVID Immunity in Old Patients), was promoted by the AP-HP (APHP210210) and approved by the ethics committee of Comité éthique Gerontopôle d’Île de France (CEGIF) on 8 June 2021 (number 42021).

**Clinical Data**

We retrospectively collected demographic and clinical data from electronic medical records (Orbis) including basal comorbidities and geriatric characteristics (state of dependence by the Katz activity of daily living scale) [37], as well as clinical (such as classical or atypical symptoms) and laboratory data regarding COVID-19. According to the World Health Organization (WHO) and Infectious Diseases Society of America guidelines, each case was classified as asymptomatic, mild (laboratory confirmed, without pneumonia), moderate (laboratory confirmed and with pneumonia), severe (respiratory frequency ≥30/minute, blood oxygen saturation ≤93%), or critical (respiratory failure requiring mechanical ventilation, shock, or other organ failure that requires intensive care) infection [38, 39] (Table 1).

**Virological Data**

SARS-CoV-2 RNA detection was performed by different RT-PCR assays according to their availability at the time of testing, including in-house assay following the WHO protocol, Abbott RealTime SARS-CoV-2 Assay (Abbott Molecular), and cobas SARS-CoV-2 test (Roche Diagnostics) [40].

Elecsys Anti-SARS-CoV-2 and Elecsys Anti-SARS-CoV-2 S immunooassays (Roche Diagnostics, Mannheim, Germany) were routinely used for the qualitative detection of anti-N antibodies and the quantitative determination of anti-S protein RBD antibodies, respectively. According to the manufacturer’s instructions, anti-N result is considered positive, when assay index is >1; the quantification range of anti-S is 0.4 to 250 U/mL, and results <0.8 U/mL are considered negative.

To evaluate the presence of NAb, we used an in vitro micro-neutralization test surrogate to viral neutralization test in cell culture (cVNT) [41, 42], the iFlash-2019-nCoV NAb assay (Shenzhen YHLO Biotech, People’s Republic of China). This test measures the inhibition of the interaction between a recombinant SARS-CoV-2 RBD protein and recombinant angiotensin-converting enzyme 2 (ACE2) receptor by NAb present in the serum sample. This test is a paramagnetic particle chemiluminescent 1-step immunoassay, run on the iFlash 1800 analyzer (Shenzhen YHLO Biotech) according to the manufacturer’s instructions. In brief, the serum samples were placed on a sample rack in the sample loading area, and a reagent pack with 2019-nCoV RBD antigen (30KD)–coated paramagnetic microparticles and acridinium ester–labeled ACE2 conjugate were placed in the reagent loading area. The iFlash system performs all functions automatically. The signal from the chemiluminescent reaction is measured, and the results are determined using a calibration curve. The cutoff value for seropositivity is

![Flowchart of the study. Abbreviations: COVID-19, coronavirus disease 2019; M, month; NAb, neutralizing antibody; PCR, polymerase chain reaction.](image-url)
PCR-Negative Results

The data characterizing the population are presented for the statistical analysis. "Indeterminate." Values between >9 and <15 were considered "indeterminate."

Statistical Analysis

The data characterizing the population are presented for the quantitative variables by their mean or median and for the qualitative variables by their numbers and percentages.

Patients with positive anti-N serology after the first wave were divided into 2 groups according to whether they had a positive PCR or a negative PCR result, and second according to severity symptoms. The characteristics of these groups were compared by Student t test for continuous variables (or Mann-Whitney tests) and χ² or Fisher exact tests for categorical variables.

Univariate and multivariate analyses were performed to investigate the relationship between patient characteristics and the antibody response rate measured twice.

A P value of <5% was considered statistically significant. The statistical analysis was carried out using R software (version 4.0.3). All statistical data are expressed according to American Psychological Association standards.

Table 1. Clinical, Biological, and Immunological Characteristics, and Comparison of Patients With Polymerase Chain Reaction (PCR)–Positive Versus PCR-Negative Results

| Clinical Characteristics | Total (N = 91) | PCR Positive (n = 74) | PCR Negative (n = 17) | P Value |
|--------------------------|---------------|-----------------------|----------------------|---------|
| Age, y, mean ± SD        | 86 ± 7        | 85.6 ± 8              | 88 ± 4               | .09     |
| Female sex               | 62 (68)       | 47 (64)               | 15 (88)              | .09     |
| Charlson score, median (IQR) | 7 (6.0–8.0) | 7 (6.0–8.0)           | 7 (6.0–7.5)          | .66     |
| No. of drugs taken, median (IQR) | 8 (6.0–10.5) | 8 (6.25–11.0)         | 8 (5.0–8.0)          | .05     |
| ADL score, mean ± SD     | 0.9 ± 1.0     | 1.0 ± 1.1             | …                   |         |
| GIR score, mean ± SD     | 2.02 ± 0.8    | 2.0 ± 0.8             | 2.12 ± 1.0           | .82     |
| BMI, kg/m², mean ± SD    | 24 ± 5        | 24.7 ± 5.6            | 24 ± 4               | .50     |
| Arterial hypertension    | 61 (67)       | 51 (70)               | 10 (59)              | .84     |
| Diabetes                 | 25 (27)       | 22 (30)               | 3 (18)               | .54     |
| Coronary disease         | 12 (13)       | 11 (15)               | 1 (6)                | .68     |
| Chronic kidney disease   | 15 (16)       | 11 (15)               | 4 (24)               | .61     |
| Chronic lung disease     | 8 (9)         | 6 (8)                 | 2 (12)               | .99     |
| Cognitive impairment     | 86 (95)       | 70 (95)               | 16 (94)              |         |
| Immunological diseases   | 2 (2)         | 2 (3)                 | 0                    |         |
| Cancer                   | 3 (3)         | 1 (1)                 | 0                    |         |
| Immunosuppressive/corticosteroid drugs | 1 (1)  | 1 (1)                 | 0                    |         |
| Asymptomatic infection   | 11 (12)       | 2 (3)                 | 9 (53)               | <.001   |
| Symptomatic infection    | 80 (88)       | 72 (97)               | 8 (47)               | <.001   |
| Mild infection           | 41 (45)       | 34 (46)               | 7 (41)               | .93     |
| Moderate infection       | 18 (20)       | 17 (23)               | 1 (6)                |         |
| Severe infection         | 19 (21)       | 19 (25)               | 0                    |         |
| Critical infection       | 2 (2)         | 2 (3)                 | 0                    |         |
| Oxygen therapy           | 28 (30)       | 25 (34)               | 0                    | .23     |
| Atypical symptoms (falls, wandering, sleepiness, agitation) | 22 (24) | 19 (26) | 3 (18) | .70 |

Biological and immunological characteristics

|                      | Total (N = 91) | PCR Positive (n = 74) | PCR Negative (n = 17) | P Value |
|----------------------|---------------|-----------------------|----------------------|---------|
| Lymphocytes, cells/µL, mean ± SD | 1397 ± 716 | 1326 ± 689            | 1708 ± 770           | .07     |
| CRP, mg/L, mean ± SD | 32 ± 42       | 33 ± 38               | 29 ± 61              | .80     |
| Serum proteins, g/L, mean ± SD | 69 ± 7   | …                     | …                    |         |
| Albumin, g/L, mean ± SD | 33 ± 4     | 32.5 ± 4              | 32.7 ± 4             | .90     |
| SARS-CoV-2 PCR positive | 74 (81)   | 74 (100)              | 0                    |         |
| Anti-S titer, U/mL, mean ± SD (M2–M4) | 206 ± 85 | 204 ± 86              | …                    |         |
| Anti-S titer, U/mL, mean ± SD (M5–M7) | 187 ± 91 | 199 ± 83              | 137 ± 107            | .04     |
| Anti-S titer, U/mL, mean ± SD (M8–M9) | 181 ± 93  | 195 ± 85              | 117 ± 100            | .01     |
| Anti-S titer, U/mL, mean ± SD (M10–M12) | 202 ± 84 | 214 ± 77              | 147 ± 105            | .03     |
| NAb titer, AU/mL, mean ± SD (M2–M4) | 293.9 ± 339.7 | 245.8 ± 288.3 | … |         |
| NAb titer, AU/mL, mean ± SD (M5–M7) | 118.1 ± 183.5 | 121.8 ± 182.0 | 102.0 ± 194.5 | .03     |
| NAb titer, AU/mL, mean ± SD (M8–M9) | 91.0 ± 168.8 | 94.9 ± 176.0 | 74.6 ± 137.7 | .02     |
| NAb titer, AU/mL, mean ± SD (M10–M12) | 101.3 ± 171.7 | 102.5 ± 166.2 | 117.4 ± 213.2 | .10     |

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: ADL, activities of daily living; BMI, body mass index; CRP, C-reactive protein; GIR, Groups Iso-Resources; M, month; NAb, neutralizing antibody; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation.

* n = 71.

* n = 68 (3 were not dosed for technical reasons).
Outcomes
The first objective of the study was to determine the presence and durability of NAb up to 12 months of COVID-19 in our frail elderly patients infected during the first outbreak. We also determined whether these patients would become reinfected after contact with infected patients and during a new epidemic.

RESULTS

Demographic and Clinical Characteristics
Among patients hospitalized in our LTCF, infected with SARS-CoV-2 during the first outbreak, 91 were monitored for up to 9 months and 68 for up to 12 months (Figure 1).

At inclusion, patients’ mean age was 86 ± 7 years, and 62 (68%) were female. Of the 91 monitored patients, 61 (67%) had arterial hypertension, 25 (27%) diabetes, 15 (16%) chronic kidney disease, and 12 (13%) coronary disease. Median Charlson index was 7 (interquartile range [IQR], 6–8) and median number of drugs taken was 8 (IQR, 6–10.5). Median activity of daily living and GIR (a French disability score according to the Iso-Resource Group) scores were 0.9 ± 1.0 and 2.02 ± 0.8, respectively (Table 1).

During the first wave (14 March to 18 May 2020), SARS-CoV-2 infection was diagnosed by a positive PCR in 74 patients (81%), 72 (97%) of whom had symptomatic infection. A first-wave SARS-CoV-2 infection was retrospectively diagnosed by a positive anti-N serology on sera sampled between March and May 2020 in 17 patients (19%), of whom only 8 (47%) had a symptomatic infection. Overall, SARS-CoV-2 infection was symptomatic in 80 (88%) patients. Among them, 41 (45%) had mild clinical manifestations and 39 (42%) had moderate, severe, or critical COVID-19; 28 (30%) required oxygen therapy.

Laboratory findings (mean) showed C-reactive protein (CRP), 32 mg/L ± 42; albumin, 33 g/L ± 4; and lymphocyte count, 1397 cells/µL ± 716 (Table 1).

Kinetics of Anti–SARS-CoV-2 Antibody Levels
Ninety-one patients were monitored for SARS-CoV-2 serology, with 2–4 available serologies during follow-up. At 12 months, 9 patients had died from causes unrelated to COVID-19, 2 had been transferred to a nursing home, 9 had been routinely vaccinated between month (M) 9 and M12 (their samples collected after vaccination were excluded), and 3 had no available serum at M12 to test NAbs. Finally, 68 patients underwent serological monitoring up to 12 months from onset of infection (Figure 1).

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Anti-S titers $>250$ U/mL were set at 260 for analysis. In our analysis, sera with Elecsys anti-S levels $>75$ U/mL or YHLO NAb levels $>50$ AU/mL were associated with a significant neutralization activity in a cVNT assay [42].

At 3, 6, 9, and 12 months of follow-up, mean anti-S titers were $206 \pm 85$, $187 \pm 91$, $181 \pm 93$ U/mL, and $202 \pm 84$. There was no significant variation of these titers over time. A single patient had undetectable anti-S antibodies at M9, and was then vaccinated following this result (Figure 2A). At M12, all 68 patients had detectable anti-S antibodies and 60 of 68 (8%) had anti-S titers $>75$ U/mL (Figure 2C). Overall, 75 of 91 patients (82%) had anti-S titers $>75$ U/mL at least once.

At 3, 6, 9, and 12 months of follow-up, mean NAb titers ($n = 68$) were $293.9 \pm 339.7$, $118.1 \pm 183.5$, $91 \pm 168.8$, and $101.3 \pm 171.7$ AU/mL, respectively. There was a significant decrease of NAb titers between M3 and M6 ($P < .0001$), but no further significant change up to M12 (Figure 2B). At 12 months of follow-up, 60 of 68 patients (88%) still had detectable NAb, and 29 of 68 (42.6%) had NAb titers $>50$ AU/mL (Figure 2D). Overall, 52 of 91 patients (57%) had NAb titers $>50$ AU/mL at least once.

**Association Between Antibody Kinetics and Clinical Characteristics**

Regarding clinical outcome, patients with symptoms had higher anti-S and NAb levels than those with asymptomatic or mild infection, except at M12 where the difference was no more significant for NAb (Table 2). In addition, patients who required oxygen (severe COVID-19) had higher anti-S levels than those who did not ($P < .001$). When comparing pauci-asymptomatic patients with patients with moderate to critical infection, we found a significant decrease of NAb at M6 and M9 in the less symptomatic patients ($P = .008$ and $P = .01$) (Table 2).

Univariate analysis showed a positive correlation between anti-S levels at M6 and body mass index (BMI) ($r [179] = 0.24$, $P = .03$), or anti-S increases with increasing BMI. On the contrary, it showed an inverse correlation between the lymphocyte count and the anti-S level at M6 ($r [179] = -0.22$, $P = .05$) and M12 ($r [160] = -0.04$, $P = .05$), either anti-S increase when lymphocytes decrease. However, univariate analysis showed a positive correlation between CRP and NAb at M6 ($r [180] = 0.30$, $P = .005$) and M12 ($r [156] = 0.33$, $P = .007$).

A mixed multivariate analysis model showed that plasma protein ($\beta = 4.19$ [95% confidence interval (CI), 1.44–6.84]; $P < .002$) and CRP ($\beta = 0.26$ [95% CI, 0.03–5.50]; $P = .03$) are positively linked to anti-S rate, but lymphocyte counts ($\beta = -0.03$ [95% CI, -.04 to -.01]; $P = .009$) are negatively correlated to the anti-S rate; high levels of CRP ($\beta = 1.84$ [95% CI, 1.24–2.44]; $P < .0001$) are positively correlated to NAb rate at M6 and M12.

**Clinical Reinfection**

From March 2020, we had 3 major outbreaks in our geriatric departments. During the second (October–December 2020) and third (January–March 2021) waves, the VOCs 20I/501Y.V1 (Alpha) and 20H/501Y.V2 (Beta) were predominantly circulating, but none of the 91 LTC patients developed symptomatic or asymptomatic reinfection after their first exposure to SARS-CoV-2, as confirmed by a negative RT-PCR from nasopharyngeal swabs each time a contact with an infected patient or infected caregiver occurred.

**DISCUSSION**

LTCF have been disproportionately affected by the COVID-19 pandemic, with high rates of infection and deaths among the frail elderly residents [5, 6]. Despite potential immunosenescence, we report a very high and long-lasting antibody response after SARS-CoV-2 infection in a cohort of very old and frail patients. Indeed, all but 1 maintained detectable anti-S titers up to 12 months after infection, including NAb in 88% and an efficient neutralizing response (NAb $>50$ UA/mL) in 43% of them.

Consistent with other studies, we found higher NAb titers in patients with more severe infections, higher BMI, higher levels of CRP and plasma proteins, and lower lymphocyte counts [13, 24, 30, 43]. After 12 months of follow-up, no patient developed clinical reinfection with wild-type virus or with Alpha and Beta variants that were actively circulating during the second and third wave, suggesting cross-neutralizing ability to these circulating variants and protection against reinfection. In our hands, NAb titers $>50$ AU/mL are associated with a serum neutralizing activity in vitro, while this is not the case for NAb titers $<50$ AU/mL [42]. In our study, absence of reinfection was observed despite NAb titers $<50$ AU/mL in $>50$% of patients. This point underlines the difficulty of establishing humoral correlates of protection and may be explained by the presence of memory B-cell repertoire and equally effective cellular immunity in our elderly patients [36].

Our data strongly suggest that natural postinfectious immunity is robust and protective also in the elderly [44], as also shown by others: Jeffery-Smith et al reported a reinfection rate, determined by RT-PCR or antibody, of 1.1% in 2 London LTCFs at 4 months after the first infection. The only reinfected people were health workers [45]. In an Italian cohort, the risk of reinfection was very low (1.1%) and occurred after a median follow-up of 9 months in patients with a previous history of mild infection, mostly with weak or absent serological response. None of the elderly (>65 years) subjects included were reinfected [46].

By contrast, in a large cohort of Danish people tested positively by PCR during the first epidemic wave, protection against repeat SARS-CoV-2 infection was 80% or more in people aged...
Abbreviations: M, month; NAb, neutralizing antibody.

<65 years while protection dropped to <50% in individuals aged >65 years [47]. However, strains' characteristics, cellular or humoral responses to SARS-CoV-2, and clinical presentation of reinfections in these older patients were not available.

Antibodies against spike and neutralizing antibodies as well as memory B-cell and interferon-γ-secreting T-cell responses can be detectable in >70% of patients for up to 1 year. In these studies the age of the patients did not affect the persistence of these immune responses, but there were no very old subjects included [48, 49].

A new study found the effectiveness of previous infection in preventing reinfection and progression to critical or fatal COVID-19 with the different variants of SARS-CoV-2 including Omicron, which seems to escape incomplete vaccination. This natural immunity is more robust regardless of vaccine-induced immunity [50].

The main limitation of this single-center, retrospective study design with a medium-sized cohort is that it does not allow to design with a medium-sized cohort is that it does not allow to
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## Table 2. Association Between Anti-Spike and Neutralizing Antibody Titers and Clinical Outcome

| Anti-S Spike Titers | Total (N = 91) | Asymptomatic or Mild Infection (n = 52) | Moderate or Severe or Critical Infection (n = 39) | P-Value |
|---------------------|---------------|----------------------------------------|------------------------------------------------|--------|
| Anti-S titer, U/mL M2–M4 | 206 ± 85 | 168.3 ± 100.6 | 238.3 ± 53.0 | .02 |
| Anti-S titer, U/mL M5–M7 | 187 ± 91 | 155.3 ± 98.1 | 223.8 ± 64.6 | <.001 |
| Anti-S titer, U/mL M8–M9 | 181 ± 93 | 144.5 ± 98.1 | 221.6 ± 65.6 | <.001 |
| Anti-S titer, U/mL M10–M12 | 202 ± 84 | 177.2 ± 91.3 | 230.6 ± 64.4 | .007 |
| Anti-S Spike and NAb Titors | | | | |
| NAb titer, AU/mL M2–M4 | 293.9 ± 339.7 | 169.9 ± 217.6 | 396.0 ± 391.6 | .06 |
| NAb titer, AU/mL M5–M7 | 118.1 ± 183.5 | 74.5 ± 124.2 | 176.2 ± 230.1 | .008 |
| NAb titer, AU/mL M8–M9 | 91.0 ± 168.8 | 51.7 ± 85.4 | 146.0 ± 232.2 | .01 |
| NAb titer, AU/mL M10–M12 | 101.3 ± 171.7 | 83.9 ± 155.3 | 129.5 ± 191.2 | .3 |

Data are presented as mean ± standard deviation unless otherwise indicated. abbreviations: NAb, neutralizing antibody.

Notes

- **Author contributions.** R. C., A. M. R.-A., and C. T. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. R. C., C. V.-F., A. A.-T., L. M., A. M. R.-A., and C. T. contributed equally to this work. Drafting of the manuscript: R. C. Laboratory testing: L. M., C. V.-F., A. A.-T., A. M. R.-A., C. T. Statistical analysis: A. A.-T. and A. M. R.-A.

- **Patient consent statement.** This study was approved by the local ethics committee CEGIF (Comité éthique Gerontopôle d’Ile de France) on 8 June 2021 (approval reference number 42021) and complies with the current standards in France. All patients gave written informed consent.

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