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ORIGINAL ARTICLE

Antibody response after two doses of the SARS-CoV-2 Comirnaty vaccine in a Covid-19 positive and Covid-19 negative Italian healthcare workers cohort

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ABSTRACT

Background: Extensive vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is now universally regarded as one of the most effective strategies for counteracting the current pandemic. The durability of the immune response of available vaccines is not known, therefore the quantitative dynamics of serum anti-S antibodies after Comirnaty vaccine in health care workers (HCW) of Desio Hospital was conducted.

Methods: 51 previously infected and 198 not infected HCW, from Desio, Italy were enrolled in the study. Comirnaty double dose schedule was completed by each subject. Specific anti-S antibodies against the SARS-CoV-2 S protein were measured by ECLIA in sequential blood samples.

Results: A significant difference was observed beginning at pre priming dose (T0) of the anti-S antibodies between the two subgroups which persisted throughout the study (4 months). A significant reduction occurred after 4 months post-priming dose (T3). Finally, a subgroup of low and late responders with an increasing trend was found.

Conclusions: Specific anti-S antibodies are significantly decreased 4 months post priming dose of Comirnaty vaccine although prior COVID-19 infection seems to escalate humoral response. Further evaluation concerning antibody persistence beyond this point, and the proportion of neutralizing antibodies with higher affinity towards SARS-CoV-2 is needed, especially in naïve and immunosuppressed subjects.

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Introduction

As of March 11, 2020, the world has been officially declared by the World Health Organization (WHO) to be under siege of coronavirus disease 2019 (COVID-19). One year later, the toll on human lives to SARS-CoV-2 has been more than 3.98 million deaths, and over 183,000,000 more infected in almost 200 countries and territories worldwide [1]. This has generated a worldwide health and economic emergency. Containment strategies have not been totally successful in keeping the virus from spreading around the globe, nor from developing variants of concern. The single most efficacious strategy in reducing the risk of infection is the use of vaccines. The US government responded to this necessity by establishing Operation Warp Speed in May of 2020 in order to develop, produce, and distribute COVID-19 vaccines [2]. As of June 18, 2021 there are a total of 287 candidate vaccines of which 102 are in various stages of clinical progress [3]. Four vaccines, so far, have received FDA emergency, European Medicines Agency (EMA), and Agenzia Italiana del Farmaco (AIFA) approval in Italy: Comirnaty (BNT162b2, Pfizer-BioNTech, BioNtech Manufacturing GmbH, Mainz, Germany) an mRNA vaccine; Spikevax (mRNA-1273, Moderna Biotech Spain, S.L., Madrid, Spain) an mRNA vaccine; Vaxzevria (ChAdOx1nCoV-19, AstraZeneca AB, Sodertalje, Sweden) an adenovirus-vectored vaccine; COVID-19 vaccine Janssen (Ad26.COV2.S, Janssen-Cilag International, Beerse, Belgium) an adenovirus serotype 26 vectored vaccine [4]. All of these vaccines have targeted the spike (S1) glycoprotein of the SARS-CoV-2 surface, eliciting an immune response against the Receptor Binding Domain (RBD) located on the S1 protein by generating functional neutralization antibodies. Antibodies against SARS-CoV-2 with strong neutralizing capacity, especially against the RBD, have been identified in COVID-19 patients [5]. This humoral immune response seems to last for 6–8 months [5]. Therefore, a logical consequence of this observation is that also the available vaccines may produce a humoral response that is at least as durable. A longitudinal appraisal of antibody response to vaccination is pertinent since neither SARS-CoV nor MERS-CoV infections, especially mild ones, seem to generate long-lived antibody responses [6]. This may also be true for COVID-19, and there is still debate on whether subjects infected with SARS-CoV-2 should be subjected to the two dose regimen. Therefore, this study points

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to determine the durability of antibodies elicited by Comirnaty vaccine in a cohort of COVID-19 positive and COVID-19 negative healthcare workers (HCW).

Materials and methods

249 COVID-19 positive ($n = 51$) and COVID-19 negative ($n = 198$) HCW, from Desio Hospital (ASST Brianza), Desio, Italy were enrolled in the study (ABCV-Brianza, Anti-Bodies-Covid-Vaccino-Brianza). COVID-19 positivity was documented by RT-PCR post nasopharyngeal swab. All subjects received two doses (priming dose and booster dose, 30 µg mRNA in 0.3 mL/dose) of the Comirnaty vaccine. The booster dose was administered exactly 21 days after the priming dose during the period from January 2021 through February 2021. Sequential blood drawings for anti SARS-CoV-2 S antibody testing were performed at: T0, before the priming dose (day 0); T1, before the booster dose (day 21); T2, exactly 14 days after the booster dose (day 35); T3, 4 months after the booster dose (day 120).

Quantitative determination of antibodies (including IgG) to the SARS-CoV-2 spike (S) protein RBD in human serum and plasma was performed with the Roche Elecsys® Anti-SARS-CoV-2 S immunoassay on the Roche Cobas c8000 platform (Roche Diagnostics GmbH, Mannheim, Germany). Assay range was 0.4 – 250 U/mL. A positive test result is obtained when it corresponds to $\geq$ 0.80 U/mL and a negative one when it corresponds to $<$ 0.80 U/mL [7]. The claimed cut-off for positivity of $\geq$ 0.80 U/mL is equivalent to $\geq$ 0.80 BAU/mL since the correlation, claimed by the manufacturer, between Roche Elecsys Anti SARS-CoV-2 S and WHO International Standards for anti-SARS-CoV-2 S is excellent ($r^2 = 0.9992$, slope = 0.972, intercept = 0.0072) [8]. After testing, values <0.4 U/mL were considered to be 0.4 and those >250 U/mL were diluted accordingly (1:100) until up to >25,000 and not beyond. A post-vaccination questionnaire was sent to all participants of the study in order to collect information concerning eventual side effects they may have had after the priming dose and after the booster dose.

The study was performed in accordance with the Declaration of Helsinki and all participants gave informed consent. The study was approved by the Local Ethical Committee

Statistical analysis

The descriptive statistics for the main characteristics of the study group were expressed as Median (1st quartile – 3rd quartile) for continuous variables and as absolute frequency (column percentage) for the categorical variables (Table 1). The Wilcoxon-signed ranks-test was used to assess the differences between the COVID-19 positive and COVID-19 negative subgroup at each time point. The repeated ANOVA test was applied to determine if there were statistically significant differences over time for immunoglobulin (Ig) anti-S concentration. A box plot was used to show the Ig anti-S concentration at each time point for all groups.

Table 1. Ig anti-S concentrations for COVID-19 negative and COVDI-19 positive subgroups evaluated at each time point.

Variable Ig anti-S (U/mL)	COVID-19 negative	COVID-19 positive	*p*-Value
T0	0.4 [0.4; 0.4]	69 [18; 239]	<.001
T1	19.5 [8; 57.25]	8215 [5499; 145,52]	<.001
T2	1576 [812.75; 2905.5]	13,625 [9786; 21,679]	<.001
T3	898.5 [542.25; 1351.75]	3826 [2615; 7505]	<.001

The *p*-values were evaluated by Wilcoxon test.

Differences were considered statistically significant if the *p*-value was lower than .05. All the statistical analyses were performed using the software Stata MP 16 (StataCorp. 2019. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC).

Results

Of the total 249 HCWs, 198 (80.2%) were classified as COVID-19 negative, or naïve of infection, and 51 (19.8%) as COVID-19 positive, or with pre-existing immunity due to infection. Infected HCWs were represented by 16% males and 84% females, whereas non infected ones by 24% males and 76% females. Mean age was 49 years (range 23–69 years with a median of 51). The mean time from the first RT-PCR positive nasopharyngeal swab to the first vaccine dose in the COVID-19 positive subgroup was 105 days with a range of 28 to 301 days. Two of the 51 COVID-19 positive individuals were excluded from statistical analysis due to the absence of antibodies at T0. Two (0.80%) COVID-19 negative HCW did not respond after the booster dose, and 32 (13%) had a delayed humoral response. A breakthrough infection was observed in other two naïve participants, one with Variant of Concern (VOC) B.1.1.7 (Alpha) and one with VOC B.1.617.2 (Delta).

Table 1 summarizes the Ig anti-S concentration for the COVID-19 negative and COVID-19 positive subgroups evaluated at each time point. At T0 the previously infected subgroup already presented antibodies confirming a pre-existing humoral immunity towards SARS-CoV-2, whereas the COVID-19 negative subgroup did not. In both subgroups the antibodies increased from T0 to T2, but the magnitude of antibody production was much higher at T1 and T2 (about 9 times as much) in the COVID-19 positive subjects when compared to the negative ones. Furthermore, overall antibody concentration dropped by about 57% and about 28% at T3 in the COVID-19 positive and COVID-19 negative subgroup, respectively. Just like the magnitude of increase, also that of decrease in humoral immune response was bigger in the positive individuals. The differences in Ig concentration between the two subgroups were statistically significant at all time points (Table 1).

Gender differences in the humoral response were evaluated and it was observed that the median antibody concentrations in naïve females vs males at T1 were 19 vs 23 U/mL ($p = .24$); at T2, 1579 vs 1561 U/mL ($p = .73$); and at T3, 929 vs 839 U/mL ($p = .22$). The median antibody concentrations in COVID-19 positive females vs males were: T0, 49 vs 325 U/mL ($p = .28$); T1, 8314 vs 5630 U/mL ($p = .10$); T2,

Table 2. Evaluation of Ig anti-S concentrations for COVID-19 negative, COVID-19 positive, and a sub-group of Late-responders from T0 to T3 (120 days after the priming dose of Cominarty vaccine).

Variable	Ig anti-S T0 (U/mL)	Ig anti-S T1 (U/mL)	Ig anti-S T2 (U/mL)	Ig anti-S T3 (U/mL)	*p*-Value	Partial SS	*df*	MS	*F*
COVID-19 negative	0.4 [0.4;0.4]	19.5 [8;57.25]	1576 [812.75;2905.5]	898.5 [542.25;1351.75]	<.001	1.55E + 13	249	62,105,267	6.7
COVID-19 positive	69 [18;239]	8215 [5499;14552]	13625 [9786;21,679]	3826 [2615;7505]	<.001	9.68E + 12	51	1.90E + 11	10.3
Late-responders	0.4 [0.4;0.4]	8 [3.5;18.5]	566.3 [317.5;1004.5]	929 [554.5;1476.5]	<.001	74,175,163	37	2,004,734.1	4.1

p-Values are results from one-way repeated measures ANOVA.

Table 3. Age-related serological response at each time point, and rate of decline in antibody concentration for COVID-19 positive and negative HCWs.

A. Positive HCWs

Time point	20–29 years (*N* = 2)	30–39 years (*N* = 13)	40–49 years (*N* = 7)	50–59 years (*N* = 25)	60–69 years (*N* = 2)	*p*-Value (ANOVA)
	Anti-S antibodies median (IQ1–IQ3) in COVID-19 positive subjects					
T0	28.5 (26–31)	44 (10–227)	49 (8–156)	99 (23–275)	5954 (56–11,852)	*p* = .01*
T1	5342 (3522–7162)	10,825 (5519–13,856)	5000 (4061–15,077)	9195 (5905–15,857)	12,897.5 (6259–19,536)	*p* = .54
T2	12,224 (8651–15,797)	14,246 (10,936–21,459)	11,856 (6135–15,982)	14,170 (9744–25,000)	17,652 (13,625–21,679)	*p* = .56
T3	3650 (3634–3666)	3640 (2483–5322)	3307 (1971–3826)	4843 (2615–10,200)	7133 (4176–10,090)	*p* = .38
% of decline (T3–T2)/T2	70%	74%	72%	66%	60%	

B. Negative HCWs.

Time point	20–29 years. (*N* = 6)	30–39 years. (*N* = 20)	40–49 years. (*N* = 55)	50–59 years. (*N* = 95)	60–69 years. (*N* = 20)	*p*-Value (ANOVA)
	Anti-S antibodies median (IQ1- IQ3) in COVID-19 negative subjects					
T0	<0.4 (0.4–0.4)	<0.4 (0.4–0.4)	<0.4 (0.4–0.4)	<0.4 (0.4–0.4)	<0.4 (0.4–0.4)	*p* = 1
T1	40.5 (15–64)	27 (19–70.5)	23 (9–63)	17 (7–50)	10.5 (5.5–24.5)	*p* = .80
T2	1935 (855–2548)	2101.5 (1197–3003)	2027 (962–3250)	1466 (704–2618)	1175 (558–1808)	*p* = .25
T3	1182 (987–2239)	1047 (766–1453)	976 (647–1515)	808 (496–1255)	616 (487–924.5)	*p* = .6
% of decline (T3–T2)/T2	39%	50%	52%	45%	48%	

14,170 vs 12,970 U/mL ($p = .78$); and T3, 3666 vs 4001 U/mL ($p = .86$). These values were not statistically significant in either group of COVID-19 HCWs.

Age-related differences were also taken into consideration (Table 2(A,B)). The most numerous subjects in the COVID-19 positive group (Table 2(A)) were found to be in the 50–59 ($N = 25$) and 30–39 ($N = 13$) age group. At T0 the 2 oldest participants of 60 and 61 years had the highest values in comparison with those found in all the younger age groups. However, this was due to the very high concentration (antibody concentration 11,852 U/mL) observed in the 60 year old individual who, although was confirmed to be positive to COVID-19 eight months prior to the first dose of Comirnaty, probably had an asymptomatic contact with the virus just before the vaccine. The 61 year old subject (antibody concentration of 56 U/mL) had been confirmed positive by RT-PCR 3 months prior to the first dose and showed a low antibody response to the virus at that time. However, at all the considered time points, no significant differences were detected among all age groups. In the COVID-19 negative group (Table 2(B)) a decreasing trend in antibody response was observed from the youngest to the oldest age group at T1 and at T3, whereas at T2, all age groups seemed to be comparable. However, no statistical differences were confirmed at any time point for any of the groups. The rate of decline in antibodies seemed to be slowest in the youngest age group, and overall it appeared to be slower in the COVID-19 negative group when compared to the COVID-19 positive one.

In Table 3 the repeated ANOVA test was utilized to determine if there were statistically significant differences over time for Ig anti-S concentrations within each group considered. This also took into account the Late responders, a third subset of 32 COVID-19 negative subjects. The results indicate that for each group, at every specific time point, the difference in increase/decrease with the preceding time point was statistically significant. Furthermore, the Median value observed at each time point for the Late responders show that there is a slow but steady increase in antibody concentration from T0 to T3 which distinguishes this group from the others in whom the T3 value shows the beginning of a diminishing trend.

The opposing trend between this subset and the other two groups is also evident from the boxplot (Figure 1). The boxplot also shows that although infected HCWs developed a significantly higher response to SARS-CoV-2, there is a much greater dispersion in the individual response in T1, further accentuated in T2, of the subjects in the COVID-19 positive group with respect to the others.

Finally, Table 4 provides the types and frequency of side effects experienced by the HCWs after each dose of the vaccine. The sample material used concerning side-effects were questions supplied in an online questionnaire format, the link of which was sent to each participant *via* e-mail. The results were collected anonymously from the online format. A total of 119 out of 249 (48%) subjects responded to the questionnaire, of these 102/119 (86%) were COVID-19 Negative and 17/119 (14%) were COVID-19 positive.

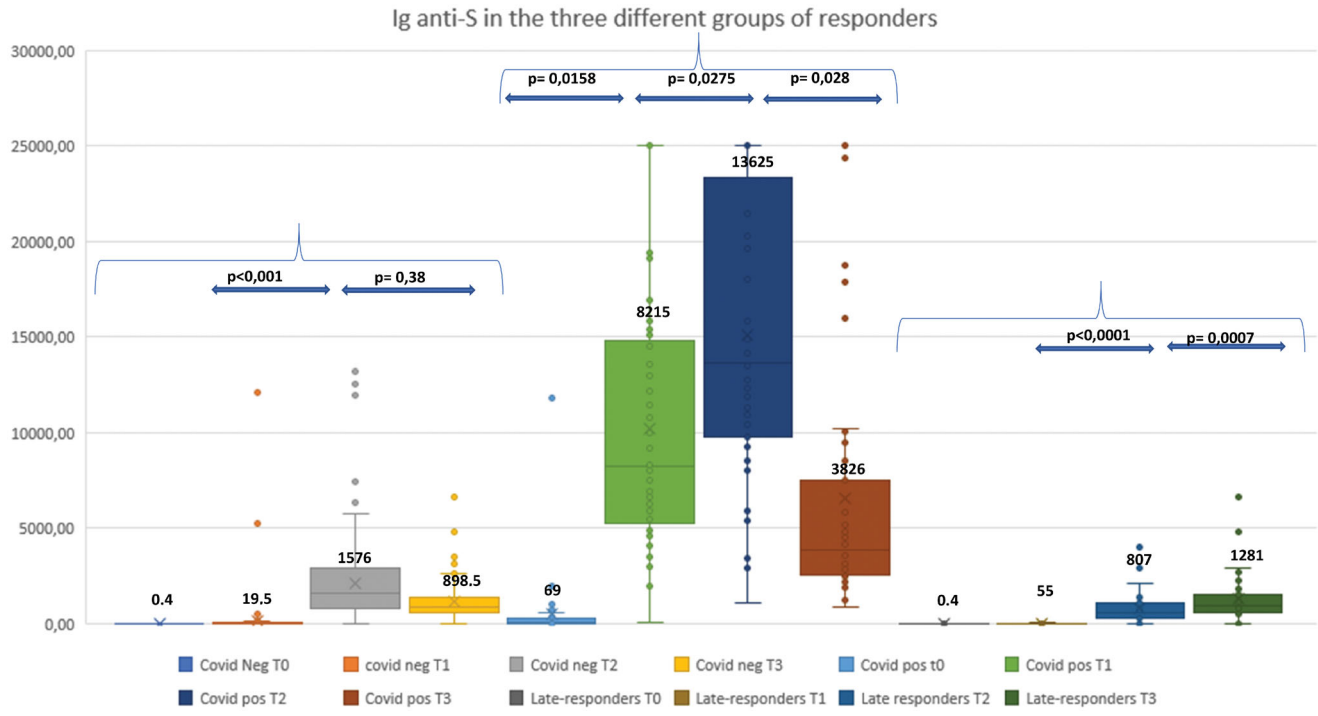

Figure 1. T0-before the priming dose; T1–21 days after the priming dose; T2–35 days after the priming dose; T3–120 days after the priming dose. Mean and standard deviation in different subgroups.

Table 4. Types and frequency of side effects in COVID-19 NEG and COVID-19 POS subjects vaccinated with Cominarty after the priming dose and after the booster.

	After priming dose		After booster dose	
Side effects	COVID-19 NEG (%)	COVID-19 POS (%)	COVID-19 NEG (%)	COVID-19 POS (%)
Local Pain	74	76	60	47
Myalgia	24	18	22	12
Arthralgia	7	18	22	6
Chills	8	18	20	18
Headache	9	24	28	24
Fever	2	12	10	12
Nausea	6	6	10	12
Any other local symptom	13	18	9	6
Any other systemic symptom	34	53	53	53

The most common specific effect for both groups documented after either vaccination was local pain, which seemed to have diminished after the second dose. The booster dose seemed to determine a greater increase in almost all the specific side effects, and especially in the systemic ones, in the COVID-19 negative subjects respect to the positive ones.

Discussion

Recent studies clearly demonstrated that HCWs who developed SARS-CoV-2 infection prior to vaccination have an enhanced antibody response to the priming dose. This generated a debate in healthcare management policies on whether to provide COVID-19 positive HCWs a second dose of vaccine or to utilize that second dose for vaccinating a greater number of naïve individuals. Indeed, part of the decision lies on the durability of the humoral immune response in the individuals with pre-existing immunity. Longitudinal SARS-CoV-2 antibody quantification allows to establish antibody persistence over time in order to better manage vaccination schedules and the prevention against COVID-19 pandemic. We aimed to establish the permanence of antibodies elicited by two doses of Comirnaty vaccine in a cohort of COVID-19 positive and COVID-19 negative HCWs.

The results from our study confirm the amplification of specific anti-S antibodies in previously infected subjects. This group of individuals had significantly higher pre-vaccination concentrations (T0) in comparison to the naïve subgroup. Beginning at the priming dose of the Comirnaty vaccine, we observed a significantly stronger response in the COVID-19 positive subgroup compared to the negative one. The median antibody concentrations of the individuals with pre-existing immunity exceeded those of naïve ones by about nine-fold at T2, 14 days after the second dose, at antibody peak. It is likely that the viral stimulation of the immune memory potentiates antibody production after the induced stimulus from the vaccination [9]. However, already at 120 days (4 months) after the priming dose there is a

downward trend in antibody concentration with a reduction of about 1/3 of the anti-S antibodies respect to the peak reached at T2 in the COVID-19 positive and about 1/2 in the COVID-19 negative individuals. Indeed, Ibarrondo et al. [10] have found that the humoral immunity obtained over a mean of 86 days after the onset of symptoms decreases by a half-life of 36 days. Therefore, it can be argued that albeit the powerful humoral immune response obtained in previously infected HCWs as soon as the first vaccination, the reducing trend already evident after just 4 months from the first dose warrants the necessity of a second dose, as otherwise this loss of the humoral immune response will be anticipated.

It remains to be established how long (in terms of months) the decline in antibody concentration will last and if a low concentration could be a determinant in reinfection or breakthrough infection (in the case of naïve individuals) with VOCs. In fact, a breakthrough infection was observed in two naïve participants, one with VOC B.1.1.7 (Alpha) and one with B.1.617.2 (Delta). The former individual was infected between T2 and T3 and had a low antibody level (384 U/mL) relative to the median level (1576 U/mL) of the subject's group, and the latter HCW was infected after T3 when the concentration was 441 U/mL. Thus, reinfection/breakthrough infection may be determined by a partial or decaying serological immunity which in turn may induce the genesis of antibody escape variants [11].

The hypothesis was formulated that the large dispersion of the quantity of anti-S antibodies at T1 and T2 observed in the COVID-19 positive subjects was correlated to the time passed between infection (first positive documented naso-pharyngeal swab) and T0: it would seem logical to hypothesize that the longer the time, the lower the basal antibody level. However, it was observed that there was no correlation between the two parameters: at the shortest time interval (28 days) the corresponding antibody concentration at T1 was 4061 U/mL and T2 11,928 U/mL, whereas at the longest time interval (301 days) T1 was >25,000 U/mL and T2 > 25,000 U/mL. Instead, the large dispersion observed may be related to the wide clinical spectrum of the infection, from asymptomatic to mild-symtptomatic. In agreement with this, is the finding of two COVID-19 positive individuals in whom the basal concentration was 0.4 U/mL and in whom the first positive naso-pharyngeal swab was documented on November 2, 2020, that is, 63 and 67 days prior to vaccination with the first dose.

During the study a subset of the COVID-19 negative subgroup (Late responders) emerged. These 32 individuals displayed a delayed humoral response with a countertrend pattern respect to the positive and negative COVID-19 groups. The Late responders showed a slow but steadily increasing trend in antibody levels. It was found that 28.1% (9/32) had diseases determining immunosuppression, 15.6% (5/32) had had a similar humoral immune response to Hepatitis B vaccine, and the remaining 56.3% (18/32) were not able to account for this type of delayed response. It will be interesting to see how the antibody concentrations will evolve in these individuals during the following months.

The findings concerning the reactogenicity to Comirnaty vaccine demonstrated that the booster dose determined a stronger presence of transient side effects associated with the vaccine in the COVID-19 negative subjects, and these were more systemic (fever, malaise, myalgia, arthralgia, chills, etc.,) than general. This is in agreement with other authors [2,9,12–14] who found similar results that were transient and mild.

Indeed a limitation of this study is the small size of the cohort (249 subjects) and also the small number of the COVID-19 positive individuals with respect to the negative ones. The results obtained should be confirmed by bigger sized groups. However, the differences observed in the humoral response between the two groups supported preceding findings in the literature [15]. Another limit of this study is the lack of a parallel investigation of the cellular response in the same cohort in order to evaluate its contribution on the durability of protection against SARS-CoV-2. This type of investigation is planned in the near future.

In conclusion, although prior COVID-19 infection seems to escalate the humoral response to Comirnaty vaccine, its durability at 4 months post priming dose appears to start waning. Further evaluation of the persistence of antibodies beyond this point and the proportion of neutralizing antibodies present needs to be carried out in order to determine how long protection against SARS-CoV-2 lasts, especially in naïve and immunosuppressed subjects, but also in seropositive ones, and if a third booster is needed in all.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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