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BRIEF REPORT

Durability of anti-spike antibodies after vaccination with mRNA SARS-CoV-2 vaccine is longer in subjects with previous infection: could the booster dose be delayed?

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Abstract

Purpose The long-term effectiveness of BNT162b2/Pfizer vaccine remains undetermined. This observational prospective study was designed to verify durability of antibodies against the viral receptor-binding domain (RBD) spike (S)-protein (RBD S-Protein IgG) after the second-dose administration of the vaccine among Health Care Workers (HCWs).

Methods In all HCWs at the Poliambulanza Foundation Hospital Brescia (Italy) were quantified the levels of RBD S-Protein IgG (Abbott Diagnostics) at 45 and 240 days after the second-dose vaccine. Previous infection was defined as antibodies against SARS-CoV-2 nucleocapsid positivity (Abbott Diagnostics) before vaccination. The Mann–Whitney *U* test was used to compare mean levels of RBD S-Protein IgG among previously infected and uninfected HCWs.

Results The mean level of the RBD S-protein IgG detected 45 days after the second dose of the vaccine was 30,041 AU/mL (95% CI 145–80,000) for the 250 previously infected HCWs and it was significantly higher ($p < 0.001$) than that observed in the 1121 previously uninfected subject with the mean level of 10,604 AU/mL (95% CI 165–62,241). Similarly, at 240 days in previously infected subjects the antibody titer was of 8145 AU/mL (95% CI 347–80,000) and significantly higher ($p < 0.001$) than that observed in the 1121 previously uninfected HCWs with a mean antibody level of 1271 AU/mL (95% CI 50–80,000). When comparing the change in mean antibody levels overtime, the previously infected HCWs presented a 72.9% reduction in RBD S-protein IgG while in the previously uninfected HCWs the reduction was 88.0%. In addition, in the HCWs group without previous infection we reported 53 new SARS-CoV-2 infections and they had a mean level of RBD S-protein IgG antibodies of 1039 AU/mL (95% CI 157–4237) at 240 days. No new infections were found in previously SARS-CoV-2 infected subjects.

Conclusions We report that the mean level of post vaccinal RBD S-protein IgG was significantly higher in the previously infected HCWs than in previously uninfected subjects at 45 and 240 days after the second-dose vaccine. Moreover, our data suggest that the risk of a new SARS-CoV-2 infection was higher in the previously uninfected HCWs than in those who had already contracted natural viral infection. The limitations of this study prevent us to draw definitive conclusions on the antibody titers and on the role of a previous SARS-CoV-2 infection in influencing the levels of post-vaccine RBD S-protein IgG. The booster dose of the vaccine could be delayed after the second dose in previously naturally infected subject and it could have an important strategic impact on the organization of the future COVID-19 vaccination campaign.

Keywords SARS-CoV-2 · Anti spike antibodies · Immunization · BNT162b2 · Vaccine

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Introduction

A two-dose regimen of the BioNTech/Pfizer mRNA BNT162b2 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine was demonstrated to be highly effective in preventing symptomatic COVID-19, both in clinical trials [1] and real-world settings [2]. However, long-term effectiveness remains undetermined, and there is evidence of waning immunity in terms of antibody

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dynamics [3]. Previous studies have found a significant waning of humoral responses within 6 months after receipt of the second dose of BNT162b2 with a continuous decrease in anti-spike IgG titers [4]. Other studies also found a modest decline in the frequency of T cells at 6 months and a robust expansion in response to antigen and recognition of spike peptides from the delta variant [5].

This observational prospective study was designed to verify durability of a protective immune response via antibodies against the viral receptor-binding domain (RBD) spike (S)-protein (RBD S-protein IgG) after the second-dose administration of BNT162b2/Pfizer vaccine among Health Care Workers (HCWs).

Methods

In April, June and November 2020, before the first dose of BNT162b2/Pfizer vaccine, the antibodies against SARS-CoV-2 nucleocapsid (SARS-CoV-2 N-protein IgG) (Abbott Diagnostics) were measured in all HCWs at the Poliambulanza Foundation Hospital Brescia, a 600-bed tertiary care hospital in Northern Italy. The positive threshold was 1.4 signal to cutoff-ratio.

Previous infection was defined as SARS-CoV-2 N-protein IgG positivity before vaccination and/or history of positive polymerase chain reaction result from a nasopharyngeal swab. The negative individuals were considered previously uninfected.

All the symptomatic and asymptomatic infections occurred prior to vaccination during the first wave (from the 21st February to the 11th June 2020) and the second wave (from 14th September to 31th December 2020) in Italy. Both the previously uninfected and previous infected HCWs received the first and second dose of BNT162b2/Pfizer vaccine between January and February 2021, after the second wave.

The levels of IgG antibodies against the spike protein receptor-binding domain were quantified using anti SARS-CoV-2 IgG II Quant assay (RBD S-protein IgG) (Abbott Diagnostics) 45 and 240 days after the second dose of the vaccine. The positive threshold was 50 Arbitrary Units per mL (AU/mL) and the upper limit was 40,000 AU/mL, extendable to 80,000 AU/mL after automatic dilution.

This study was approved by the Provincial Ethical Committee (NP4478). All participants that volunteered in this study provided explicit and informed written consent.

The Mann–Whitney *U* test was used to compare mean levels of RBD S-protein IgG among previously infected and uninfected HCWs. The test was performed as two-tailed, and a p value < 0.001 was considered statistically significant.

Results

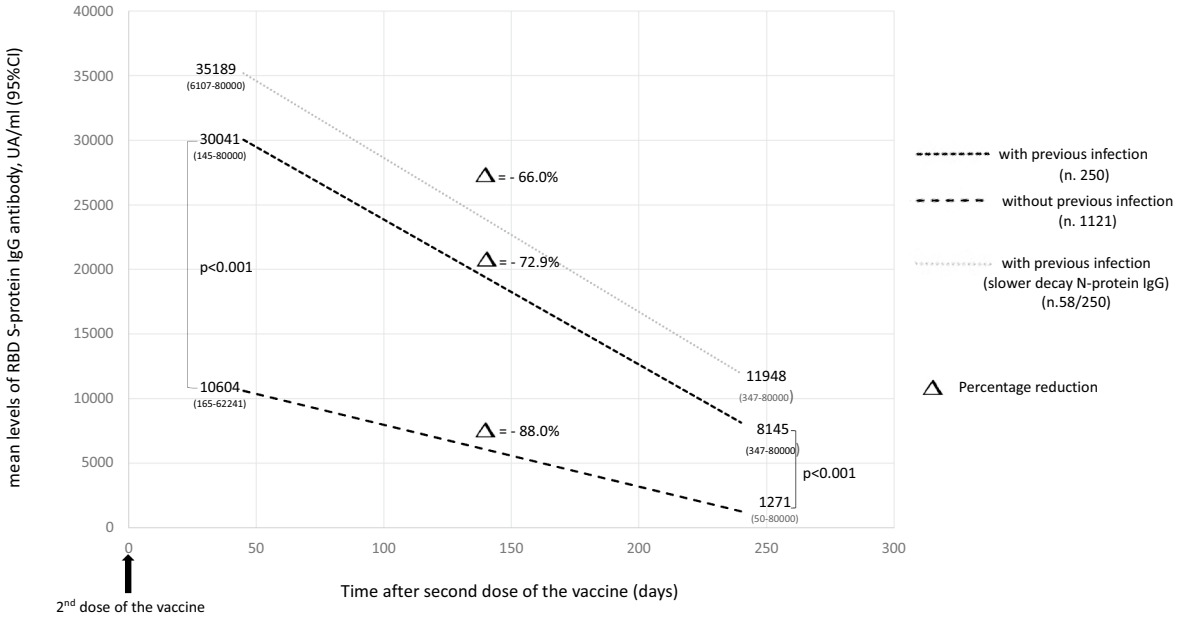
In our previously retrospective observational cohort study we enrolled 1510 HCWs (71.8% female, mean age 44 (20–75 years). This research demonstrated a robust vaccine response with a highly significant increase level of RBD S-protein IgGin subjects with prior SARS-CoV-2 infection compared to previously uninfected individuals at 45 days after the second dose administration of BNT162b2/Pfizer vaccine [6].

In this new study we have conducted serologically follow-up with 1371 subjects at 240 days after the second dose of the vaccine; 1121 had no prior SARS-CoV-2 infection while 250 had an infection previously documented during the first and second wave in Italy.

The mean RBD S-protein IgG level detected 45 days after the second dose of the vaccine in previously infected HCWs was 30,041 AU/mL (95% CI 145–80,000), and in uninfected subjects the mean antibodies titer was 10,604 AU/mL (95% CI 165–62,241). The *U* test suggests that the antibodies titer was significantly higher in previously infected HCWs than in the uninfected subjects (*U* value 15,748, *z*-score—16,697; $p < 0.001$). At 240 days after the administration of the second dose of the vaccine, the previously infected HCWs presented a mean level of post vaccinal RBD S-protein IgG of 8145 AU/mL (95% CI 347–80,000). In comparison, the mean antibody levels observed in previously uninfected HCWs was significantly lower (*U*-value 5829, *z*-score − 20,255 $p < 0.001$) at 1271 AU/mL (95% CI 50–80,000). When comparing the change in mean antibody levels overtime (Fig. 1), the previously uninfected HCWs presented a 72.9% reduction in RBD S-protein IgG, while in previously uninfected HCWs the reduction was 88.8%.

In November and December 2021, during the periodic (twice a month) monitoring of our HCWs with molecular nasopharyngeal swabs, we reported 53 new acute acquired SARS-CoV-2 infections in the group without prior infection. The mean level of antibodies for these 53 HCWs was 10,666 AU/mL (95% CI 1877–35,374) at 45 days and 1029 AU/mL (95% CI 157–4237) at 240 days after the second dose of the vaccine which represent a 90% reduction of the titer. No new infections have been found in previously SARS-CoV-2 infected subjects.

The N-protein IgG antibodies were detected in all HCWs enrolled in the study: 250 were positive (previous SARS-CoV-2 infection) and 1121 were negative (uninfected). The positive threshold was 1.4 signal to cutoff-ratio and it is not possible to have the titers for the N-protein IgG. Of the 250 previously infected HCWs, only a subset of 58 were persistently seropositive for



Time after 2nd dose of the vaccine[a] (days)	Mean levels of RBD S-protein IgG antibody, AU/mL (95%CI)		p-value[d]
	Prior SARS-CoV-2 infection		
	Yes (n. 250)[b]	No (n. 1121)[c]	
45	30041 (145-80000)	10604 (165-62241)	p <0.001
240	8145 (347-80000)	1271 (50-80000)	p <0.001

Abbreviations: RBD, receptor binding domain; S-protein, Spike-protein; IgG, type G immunoglobulin; AU, arbitrary units; 95% CI, 95% confidence interval; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.

[a] BNT162b2 mRNA Covid-19 Vaccine/Pfizer-BioNTech [b] Seropositive for SARS-CoV-2 Nucleocapsid-protein IgG

[c] Seronegative for SARS-CoV-2 Nucleocapsid-protein IgG [d] Mann Whitney U test

Fig. 1 SARS-CoV-2 anti-spike IgG antibody levels after the second dose of the mRNA vaccine

SARS-CoV-2 N-protein IgG in all three repeated antibodies test (April, June and November 2020) and presented a slower decay of antibody titers. The interval time between SARS-CoV-2 infection (April 2020) and first vaccine dose (January 2021) was at least 10 months. All 58 slower decay N-protein IgG subjects had negative molecular nasopharyngeal swabs. The mean RBD S-protein IgG level was 35,189 AU/mL (95% CI 6107–80,000) at 45 days after the second dose of the vaccine and 11,948 AU/mL (95% CI 347–80,000) at 240 days after the complete vaccination with a percentage reduction of 66.0%. In uninfected subjects the mean antibodies titer was 10,604 AU/mL (95% CI 165–62,241) at 45 days after the second dose of the vaccine. In the previously infected HCWs subgroup the mean RBD S-protein IgG level was 11,948 AU/mL (95% CI 347–80,000) at 240 days after the second dose of the vaccine (*U* value 12,633, *z*-score 1583; $p = \text{ns}$).

Discussion

In our previous study [6], 45 days after the second dose of BNT162b2/Pfizer vaccine, we confirmed that naturally infected individuals with SARS-CoV-2 had a highly significant increase in the level of RBD S-protein IgG. At 240 days after the vaccine there was a significant difference in mean level of RBD S-protein IgG in previously SARS-CoV-2 infected subjects than in the uninfected individuals. This robust and persistent immunological response was probably the consequence of the memory B-cell and T-cell response to natural viral infection [7]. In this moment, it is unclear what the extent of antibody titers means for the risk of a future infection. Moreover, even if the two small groups considered in the present study were numerically unbalanced and the follow-up was only two months, there was a significantly different risk of infection

in previously uninfected subjects compared to previously infected individuals (53/1121 vs 0/250, $p < 0.05$). These results suggest that, at least within the first 240 days after vaccination, receiving a double dose of the vaccine after natural viral infection leads to better protection than just two initial vaccinations without previous infection.

Both naturally immune and vaccinated individuals showed a high degree of concordance between anti-RBD antibodies and microneutralization test (MNT) titers. The level of RBD S-protein IgG may represent a good surrogate marker of protective response [8]. In our study the mean RBD S-protein IgG level could be defined as protective in previously uninfected subjects after 45 days after the second dose of BNT162b2/Pfizer. At 240 days, we could hypothesize a similar protection in previously infected subjects with slower decay of SARS-CoV-2 N-Protein IgG because the average antibodies level of these subjects was similar to that of previously uninfected at 45 days after the second dose of the vaccine (respectively 11,948 AU/mL vs 10,604 AU/mL, $p =$ ns). These data could allow us to hypothesize that a booster dose of the vaccine may be delayed by at least 240 days from the second dose in previously naturally infected subjects. The memory B-cells specific for the RBD S-protein were detected in almost all SARS-CoV-2 infections, and immunological memory assessed for up to 8 months after infection [9]. The N-protein IgG is a representative antigen for the T-cell response in natural SARS-CoV-2 infection and induces specific T-cell proliferation and cytotoxic activity. A slower decay of N-protein IgG level could induce a longer immune response [10].

The current spread of the Omicron SARS-CoV-2 variant in the World is causing a significant increase of subjects with previous natural viral infections. Thus, if our present results are confirmed by other studies, the delayed booster dose of the vaccine could have an important strategic impact on the organization of the COVID-19 vaccination campaign. Furthermore preliminary data showed that the Omicron variant had more extensive escape from vaccine elicited immunity. However, escape was incomplete in individuals with previous natural infection. Perhaps previous infection, followed by vaccination or booster is likely to increase the neutralization level and likely confer protection from severe disease in Omicron infection [11]. A recent study reports that two doses of BNT162b2 vaccine administered after previous infection appeared to boost and extend immunity. Infection-acquired immunity boosted with vaccination remained highest and most durable in participants who received two doses of vaccine after a primary infection and there was no indication of waning of this immunity even more than 1 year after previous infection [12]. There are many persons who get infected by Omicron variant and its sub-variants despite being vaccinated but in previous infection subjects the infection-acquired immunity boosted with vaccination remains most durable. Furthermore, the protection against symptomatic infection in the cohort of vaccinated subjects after previous infection was similar to that reported after a three-course vaccination (two doses and a booster dose) [13].

In summary, conclusions cannot be drawn from protective RBD S-protein IgG antibodies titer but we have reported that the durability of anti-spike antibodies after vaccination with mRNA SARS-CoV-2 vaccine is longer in subjects with previous infections. Furthermore the risk of a new SARS-CoV-2 infection was higher in the previously uninfected individuals. The waning immunity and viral diversification create a need for a third dose of the vaccine but this could be delayed in previously infected subjects. Further studies will be needed to define a quantitative protection threshold, the rate of decline of antiviral antibodies and the timing of vaccine booster dose administration in prior SARS-CoV-2 infected subjects.

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Author contributions RS conceived and designed the study. RG collected data and performed the analysis. RS and RG wrote the first draft. RS, RG and WG provided substantial scientific input to the manuscript. All authors revised and agreed upon the final version of the manuscript.

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Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval Ethical approval was obtained from Provincial Ethic Committee (NP4478).

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