

# Anti-SARS-CoV-2 IgG antibodies induced by the BNT162b2 mRNA vaccine is age-dependent and influenced by a previous natural SARS-CoV-2 infection

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**Abstract.** Background and aim: our study aimed to investigate the association between anti-SARS-CoV-2 IgG level after two doses of BNT162b2 vaccine and the previously infected/infection-naïve status, age, and gender in a population of health care workers (HCWs). Methods: all the population of immunocompetent HCWs were vaccinated with two doses of BNT162b2 based on a technical data sheet. SARS-CoV-2 IgG assay was performed 25 to 32 days after the second dose. Anti-SARS-CoV-2 IgG level was used as a categorical variable, since 2080 BAU/ml was the median IgG value. The multivariate logistic regression model included the previously infected/infection-naïve status, age groups, and gender. Results: All HCWs tested were seropositive. The odds ratio (OR) for anti-SARS-CoV-2 IgG > 2080 BAU / ml between previously infected and infection-naïve HCWs was 2.05 [95% CI 1.1-3.8]. Older age groups had lower percentage of HCWs with anti-SARS-CoV-2 IgG > 2080 BAU / mL than younger groups. Finally, no association between gender and IgG level was found. Conclusions: our study showed an excellent antibody response to vaccination with BNT162b2 after two doses. A significant difference was observed between anti-SARS-CoV-2 IgG level with age and previous SARS-CoV-2 infection status.

**Key words:** SARS, antibody, vaccine, BNT162b2, booster, third

## Introduction

Limited data are available about anti-SARS-CoV-2 IgG neutralizing antibodies (NAbs) responses to BNT162b2 mRNA vaccine (Pfizer/BioNTech's Comirnaty) with two doses in health care workers (HCWs) previously infected and whether this NAbs titer were influenced by age and sex. One of the most numerous studies available investigated the serology of 237 vaccinated HCWs and reported that NAbs titer following a single dose (n=216) in those previously infected were higher than naïve individuals given two

doses (n=21) or one dose (1). Moreover, a recent study from the UK about 72 HCWs reported that serological response to BNT162b2 inversely correlated with age, as evidenced in published reports of other vaccine (2,3).

BNT162b2 elicits both NAbs and cellular immune responses to the spike (S) protein, which may help protect against SARS-CoV-2 infection. The S protein is a glycoprotein comprising two subunits, S1 and S2. NAbs are defined as antibodies that defend a cell from a pathogen or infectious particle by neutralizing any biological effects. NAbs interfere with the mechanisms by which the virus attacks target cells.

NABs mainly interfere with the S proteins present on the surface of the viral capsid by preventing the binding of the S1 subunit with the angiotensin-converting enzyme 2 receptor present on the target cells, or by blocking the conformational changes of the S2 subunit, thus preventing the virus from entering the target cell (4–7). The level of IgG anti-S1/S2 correlates with virus neutralization (8,9). The presence of NABs is commonly considered as a sign of protection against a pathogen, even though it should be noted that lack of scientific data at this time does not allow the determination of whether neutralizing IgG antibodies against SARS-CoV-2 provide long-term immunity against the virus or if they protect patients against re-infection.

Italy was one of the hardest-hit areas in Europe during the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic in 2020 (10,11). Rapid vaccine-induced population immunity is a key global strategy for controlling coronavirus disease-2019 (COVID-19). The vaccination campaign in Europe was launched on December 27, 2020, with the first available vaccine, BNT162b2 mRNA vaccine. Anti-COVID-19 vaccination campaign was associated with a significantly lower incidence of SARS-CoV-2 infection among HCWs (12). Several ongoing follow-up programs in Italy are monitoring the long-term humoral immune response to vaccination with BNT162b2 and its clinical significance.

Our study aimed to investigate the association between anti-SARS-CoV-2 IgG level after two doses of BNT162b2 and the previously infected/infection-naïve status, age, and gender in a population of HCWs.

## Methods

In February–March 2021, all the population of immunocompetent HCWs who worked with direct patient contact in a hospital in Northern Italy were tested for anti-SARS-CoV-2 IgG level after vaccination against SARS-CoV-2. HCWs were vaccinated with two doses of BNT162b2 based on a technical data sheet, from December 2020 to February 2021. SARS-CoV-2 IgG assay was performed 25 to 32 days after the second dose of the BNT162b2 vaccine. To avoid overestimation of IgG level attributable to the

vaccine, HCWs who were infected with SARS-CoV-2 after the first dose of the vaccine were excluded. On the other hand, a previous SARS-CoV-2 infection that occurred before vaccination was not an exclusion criterion.

Anti-SARS-CoV-2 IgG were measured with “LIAISON® SARS-CoV-2 Trimeric IgG” assay by DiaSorin, showing a sensitivity of 98.7% and a specificity of 99.5%, as declared by the manufacturer (13). LIAISON® SARS-CoV-2 TrimericS IgG assay is a new generation of chemiluminescence immunoassay (CLIA), for the quantitative determination of anti-trimeric spike protein specific IgG antibodies to SARS-CoV-2 in human serum or plasma samples. The assay can support the study of the immune status of infected patients by providing an indication of the presence of NABs against SARS-CoV-2, by showing positive percent agreement of 100% (95%CI: 97.8% - 100.0%) and negative percent agreement of 96.9% (95%CI: 92.9% - 98.7%) at the Microneutralization Correlation test (13). To assess anti-SARS-CoV-2 IgG level differences by age, the study population has been divided in age groups, 21–30, 31–40, 41–50, 51–60 and 61–70 years old. Anti-SARS-CoV-2 IgG level was used as a categorical variable, since 2080 BAU/ml was the median IgG value. The data were analyzed using SAS (SAS institute, NC, USA) statistical software and GraphPad Prism 9 (GraphPad Software, CA, USA). Data were analyzed using Mann-Whitney U test and a multivariate logistic regression. The multivariate logistic regression model included the previously infected/infection-naïve status, age groups, and gender. P-values < .05 were considered significant.

## Results

Characteristics of the 583 HCWs are shown in Table 1. A total of 109 (19%) HCWs had a diagnosis of COVID-19 before getting the vaccine. The median age of HCWs included in the study was 40 (range 22–70), with a high prevalence of females (503/583, 86%). Considering a previous diagnosis of COVID-19, the median age of HCWs in the previously infected HCWs group was 38 years (range: 24–70 years), and a majority were females (97/109,

**Table 1.** Characteristics of HCWs and odds ratio between anti-SARS-CoV-2 IgG level and previously infected/infection-naïve status, age, and gender

Age groups (years)	Total HCWs	Subgroup of SARS-CoV-2 infection-naïve HCWs	Subgroup of previously infected HCWs
21-30, N (%)	164	128 (78%)	36 (22%)
31-40, N (%)	137	114 (83%)	23 (17%)
41-50, N (%)	125	99 (79%)	26 (21%)
51-60, N (%)	119	98 (82%)	21 (18%)
61-70, N (%)	38	35 (92%)	3 (8%)
TOTAL, N (%)	583	474 (81%)	109 (19%)
Women /Men (N)	503/80	406/68	97/12
Dosing interval, median days (range)	21 (21 – 24)	21 (21 – 24)	21 (21 – 24)
Months from infection to vaccine, median (range)	–	–	2.5 (0 – 10.6)
Days from second dose to sampling, median (range)	28 (25 – 32)	28 (25 – 32)	28 (25 – 32)
<b>Multivariate logistic regression (anti-SARS-CoV-2 IgG &gt; 2080 BAU/ml)</b>			
21-30 years	Ref.	–	–
31-40 years, OR [95% CI]	0.81 [0.43-1.54]	–	–
41-50 years, OR [95% CI]	0.49 [0.27-0.89]	–	–
51-60 years, OR [95% CI]	0.39 [0.22-0.71]	–	–
61-70 years, OR [95% CI]	0.44 [0.18-1.07]	–	–
Women	Ref.	–	–
Men, OR [95% CI]	0.69 [0.39-1.23]	–	–
Infection-naïve	Ref.	–	–
Previously infected, OR [95% CI]	2.05 [1.1-3.8]	–	–

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2;

HCWs: health care workers;

Ref.: reference category;

OR: odds ratio

CI: confidence interval

89%). In the infection-naïve HCWs group, the median age was 40 years (range: 22-70 years), and a majority were females (406/474, 86%). In both groups, SARS-CoV-2 IgG assay was performed after a median of 28 days (range 25-32 days) from the second dose of BNT162b2 vaccine. A serological test result  $\geq 33.8$  BAU/ml was considered positive, according to the datasheet of the test (13). All HCWs tested were seropositive. The upper limit of quantification was 2080 BAU/ml. A total of 464/583 (80%) participants had a serological test result above the upper limit of quantification ( $\geq 2080$  BAU/ml).

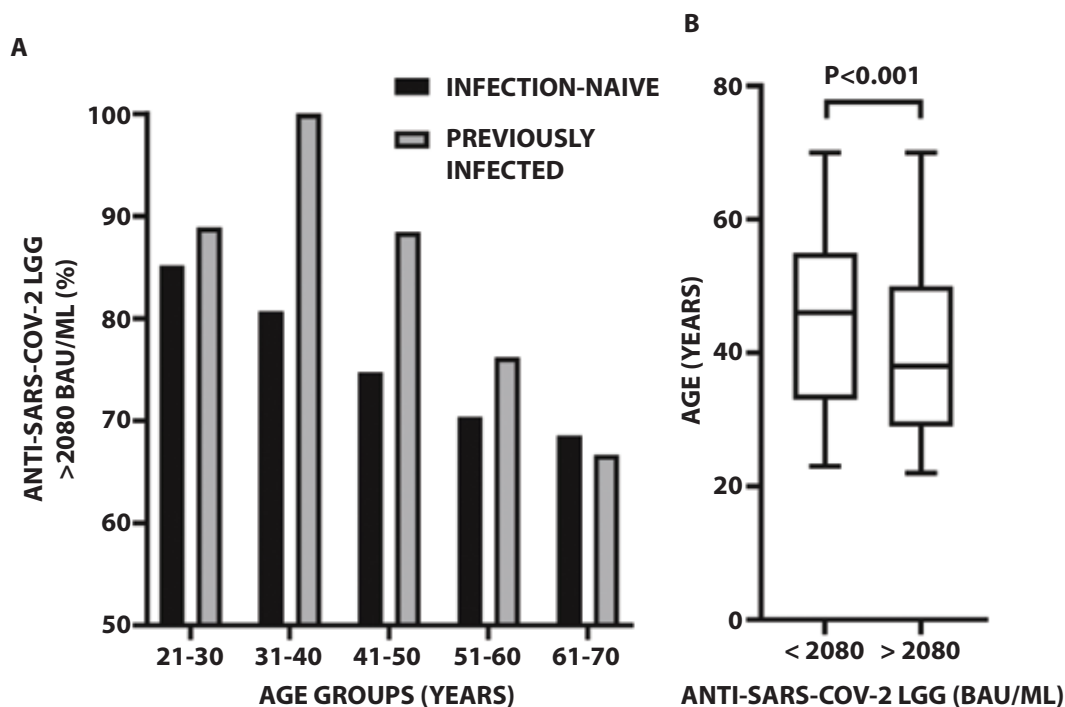
Results on the relationships between the anti-SARS-CoV-2 IgG level and the previously infected/infection-naïve status, age groups, and gender are reported in Table 1. The odds ratio (OR) for anti-SARS-CoV-2 IgG > 2080 BAU / ml between previously infected and infection-naïve HCWs was 2.05 [95% CI 1.1-3.8]. The OR by age groups was 0.81 [95% CI 0.43-1.54), 0.49 [95% CI 0.27-0.89), 0.39 [95% CI 0.22-0.71), 0.44 [95% CI 0.18-1.07), for the age groups of 31-40, 41-50, 51-60 and 61-70 years, respectively. The relationship between the anti-SARS-CoV-2 IgG level and age is also shown in

Figure 1A and Figure 1B. In details, Figure 1A reports the results by age groups and previously infected/infection-naïve status, while Figure 1B reports the relationship between IgG level and age, as a continuous variable. Finally, no association between gender and IgG level was found, OR 0.69 [95% CI 0.39-1.23] (Table 1).

## Discussion

Our study showed that all HCWs tested seropositive for anti-SARS-CoV-2 IgG antibodies after vaccination with BNT162b2. The median antibody level was approximately 100 times higher than the test-positivity cut-off. We found that the IgG level after two doses of BNT162b2 was higher in the group of HCWs previously infected with SARS-CoV-2 and there was a progressive decrease in OR for anti-SARS-CoV-2 IgG > 2080 BAU / ml with increasing age (Table 1). Older age groups had lower percentage of HCWs with anti-SARS-CoV-2 IgG >

2080 BAU / mL than younger groups (Figure 1A). Furthermore, the same figure shows that the percentage of individuals with IgG > 2080 BAU / ml was higher in the subgroups with previous infection. Figure 1B shows that individuals with anti-SARS-CoV-2 IgG > 2080 BAU / mL were younger than HCWs with lower IgG levels. Our study is consistent with what has already been reported in the literature in others preprint studies (2,14). Some authors have already described that the immune response to vaccination is less effective in extremely elderly individuals (>80 years old) than in those younger than 60 years (15). This effect is likely due to senescence of the immune system caused by aging (16–18). For this reason, in Italy, a large and multi-centre ongoing study called GeroVAX, coordinated by the National Institute of Health, is evaluating the efficacy, safety, and duration of anti-COVID-19 vaccinations in long-term care facilities, among extremely elderly individuals in whom the immune response could be altered or less effective. No statistically significant difference was observed between males and females, as expected.



**Figure 1.** (A) Percentage of HCWs with anti-SARS-CoV-2 IgG > 2080 BAU/ml by age groups and previously infected/infection-naïve status; (B) Anti-SARS-CoV-2 IgG levels by age (continuous).

The limitations of this study include the possible selection bias and lack of clinical outcomes. Another possible bias may exist due to missing antibody serological data before vaccination. Thus, infection-naïve individuals could have COVID-19 seropositivity due to undiagnosed SARS-CoV-2 infection (e.g. asymptomatic infections). However, nasal swab tests with RT-PCR were routinely performed every two weeks as a screening in HCWs. Hence, the probability of undiagnosed SAR-CoV-2 should be very low.

In conclusion, our study showed an excellent antibody response to vaccination with BNT162b2 after two doses. A significant difference was observed between anti-SARS-CoV-2 IgG level with age and previous SARS-CoV-2 infection status. However, other studies are needed to monitor the evolution of IgG level overtime in immunized subjects, to assess if a booster dose of COVID-19 vaccine should be recommended in some groups of people.

**Conflict of Interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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