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A cohort analysis of SARS-CoV-2 anti-spike protein receptor binding domain (RBD) IgG levels and neutralizing antibodies in fully vaccinated healthcare workers

Abstract

Objectives: The waning of humoral immunity after COVID-19 vaccine booster (third dose) has not yet been fully evaluated. This study updates data on anti-SARS-CoV-2 spike protein receptor binding domain (S-RBD) binding antibodies (bAb) and neutralizing antibodies (NAb) levels in individuals with homologous vaccination 3–4 months after receiving the booster dose.

Methods: Fifty-five healthcare workers (HCW) from Padova University-Hospital were asked to collect serum samples for determining antibodies (Ab) at 12 (t_{12}) and 28 (t_{28}) days, at 6 months (t_{6m}) after their first Comirnaty/BNT162b2 inoculation, and 3–4 months after receiving the 3rd homologous booster dose. HCW were monitored weekly for SARS-CoV-2 infection. Ab titers were measured by two chemiluminescent immunoassays, one targeting the S-RBD immunoglobulin G (IgG), and one surrogate viral neutralization test (sVNT), measuring NAb.

Results: Twenty of the HCW had natural COVID-19 infection (COVID+) at different times, before either the first or the second vaccination. Median S-RBD IgG and NAb levels and their interquartile ranges 3–4 months after the 3rd dose were 1,076 (529–3,409) kBAU/L and 15.8 (11.3–38.3) mg/L, respectively, for COVID–, and 1,373 (700–1,373) kBAU/L and 21 (12.8–53.9) mg/L, respectively, for COVID+. At multivariate regression analyses, with age and gender included as covariates, S-RBD IgG bAb and sVNT NAb levels were closely associated with the time interval between serological determination and the 3rd vaccine dose ($\log_{10} \beta_{coeff}=-0.013$, p=0.012 and $\log_{10} \beta_{coeff}=-0.010$, p=0.025) for COVID+, whereas no such association was found in COVID– individuals.

Conclusions: The third booster dose increases anti-SARS-CoV-2 Ab levels, elevated levels persisting for up to 3–4 months. Waning of Ab levels appears to be less pronounced for COVID+ individuals.

Keywords: 3 months after second dose; antibodies kinetic; antibody; BNT162b2; neutralizing antibodies; SARS-CoV-2; SARS-CoV-2 mRNA vaccine; serology; vaccine third dose.

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Introduction

In Europe, the dynamic of the fourth SARS-CoV-2 pandemic wave is showing an upswing in cases in the late tail, fueling concerns about a new global surge. The rise in cases has been attributed to several factors, including the reduced virus prevention measures, the waning of vaccine induced immunity (humoral and cellular-response) to SARS-CoV-2 and new mutated variants (e.g. Omicron). Although it has been clearly established that COVID-19 vaccines provide a significant degree of protection against severe and/or any type of SARS-CoV-2 infection, the duration of protective immunity remains controversial [1]. The waning of

antibodies (Ab) titers contributes to the time-dependent reduction of immune protection, confirmatory analyses being obtained by measuring neutralizing antibodies (NAb), demonstrating that a remarkable decrease in Ab serum concentration closely correlates with reduced protection [2–5]. Studies by our team and other authors have reported a significant antibody decrease 6 months post-vaccination in individuals who completed the two-dose regimen of mRNA vaccines (such as BNT162b2) [6–10]. Further findings suggest that the dynamics of decrease in Ab elicited by vaccines might not be homogenous across individuals [2, 11]. A measure to overcome waning immunity, the administration of an additional vaccine dose, the so-called booster dose [1], first undertaken in Israel, with the vaccination program at the end of July 2021 for people aged >60 years, was followed later by other countries [12]. In Italy, administration of the third dose was started in September 2021. Current studies have demonstrated that the third booster dose can restore protective Ab, although its reduction kinetic is not well understood [13, 14]. Despite the somewhat limited evidence, the scientific debate on the utility of a fourth dose, not only for immunocompromised patients [15], is now underway.

Aim of this study was to provide an update of SARS-CoV-2 spike protein receptor binding domain (S-RBD) IgG binding antibody (bAb) and NAb levels in a series of healthcare workers (HCW) who participated in a primary homologous vaccination cycle with Comirnaty (BNT162b2). The results were then compared with those previously obtained in the same individuals after the second dose.

Materials and methods

The study cohort comprised 55 Padova University Hospital HCW who underwent a primary cycle of vaccination (first dose, followed by a second, after 21 days) with Comirnaty (BNT162b2 mRNA, BioNTech-Pfizer, Mainz, Germany/New York, USA) between December 26, 2020 and March 10, 2021, and a third dose between November 2021 and December 2021. HCW were consecutively enrolled from the Emergency Department, and the Infectious Disease and the Laboratory Medicine wards of the University-Hospital of Padova. All subjects underwent weekly nasopharyngeal swab testing from March 2020 to March 2022, while their immunological status for SARS-CoV-2 was determined weekly between April 8th and May 29th, 2020, as described elsewhere [16]. A total of 20/55 (36.4%) HCW had a previous diagnosis of COVID-19 natural infection (COVID+), made on the basis of the positivity to nasopharyngeal swab test. COVID+ individuals had contracted the disease at different time points, either before the first or second vaccine dose. Overall, the numbers and percentages of subjects within the age classes <30 years, 30/40 years, 40/50 years, 50/60 years, and >60 years were: 11 (20.0%), 21 (38.2%), 14 (25.5%), 4 (7.2%) and 5 (9.1%), respectively.

Binding IgG antibodies (bAb) against the RBD portion of the SARS-CoV-2 spike protein were measured by chemiluminescent immunoassays (CLIA), the SARS-CoV-2 S-RBD IgG on Maglumi 2000 plus (Snibe Diagnostics, Shenzhen, China), validated elsewhere [17]. Furthermore, NAb were measured by a CLIA surrogate virus neutralization test (sVNT), designed to mimic the virus–host interaction, by direct RBD protein–hACE2 protein binding, the SARS-CoV-2 NAb on Maglumi 2000 plus (Snibe Diagnostics, Shenzhen, China). This sVNT was demonstrated to closely correlate with the plaque reduction neutralization test (PRNT) in a previous study [6]. Due to the limited reagent availability, not all samples were assessed at each time point. The numbers of samples evaluated were: 25/55 (45.4%) at t_0, 49/55 (89.1%) at t_{12}, 48/55 (87.3%) at t_{28}, 54/55 (98.2%) at t_{6m}, and 54/55 (98.2%) for the time point of 3 months after the third dose. GraphPad Prism version 9.1 for Windows (GraphPad Software, LLC) was employed for descriptive statistics, and for non-parametric tests (Kruskal–Wallis test and Spearman's correlation analysis). Stata 16.1 (Statacorp, Lakeway Drive, TX, USA) was employed for Fisher's exact test, Spearman's correlations, and multivariate regression analyses. All subjects gave their fully informed written consent to participate in the study, which was conducted in accordance with the Declaration of Helsinki, and the Institutional Review Board of the University of Padova (protocol no. 7862).

Results

Among the HCW included in this study, 33 (60.0%) were females, and 22 (40.0%), males. The overall mean value for age, which did not significantly differ by gender (χ^2=0.274, p=0.607), was 39.7 (range, 25–67) years with a standard deviation (SD) of ±11.3 years. Gender was not associated with SARS-CoV-2 previous natural infection (Fisher's exact test, p=0.568), the females and males who had COVID-19 infection being 11 (55.0%) and 9 (45.0%), respectively. The individuals were included in this study within a timeframe of 3–4 months after the third dose, and the mean time interval between serological determination and the vaccine boost was 99.5 days (SD, ±23.6 days). The time interval between serological determination and the third vaccine dose did not differ significantly by gender (χ^2=0.343, p=0.558); nor was it correlated with age (Spearman's r=−0.183, p=0.184).

Figure 1 shows that neither S-RBD IgG (χ^2=0.443, p=0.506) nor NAb (χ^2=0.135, p=0.713) differed between males and females (panels E and F); non-significant associations were also found on examining the relationship between age and S-RBD IgG (Spearman's r=0.125, p=0.364) or NAb and age (Spearman's r=0.125, p=0.366) (panels C and D, respectively). Figure 1 also reports the S-RBD IgG (panel A) and NAb (panel B) levels measured: (1) before the first dose, and (2) 12 days, (3) 28 days, and (4) 6 months after the second dose, and (5) 3–4 months after the third dose. Median S-RBD IgG and NAb levels, and interquartile ranges (IQR) are shown in Table 1. The scatterplot of the correlation between S-RBD IgG and NAb level is reported in Figure 1 (panel G).
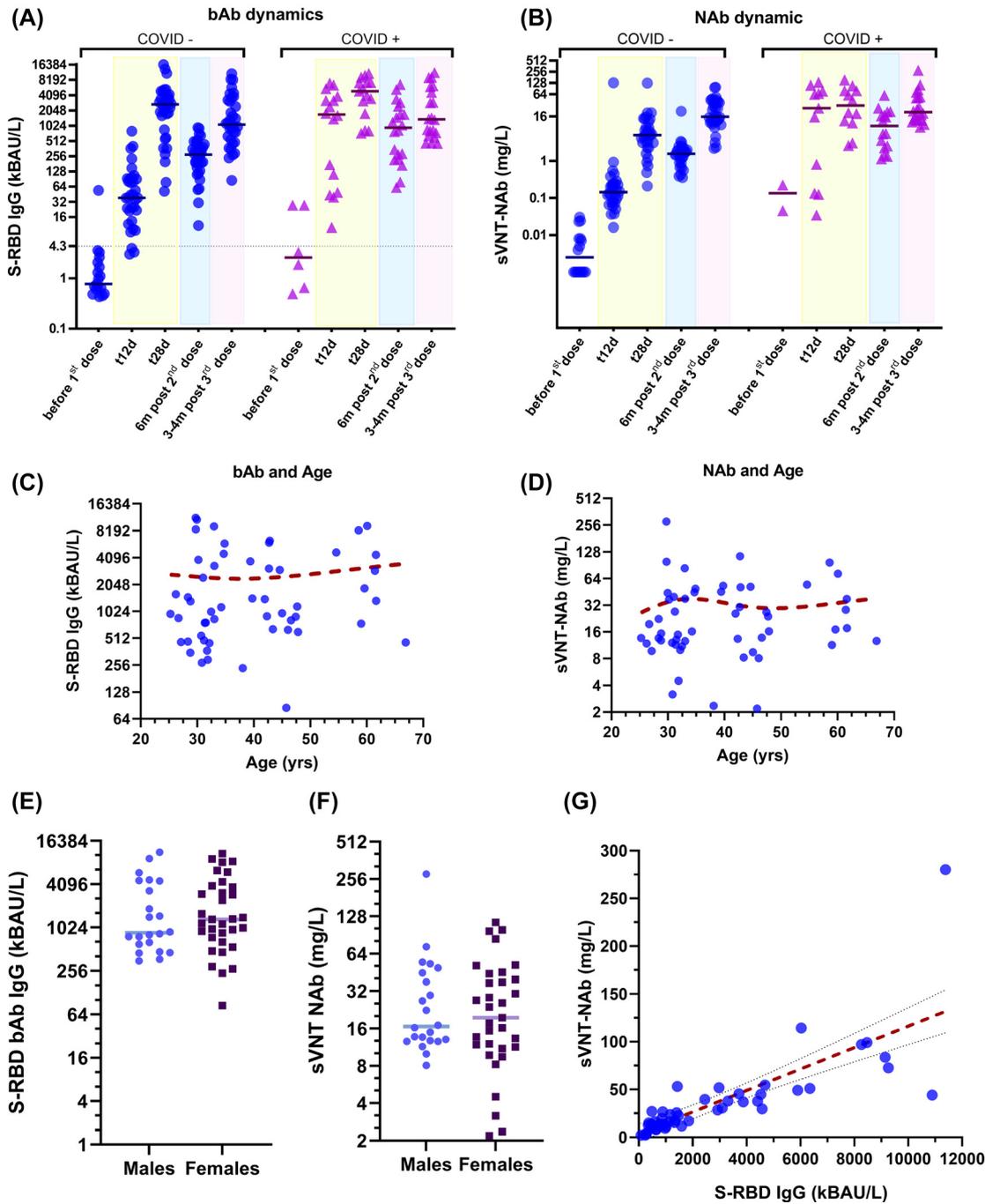
Figure 1: Levels of anti-SARS-CoV-2 spike protein receptor binding domain (S-RBD) binding antibodies (bAb) and neutralizing antibodies (NAb), measured by surrogate virus neutralization test.
(A) S-RBD IgG levels and (B) NAb levels at different time points: before the first dose, and 12 days, 28 days, and 6 months after the second dose, and 3–4 months after the third dose. Scatterplots of S-RBD IgG levels (C) and NAb levels (D) measured at 3–4 months after 3rd dose and age, with cubic splines superimposed. (E) S-RBD IgG subdivided by gender, with line indicating the median value (F) NAb subdivided by gender, with line indicating the median value (G) correlation between NAb and S-RBD levels.

Figure 2 shows the S-RBD IgG bAb levels and sVNT NAb levels with respect to the time interval between serological determination and the third vaccine dose (days). At multivariate regression analyses, with age and gender included as covariates, S-RBD IgG bAb and sVNT NAb levels were closely related to the time interval between

Table 1: Median and interquartile range statistics for S-RBD IgG binding antibodies (bAb) and for NAb levels measured at each time point, subdivided by individuals without (COVID−) and with (COVID+) previous natural SARS-CoV-2 infection.

Subjects	Before 1st dose	t_{12d}	t_{28d}	6$_m$ post 2nd dose	3–4$_m$ post 3rd dose
			COVID−		
S-RBD IgG bAb, kBAU/L	0.8 (0.5–2.1)	38.5 (17.3–91.4)	2719.0 (723.3–4154.0)	274.4 (148.2–442.9)	1076.0 (529.3–3409.0)
NAb, mg/L	0.01 (0.01–0.02)	0.14 (0.07–0.24)	5.01 (2.29–11.48)	1.56 (0.87–1.97)	15.76 (11.34–38.33)
			COVID+		
S-RBD IgG bAb, kBAU/L	2.6 (0.6–27.45)	1713.0 (64.9–3743.0)	4932.0 (1786.0–7261.0)	941.8 (237.8–2294.0)	1373.0 (669.9–5692.0)
NAb, mg/L	0.13 (0.04–0.22)	27.03 (0.13–77.63)	31.96 (11.05–79.76)	8.87 (2.33–20.40)	21.00 (12.85–53.95)

NAb, neutralizing antibodies; S-RBD IgG bAb, spike protein receptor binding domain IgG binding antibodies.
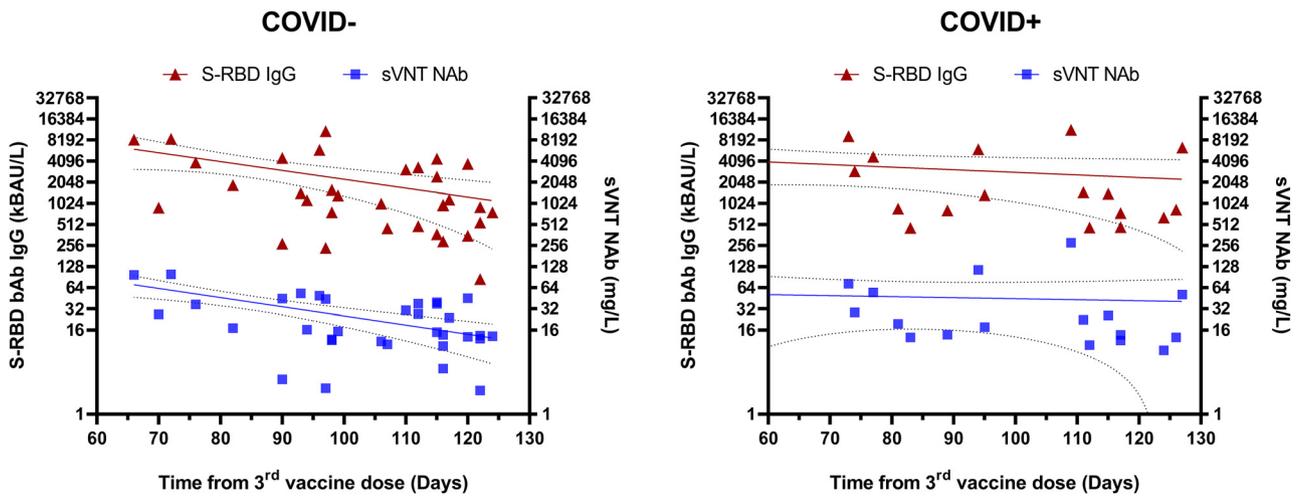
Figure 2: Scatterplots showing the correlations between anti-SARS-CoV-2 spike protein receptor binding domain binding antibodies (S-RBD IgG bAb), neutralizing antibodies (sVNT Nab), and the time from the 3rd vaccine dose (in days) in subjects without previous natural infection (COVID−, left panel) and with previous natural infection (COVID+, right panel).

serological determination and the third vaccine dose ($\log_{10}$ β_{coeff}=−0.013, p=0.012 and $\log_{10}$ β_{coeff}=−0.010, p=0.025) in COVID+, whereas no such association was found in COVID− individuals.

On comparing S-RBD IgG 6 months after the second dose with that 3–4 months after the third dose, the median increase in bAb levels was 368%, with an IQR of 222.7–1062.7% for COVID− individuals, and 51.8%, IQR from −30.9 to 293.6% in COVID+ subjects, this difference being highly significant (χ^2=7.31, p=0.007). The same comparison of sVNT NAb resulted in a median increase between these two periods of 1,369% with IQR of 656–2,186% for COVID− individuals, being 203% with an IQR of 17.1–733% for COVID+ subjects. The median increase in S-RBD IgG and sVNT NAb was neither associated with age (Spearmans' r=−0.033, p=0.829) nor with gender (Spearmans' r=0.182, p=0.235).

Discussion

The present study was conducted on a series of HCW who participated in a primary vaccination cycle and received a further third homologous booster dose of BNT162b2. Anti-SARS-CoV-2 S-RBD IgG (bAb) and NAb (sVNT) were measured by chemiluminescent assay 3–4 months after the 3rd dose. The levels of Anti-SARS-CoV-2 S-RBD IgG (bAb) and NAb in these individuals were compared at different time points (i.e., 12 and 28 days after the first dose, 6 months after the second, and 3–4 months after the 3rd).

The findings made in this study reveal that the third vaccine dose was effective in boosting both bAb and NAb in patients with or without previous natural SARS-CoV-2 infection. No gender related differences were found in bAb and NAb levels; nor were Ab levels correlated with age.

Considering the subgroup of COVID– subjects, the boost dose after 3–4 months is highly effective in increasing Ab, with respect to the levels reached 6 months after the second dose (Figure 1). These results were consistent with findings made by authors who used other analytical methods 1 month after the 3rd dose. For example, in a seronegative individual, Salvagno et al. [18] found a 39-fold increase in the antibody levels after 3rd dose with respect to that measured before receiving the vaccine booster. Similar results were also found 21–28 days after the third dose by Romero-Ibarguengoitia et al., who reported a similar quantitative 10-times increase in Ab in individuals with, and those without, a history of SARS-CoV-2 infection [14].

In our study, the levels of NAb 3–4 months after the 3rd dose were similar (in COVID+) or increased (in COVID–) with respect to the Ab levels determined after the primary vaccination cycle (i.e., 28 days after the 2nd dose). On the contrary, bAb levels after the 3rd dose were lower than those measured after the primary vaccination cycle, in both subgroups of individuals. These findings are in partial disagreement with results reported by Salvagno et al., and Romero-Ibarguengoitia et al., who found a large increase in Ab titers 1 month after the booster dose with respect to 1 month after the primary cycle [13, 14, 18]. This discrepancy might be explained in part by the different time points used for measuring Ab after the primary cycle (28 days vs. up to 1 month after the primary cycle) and after the booster dose (3–4 vs. 1 month).

The persistently elevated titers obtained after 3–4 months from the 3rd dose, with respect to 6 months after the second dose, both for COVID– (median levels 1,369 vs. 368%, respectively), and for COVID+ (median levels 203 vs. 51.8%, respectively) individuals, support the evidence of vaccine efficacy, measurable 4 months after the administration of the 3rd dose of mRNA vaccination, as reported by Ferdinands et al., on studying a cohort of 105,193 vaccinated individuals [19].

Another relevant finding made in the present study concerns the waning of NAb and bAb, studied by multivariate analyses including the time from 3rd vaccine dose, age and gender, shown in Figure 2. The results obtained highlight the fact that the waning of Ab levels after 3rd dose can be more consistent in COVID– than in COVID+ individuals, a significant decrease being measurable after 3–4 months only in individuals without previous infection. This finding could explain why in COVID+ individuals the high Ab levels persisted after the 2nd and 3rd doses.

Our study has limitations. One is the lack of a gold standard for measuring NAb: the method we used has been shown elsewhere to highly correlate with the PRNT [6], despite this evaluation was performed against wild-type virus only, and no other variants of concern (VOCs). Other potential limitations are the limited number of individuals, the unavailability of results on the cellular-mediated immune response, and the reduced specificity of these assays for new SARS-CoV-2 variants [20]. On the other hand, the strength of this study lies in its well-characterized cohort of individuals, who were followed-up for SARS-CoV-2 infection by weekly nasopharyngeal swab testing from March 2020 to March 2022.

In conclusion, the third vaccine dose has proven to increase anti-SARS-CoV-2 serum Ab, with levels that were persistently high for up to 3–4 months after the inoculum. Despite this high response in all individuals, waning of Ab levels might be less pronounced for individuals with previous natural infection to COVID-19. Overall, our results support the utility of the 3rd booster dose in reinforcing humoral immunity, especially in individuals at a higher risk of developing severe disease. Further research is required to characterize time-dependent antibody dynamics after the 3rd dose, and to provide a prolonged persistence of both humoral and cellular-mediated immunity with a view to finally controlling the SARS-CoV-2 pandemic.

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