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Letter to the Editor

Antibody response in individuals infected with SARS-CoV-2 early after the first dose of the BNT162b2 mRNA vaccine

Dear editor,

We read with interest the study recently published by Tré-Hardy et al. which analyzed the antibody response to the mRNA-1273 vaccine in health care workers (HCWs) according to their serological status before vaccination.[1] In agreement with previous reports and with our study on HCWs vaccinated with the BNT162b2 mRNA vaccine,[2–4] they showed that a single vaccine dose acts as booster in individuals with previous SARS-CoV-2 infection and rapidly induces high antibody titres, even higher than those achieved after two doses in naïve individuals.[1] Follow-up evaluation at 3 months showed a drop of antibody levels in some vaccinees who were seronegative at baseline, but not in those who were seropositive.[1] These findings support the recommendation of a single vaccine dose for individuals with prior SARS-CoV-2 infection, while suggesting the need of an additional dose in poor responders.

Conceivably, SARS-CoV-2 infection following the first vaccine dose might also act as a booster. However, information about the levels of protective antibodies in these individuals are lacking and there are no indications about the appropriateness of a second dose of vaccine in individuals who were infected with SARS-CoV-2 after having received the first dose. Here, we investigated the dynamics of antibody response to SARS-CoV-2 in HCWs who were infected within 14 days after the first dose of BNT162b2 mRNA vaccine in comparison with the response to vaccination in naïve HCWs and in those with prior infection.

In our prospective cohort study, which included 1958 HCWs vaccinated with the BNT162b2 mRNA vaccine between January 1 and March 30, 2021, 22 HCWs were infected with SARS-CoV-2 $\leq$ 14 days after the first vaccine dose and had the second dose postponed >2 months. The anti-SARS-CoV-2 antibody response in this group of HCWs (group A: concomitant infection) was compared with that observed in other groups: i.e., HCWs who got infected from March 2020 to November 2020 and were vaccinated in January 2021 (group B: prior infection, $\geq$ 2 months, $n = 55$); HCWs who got infected in December 2020 and had vaccination postponed > 1 month (group C: prior infection, < 2 months, $n = 26$), and naïve HCWs, who were regularly vaccinated in January 2021 (group D: naïve, $n = 55$). Group A received the second vaccine dose a median of 75 days after dose 1; groups B, C, and D received the second dose 21 days after the first dose (Table 1).

Median age was similar among groups; group C included a higher percentage of males; group A reported less frequently adverse events to vaccination than the other groups (Table 1). All HCWs in groups A, B and C had asymptomatic infection or mild symptoms, with the exception of one in group C who required hospitalization. In group A, SARS-CoV-2 infection was diagnosed a median of 8 days after the first vaccine dose (Table 1).

All study subjects were tested for anti-SARS-CoV-2 spike receptor-binding domain (RBD) IgG antibodies and neutralizing antibodies, as previously reported.[6] Testing was performed upon administration of the first (T0) and the second (T1) vaccine doses, and 2 to 3 weeks after the second dose (T2). For group A, T1 was set on day 38 after the first vaccine dose.

In group A, geometric mean titre (GMT) of RBD-binding IgG antibodies, measured after recovery and at median 38 days (IQR 37–38) after the first vaccine dose, was about 15-fold and 6-fold lower than that observed 21 days after the first dose in groups B and C ($p < 0.0001$). Conversely, it was 3-fold higher than the peak antibody titer measured after natural infection, i.e., at T0 in group C HCWs in whom antibodies were measured 46 days (IQR 42–48) after diagnosis ($p < 0.001$), and 2-fold higher than in naïve group D HCWs 21 days after the first vaccine dose ($p < 0.01$) (Table 1 and Fig. 1A). Following two vaccine doses, GMT of RBD-binding IgG in group A was similar to GMT in naïve HCWs after two vaccine doses, but significantly lower than in fully vaccinated group B and C HCWs with prior SARS-CoV-2 infection (Table 1 and Fig. 1A). Accordingly, in group A, neutralizing antibody GMT after the first vaccine dose was similar to that observed after natural infection, significantly higher than in naïve HCWs after the first vaccine dose, but lower than the neutralizing antibody titer observed in HCWs with prior infection who received 1 vaccine dose and in fully vaccinated HCWs (Fig. 1B). In addition, after the first vaccine dose, neutralizing antibodies were detected in all group A and B HCWs and in 85% of naïve HCWs (Table 1). A second vaccine dose induced significantly higher neutralizing antibody titers in group A than in naïve HCWs, but significantly lower than in HCWs with prior infection (Fig. 1B).

In conclusion, this study demonstrated that the titers of SARS-CoV-2 RBD-binding IgG and neutralizing antibodies induced by vaccination with BNT162b2 were significantly higher in HCWs infected with SARS-CoV-2 $\leq$ 14 days after the first vaccine dose than in naïve subjects, but significantly lower than in HCWs infected before vaccination. In addition, the relatively high levels of RBD-binding IgG and neutralizing antibodies in HCWs infected after vaccination were similar to those achieved after natural infection. This level of immunity probably confers protection against symptomatic SARS-CoV-2 infection and disease, according with data from the literature which showed that the levels of neutralizing antibodies detected in convalescent serum prevent severe infection.[5] However, as the minimum level of antibodies associated with protection has not been defined,[6] a cautionary approach is

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Table 1
Baseline characteristics and response to the BTN162b2 mRNA vaccine in health care workers with (groups A–C) or without (group D) SARS-CoV-2 infection.

	Group A Infection 1–14 days after 1 vaccine dose ($n = 22$)	Group B Infection ≥ 2 months before vaccination ($n = 55$)	Group C Infection < 2 months before vaccination ($n = 26$)	Group D Naïve($n = 55$)
Baseline characteristics				
Males, n. (%)	4 (12)	8 (15)	12 (46)	10 (18)
Females, n. (%)	18 (82)	47 (85)	14 (54)	45 (82)
Age at vaccination, median years (IQR)	42 (28–53)	46 (31–53)	43 (31–50)	47 (34–53)
SARS-CoV-2 infection				
Asymptomatic, n. (%)	3 (14)	6 (11)	6 (23)	NA
Mild symptoms, n. (%)	19 (86)	46 (84)	19 (73)	NA
Hospitalization, n. (%)	0 (0)	3 (5)	1 (4)	NA
BTN162b2 vaccination				
Days between infection and dose 1, median (IQR)	- 8 (4–11)	273 (68–291)	46 (42–48)	NA
Days between doses 1 and 2, median (IQR)	75 (72–76)	21 (21–21)	21 (21–21)	21 (21–21)
AE after dose 1, no. (%)	14 (64)	53 (96)	21 (81)	48 (87)
AE after dose 2, no. (%)	16 (73)	50 (91)	24 (92)	50 (91)
Anti-S RBD IgG				
Total positive, T0 (%)	0	52 (95)	21 (81)	0 (0)
Total positive, T1 (%)	22 (100)	55 (100)	26 (100)	54 (98)
Total positive, T2 (%)	22 (100)	55 (100)	26 (100)	55 (100)
Anti-S RBD IgG titre				
T0, GMT (95% CI)	4 (1–11)	371 (250–553)	521 (298–909)	0.8 (0.5–1.0)
T1, GMT (95% CI)	1553 (1151–2097)	23,974 (19,531–29,428)	9687 (5568–16,853)	690 (517–921)
T2, GMT (95% CI)	8997 (5864–13,802)	32,056 (28,088–36,583)	24,476 (18,644–32,131)	14,492 (11,919–17,621)
NT antibodies				
Total positive, T0 (%)	0 (0)	53 (96)	ND	0 (0)
Total positive, T1 (%)	22 (100)	55 (100)	ND	47 (85)
Total positive, T2 (%)	22 (100)	55 (100)	ND	55 (100)
NT antibody titre				
T0, GMT (95% CI)	1 (1–1)	102 (65–160)	ND	1 (1–1)
T1, GMT (95% CI)	96 (64–145)	1769 (1482–2111)	ND	18 (12–27)
T2, GMT (95% CI)	682 (455–1023)	2832 (2369–3384)	ND	382 (318–458)

NA: not applicable; ND: not done; AE: one or more adverse events following vaccine doses; NT antibodies: neutralizing antibodies; T0: day of first vaccine dose; T1: day of second vaccine dose (day 21 after first vaccine dose) in group B, C, D and day 38 after first vaccine dose in group A; T2: 2,3 weeks after second vaccine dose; GMT: geometric mean titre; 95% CI: 95% confidence interval.

preferable. Thus, while recommending a single dose for individuals who were infected months before vaccination, the same approach might not be appropriate for those who are diagnosed with the infection soon after the first dose of vaccine, especially in the context of the emergence and spread of variants of concern which escape antibody neutralization.[7] In our study, the strategy to postpone the second dose of two months in this group of HCWs allowed to rapidly achieve an optimal antibody response. This is crucial for elderly and immunosuppressed individuals (not included in our study population), since they mount significantly lower antibody responses than younger and healthy adults and are at risk of breakthrough infections. [8]

Declaration of Competing Interest

The authors have no relevant competing interest to disclose in relation to this work.

CRediT authorship contribution statement

Federico Gobbi: Funding acquisition, Visualization, Investigation, Supervision, Writing – original draft, Writing – review & editing. **Dora Buonfrate:** Visualization, Investigation, Supervision, Writing – original draft, Writing – review & editing. **Ronaldo Silva:** Formal analysis, Data curation, Visualization, Writing – review & editing. **Davide Martini:** Investigation, Writing – review & editing. **Zeno Bisoffi:** Funding acquisition, Investigation, Writing – review & editing. **Chiara Piubelli:** Investigation, Writing – review & editing. **Silvia Riccetti:** Investigation, Writing – review & editing. **Alessandro Sinigaglia:** Investigation, Writing – review & editing. **Luisa Barzon:** Visualization, Investigation, Funding acquisition, Formal analysis, Data curation, Visualization, Supervision, Writing – original draft, Writing – review & editing.

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Ethical approval

The study protocol received ethical clearance by the local Ethics Committee (Comitato Etico per la Sperimentazione Clinica delle Province di Verona e Rovigo) on January 13th, 2021 (study protocol n. 17985). All participants gave their written informed consent to participate in this study.

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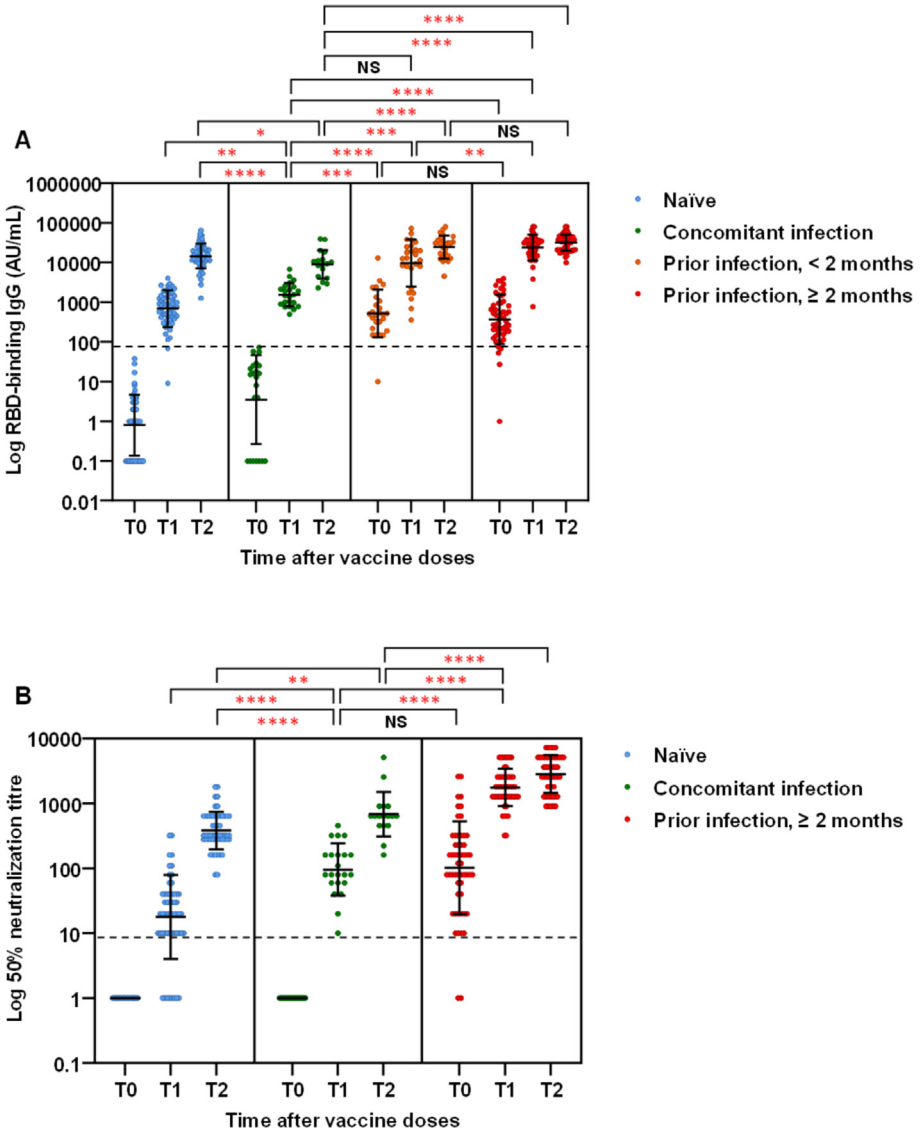

Fig. 1. shows serum anti-SARS-CoV-2 RBD IgG and neutralizing antibody titres in the different study groups.
Group A was tested at the time of the first dose of BNT162b2 mRNA vaccine (T0), at about 38 days after the first vaccine dose (T1) and 2,3 weeks after the second vaccine dose (T2); groups B, C and D were tested on the days of the first (T0) and second (T1, i.e. at 21 days after the first dose) vaccine doses and 2,3 weeks after the second dose (T2). **(A)** Anti-SARS-CoV-2 RBD IgG titers were measured by quantitative CMIA and reported as in arbitrary units (AU)/mL; **(B)** SARS-CoV-2 neutralizing antibody titers were measured by microneutralization assays with live virus and reported as IC50 (50% neutralization titre). The dashed lines indicate the cutoff level of positive antibodies (AU/mL $\geq$ 50) and neutralizing concentrations (IC50 $>$ 10). Each coloured dot represents raw values of one serum sample; solid lines indicate geometric means and standard deviation. * $p < 0.5$, ** $p < 0.01$; *** $p < 0.001$, **** $p < 0.0001$ (Mann-Whitney test). Statistical analysis was done using GrapPad Prism 9.1.2.

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