

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

Running title: SARS-CoV-2 infection early after the first vaccine dose

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Dear Editor,

Results from clinical trials and real-world studies showed the high efficacy of the BNT162b2 COVID-19 vaccine in reducing SARS-CoV-2 infection and associated morbidity and mortality [1,2]. Vaccine efficacy starts from >14 days after the first dose, is highest after completion of two doses [3], and is associated with induction of a robust neutralizing antibody response [4]. We and other authors demonstrated that a single vaccine dose acts as booster in individuals with previous SARS-CoV-2 infection and rapidly induces high levels of neutralizing antibodies, even higher than those achieved after two doses in naïve individuals [5-7]. Thus, a single vaccine dose is recommended for this target population. Conversely, there are no indications on the appropriateness of a second dose of vaccine in individuals who were infected with SARS-CoV-2 early after having received the first dose. Actually, information about the levels of protective antibodies in these individuals are lacking. Here, we investigated the dynamics of antibody response to SARS-CoV-2 in health care workers (HCWs) who were infected within 14 days after the first dose of BNT162b2 mRNA vaccine in comparison with the response to vaccination in naïve individuals and in those with prior infection.

In our prospective cohort study, which included 1,958 HCWs vaccinated with the BNT162b2 mRNA vaccine between January 1 and March 30, 2021, 22 HCWs were infected with SARS-CoV-2 ≤ 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, ≥ 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., 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 Figure 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 Figure 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 (Figure 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 (Figure 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 ≤ 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 [8]. However, as the minimum level of antibodies associated with protection has not been defined [9], a cautionary approach is 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 [10]. 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 [4].

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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 13th January 2021 (study protocol n. 17985). All participants gave their written informed consent to participate in this study.

Authorship

Study concept and design: FG, DB, LB; *investigation:* FG, DB, DM, ZB, CP, SR, AS, LB; *acquisition, analysis, interpretation of data and visualization:* RS, LB; *supervision and manuscript - first draft:* FG, DB, LB; *critical revision of the manuscript:* All.

Declaration of competing interests

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

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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	Group B	Group C	Group D
	Infection 1-14 days after 1 vaccine dose (n=22)	Infection ≥2 months before vaccination (n=55)	Infection <2 months before vaccination (n=26)	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
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 (SD)	4 (1-11)	371 (250-553)	521 (298-909)	0.8 (0.5-1.0)
T1, GMT (SD)	1553 (1151-2097)	23974 (19531-29428)	9687 (5568-16853)	690 (517-921)
T2, GMT (SD)	8997 (5864-13802)	32056 (28088-36583)	24476 (18644-32131)	14492 (11919-17621)
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.

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 vaccine dose (T0), at about 38 days after the first dose of BNT162b2 mRNA vaccine (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 ($\text{AU/mL} \geq 50$) and neutralizing concentrations ($\text{IC}_{50} > 10$). Each coloured dot represents raw values of one serum sample; solid lines indicate geometric means and standard deviation. * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$, **** $p < 0.0001$ (Mann-Whitney test). Statistical analysis was done using GraphPad Prism 9.1.2.