Letter to the Editor

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Monitoring of the immunogenic response to Pfizer BNT162b2 mRNA COVID-19 vaccination in healthcare workers with Snibe SARS-CoV-2 S-RBD IgG chemiluminescent immunoassay

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To the Editor,

We read with interest the article of Padoan and colleagues [1], who thoughtfully assessed the analytical and clinical characteristics of the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) receptor binding domain (RBD) IgG chemiluminescent immunoassay recently commercialized by Snibe diagnostics (New Industries Biomedical Engineering Co., Ltd [Snibe], Shenzhen, China). Notably, the authors of this work concluded that this novel method not only displays good analytical and clinical performance for diagnosing SARS-CoV-2 infection, but also exhibits highly significant correlation with sera neutralizing activity, as assayed with a plaque reduction neutralization (PRNT) assay [1]. Since monitoring anti-SARS-CoV-2 neutralizing antibodies after coronavirus disease 2019 (COVID-19) vaccination is highly advisable for deciphering the individual immunogenic response, and thereby predicting vaccine

efficacy [2, 3], we used this novel chemiluminescent immunoassay for analyzing the serum levels of anti-SARS-CoV-2 neutralizing antibodies in healthcare workers undergoing vaccination with Pfizer BNT162b2.

Our study population consisted of a series of consecutive healthcare workers who voluntarily received a complete cycle (i.e., two 30 µg vaccine doses, exactly 21 days one from the other) of Pfizer BNT162b2 mRNA COVID-19 vaccine (Comirnaty; Pfizer Inc, NY, USA) at the hospital of Peschiera del Garda (Italy). Blood was drawn from all subjects within evacuated blood tubes with clot activator and gel (Greiner Bio-One, Kremsmünster, Austria) at baseline (i.e., before the first vaccine dose; T0), as well as immediately before the second vaccine dose (21 days after the first dose; T1) and 30 days after the second COVID-19 vaccine dose (T2). Serum was obtained by sample centrifugation for 15 min at 1,500×g (room temperature), divided in identical aliquots and stored at –70 °C until measurement in full batch with Snibe S-RBD IgG. The analytical and clinical characteristics of this chemiluminescent immunoassay in patients with COVID-19 were comprehensively reported in the previous study of Padoan et al. [1]. Briefly, the cut-off is 1.0 kU/L, the imprecision was comprised between 4.0 and 12.2%, the cumulative sensitivity and specificity for diagnosing COVID-19 were as high as 99.0 and 92.5%, respectively, whilst the correlation with PRNT₅₀ titer was 0.712. We also assayed total anti-SARS-CoV-2 antibodies serum level with the Roche Elecsys Anti-SARS-CoV-2 S chemiluminescent immunoassay, on a Roche Cobas 6000 (Roche Diagnostics, Basel, Switzerland).

The statistical analysis was conducted using Analyse-it (Analyse-it Software Ltd, Leeds, UK), with results of measurements expressed as median and interquartile range (IQR). Correlations were performed using Spearman's rank correlation coefficient, whilst comparisons between groups were performed using the Mann–Whitney test, with statistical significance set at $p < 0.05$. All study participants

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Figure 1: Snibe IgG anti-S-RBD response in a cohort of healthcare workers undergoing after Pfizer BNT162b2 mRNA vaccination. Serum samples were drawn at baseline (T0), 21 days after the first Pfizer BNT162b2 mRNA vaccine dose (T1) and 30 days after the second vaccine dose (T2) in SARS-CoV-2 baseline seronegative (NEG) and seropositive (POS) subjects. RBD, receptor binding domain.

provided written informed consents for undergoing COVID-19 vaccination and anti-SARS-CoV-2 serological monitoring. The study was conducted in accordance with the Declaration of Helsinki and cleared by the Ethics Committee of Verona and Rovigo Provinces (3246CESC).

The final study population consisted of 194 consecutive healthcare workers, who received the complete vaccine cycle (median age 42 years, IQR 30–52 years; 59.3% females), 30 (15.5%) of whom were considered SARS-CoV-2 positive at baseline for having Snibe IgG anti-S-RBD levels >1.0 kU/L (median age 44 years, IQR 33–52 years; 50.0% females). We used this positivity cut-off indicated by the manufacturer since neither clinical nor molecular data reflecting the likelihood of a previous SARS-CoV-2 infection were available (results could be converted into BAU/mL by multiplying the value for 4.33, as specified by the manufacturer). No significant differences in age ($p=0.261$) or sex ($p=0.109$) were found between the baseline seronegative and seropositive cohorts. The kinetics of Snibe IgG anti-S-RBD levels at the three time points is shown in Figure 1. As predictable, Pfizer BNT162b2 mRNA vaccination was effective to elicit an increase of IgG anti-S-RBD levels in both cohorts of baseline SARS-CoV-2 seronegative and seropositive subjects. More specifically, in baseline SARS-CoV-2 seronegative subjects the median IgG anti-S-RBD levels were 0.35 (IQR, 0.24–0.51) kU/L before vaccination, increasing to 44.15 (IQR, 25.55–80.62) kU/L at T1 and 513.15 (IQR, 266.84–778.43) kU/L at T2, respectively. The corresponding median levels in baseline seropositive subjects were 1.70 (IQR, 1.38–11.06) kU/L before vaccination, increasing to 74.65 (IQR, 27.50–2,000) kU/L at T1 and 718.35 (IQR, 311.20–1,168.16) kU/L at T2, respectively. The median IgG anti-S-RBD levels elicited by COVID-19 vaccination was significantly higher in baseline SARS-CoV-2 seropositive than seronegative subjects at T0, ($p<0.001$), T1 ($p<0.001$) and also T2 ($p=0.001$) time points (Figure 1). Notably, seropositivization occurred in all except one baseline SARS-CoV-2 seronegative subjects at T1 (163/164; 99.4%), and in all subjects at

T2 (164/164; 100%). The full Snibe IgG anti-S-RBD antibodies response at T2 (expressed as T2/T0 ratio) did not significantly correlate with age (Spearman's correlation: $r=-0.14$; 95% CI, -0.29 to 0.01 ; $p=0.076$) or sex (Spearman's correlation: $r=-0.06$; 95% CI, -0.21 to 0.99 ; $p=0.436$). The Spearman's correlation between Snibe IgG anti-S-RBD and Roche total anti-SARS-CoV-2 S serum levels at T1 and T2 is summarized in Table 1, showing coefficients between 0.70 and 0.96.

In a previous study [4], Padoan et al. used this same Snibe IgG anti-S-RBD chemiluminescent immunoassay to monitor the immunological responses after Pfizer BNT162b2 mRNA vaccination in healthcare workers, measuring antibodies levels 12 days after the first vaccine dose and 7 days after the second vaccine dose, thus at different time points compared to our investigation. The values achieved in their report in baseline SARS-CoV-2 seronegative cohort were considerably lower than those obtained in our study, both after the first (6.6 vs. 44 kU/L) and second (382 vs. 513 kU/L) vaccine doses. An opposite trend was noted in the baseline SARS-CoV-2 seropositive cohort, since Padoan et al. found that Snibe IgG anti-S-RBD levels were substantially

Table 1: Spearman's correlation between Snibe IgG anti-S-RBD and Roche total anti-SARS-CoV-2 S antibodies serum levels 21 days after the first Pfizer BNT162b2 mRNA vaccine dose (T1) and 30 days after the second vaccine dose (T2) in the whole cohort as well as in SARS-CoV-2 baseline seronegative (NEG) and seropositive (POS) subjects.

Vaccine recipients	T1	T2
Whole cohort	$r=0.85$ (95%CI, 0.81–0.89; $p<0.001$)	$r=0.78$ (95% CI, 0.72–0.83; $p<0.001$)
Baseline SARS-CoV-2 NEG	$r=0.81$ (95% CI, 0.75–0.86; $p<0.001$)	$r=0.80$ (95% CI, 0.74–0.85; $p<0.001$)
Baseline SARS-CoV-2 POS	$r=0.96$ (95% CI, 0.91–0.98; $p<0.001$)	$r=0.70$ (95% CI, 0.46–0.85; $p<0.001$)

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RBD, receptor binding domain.

higher than those achieved in our SARS-CoV-2 seropositive subjects after both the first (746 vs. 75 kU/L) and second (1713 vs. 718 kU/L) vaccine doses. Importantly, in both these studies, the immunogenic response was greater in baseline SARS-CoV-2 seropositive than seronegative cohort after the first Pfizer BNT162b2 mRNA vaccine dose. However, after the second dose, contrary to the data presented by Padoan et al. [4], we found that such difference persisted ($p=0.001$). These important discrepancies suggest that different populations, even within a homogenous geographical area (i.e., the region of Veneto, in Northern-East Italy) may display heterogeneous immunogenic response for multiple biological, demographic or clinical reasons, thus reemphasizing the importance of serial and individualized anti-SARS-CoV-2 antibodies monitoring [2, 3]. As in Padoan's study [4], Snibe IgG anti-S-RBD response did not correlate with age or sex of recipients, thus suggesting that these demographic variables may not be significant determinants or predictors of anti-S-RBD response in cohorts consisting mostly of relatively young healthcare workers (42 ± 12 years in Padoan's study vs. 42 ± 13 years in our serological survey, respectively).

In conclusion, the results of this study underpin the importance of serological surveys in assessing the immunogenic response after vaccination and pave the way to considering personalization of COVID-19 vaccine administration.

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Ethical approval: The study protocol (number 3246CESC) was cleared by the Ethics Committee of the Provinces of Verona and Rovigo. All subjects were informed of the study and voluntarily agreed to participate, providing a written consent.

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