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## Research Article

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# **Effect of BNT162b2 booster dose on anti-SARS-CoV-2 spike trimeric IgG antibodies in seronegative individuals**

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**Short title:** BNT162b2 booster and anti-SARS-CoV-2 spike trimeric IgG

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**Background:** We provide here an updated analysis of an ongoing serosurveillance study, presenting data on the effect of a third dose of Pfizer/BioNTech BNT162b2 vaccine on serum anti-SARS-CoV-2 IgG antibodies.

**Methods:** We tested baseline SARS-CoV-2 seronegative healthcare workers undergoing primary vaccination with the mRNA-based COVID-19 Comirnaty vaccine, followed by administration of homologous vaccine booster (third dose). Venous blood was collected before either dose of primary vaccination, at 1, 3 and 6 months afterwards, as well as before and 1 month after receiving the booster. The serum concentration of anti-SARS-CoV-2 spike trimeric IgG was assayed with DiaSorin Trimeric spike IgG immunoassay.

**Results:** The final study population included 53 SARS-CoV-2 seronegative healthcare workers (median age 46 years; 60% females). A first peak of anti-SARS-CoV-2 spike trimeric IgG values was reached 1 month after completing primary vaccination, after which the levels gradually declined until before receiving the vaccine booster. A second peak of anti-SARS-CoV-2 spike trimeric IgG concentration was observed 1 month after receiving the vaccine booster dose (8700 kBAU/L), which was 39-fold higher than before receiving the vaccine booster (221 kBAU/L;  $p < 0.001$ ), but was also nearly 3-fold higher compared to values seen at the first peak (2990 kBAU/L;  $p < 0.001$ ). The rate of subjects with protective anti-SARS-CoV-2 spike trimeric IgG values (i.e.,  $> 264$  kBAU/L) increased from 47.2% to 100% after 1 month from vaccine booster.

**Conclusion:** These results support current policies fostering COVID-19 vaccine boosters to reinforce humoral immunity against SARS-CoV-2.

**Keywords:** COVID-19; SARS-CoV-2; Vaccination; Antibodies; Immune response

## **Introduction**

Several lines of evidence now attest that laboratory medicine plays an essential role not only in diagnosing acute SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) infections, but also for predicting and monitoring the efficacy of coronavirus disease 2019 (COVID-19) vaccination [1]. Although it has been clearly established that COVID-19 vaccination provides a considerably high protection against the risk of developing symptomatic and/or severe illness on the short-term, the efficacy of all types of COVID-19 vaccines manufactured so far declines significantly over time [2], and this reduced efficiency is mirrored by a remarkable decrease in the serum concentration of anti-SARS-CoV-2 IgG neutralizing antibodies [3,4].

The administration of booster vaccine doses is now regarded as the most reliable approach to restore vaccine efficacy to adequate levels (i.e., typically over 50%) especially in population of “low responders” and to more efficiently neutralize highly mutated SARS-CoV-2 variants [5]. Since such favourable effects of COVID-19 vaccine boosters seems to be reliably mirrored by a concomitant boost of serum anti-SARS-CoV-2 IgG neutralizing antibodies [6], we provide here an updated analysis of an ongoing serosurveillance study, presenting data on the effect of a third dose of Pfizer/BioNTech BNT162b2 vaccine after the primary homologous vaccination cycle on serum anti-SARS-CoV-2 IgG antibodies.

## **Materials and Methods**

The main characteristics of this study have been comprehensively described elsewhere [7]. Briefly, the initial sample included 100 baseline SARS-CoV-2 seronegative healthcare workers, who were recruited from the personnel of the Hospital of Peschiera del Garda hospital (Italy). All subjects volunteered to undergo primary vaccination with the mRNA-based COVID-19 Comirnaty vaccine (Pfizer/BioNTech

BNT162b2; Pfizer Inc., New York, NY, US; two 30 µg doses, separated by 3 weeks), followed by administration of a vaccine booster (single 30 µg dose) >8 months from completing the primary vaccination cycle. Molecular screening for identifying both symptomatic and asymptomatic SARS-CoV-2 infections (Seegene Allplex SARS-CoV-2 Assay; Seegene Inc., South Korea or Altona Diagnostics RealStar SARS-CoV-2 RT-PCR Kit; Altona Diagnostics GmbH, Hamburg, Germany) was conducted with 2-4 weeks intervals, until the end of the study. Venous blood was sequentially collected before either dose of primary vaccination cycle, was then drawn again at 1, 3 and 6 months after completing the primary vaccination cycle, as well as before and 1 month after receiving the homologous booster vaccine dose. The serum concentration of anti-SARS-CoV-2 spike trimeric IgG was measured with DiaSorin Trimeric spike IgG immunoassay on Liaison XL (DiaSorin, Saluggia, Italy). The analytical and clinical performance of this test have been thoughtfully reviewed elsewhere [8]. Briefly, the cumulative agreement and area under the curve (AUC) with the gold standard plaque reduction neutralization test (PRNT) were found to be 87% and 0.95, respectively, with diagnostic sensitivity and specificity as high as 96% and 85%, respectively. Test results were expressed as kilo Binding Antibodies Units per litre (kBAU/L), and thus traceable to the international standard for anti-SARS-CoV-2 immunoglobulins WHO/NIBSC 20-136. The minimum sample volume used for this assay is 160 µL (10 µL of test sample plus 150 µL of dead volume), the limit of detection is 4.81 kBAU/L, whilst the diagnostic cut-off is set at 33.8 kBAU/L. Results of measurements were presented as median and interquartile range (IQR). Anti-SARS-CoV-2 spike trimeric immunoglobulin values were also compared with the 80% limit of COVID-19 vaccine efficacy against symptomatic disease (i.e., 264 kBAU/L), as earlier calculated by Feng et al [9]. Written informed consent for receiving homologous Pfizer/BioNTech BNT162b2 vaccination (both primary cycle and booster dose) and participating to

longitudinal serological monitoring was obtained by all participants. Statistical analysis was carried out with Analyse-it software (Analyse-it Software Ltd, Leeds, UK). This observational retrospective study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Verona and Rovigo Provinces (59COVIDCESC; November 3, 2021).

## Results

Out of the 100 original participants, 18 subjects were excluded since they tested positive for SARS-CoV-2 mRNA during the study period, whilst 29 subjects were lost on follow-up. Therefore, our final sample consisted of 53 SARS-CoV-2 baseline seronegative healthcare workers (median age 46 years, IQR, 34-53 years; 32/53 females). The variation of anti-SARS-CoV-2 spike trimeric IgG is summarized in Figure 1. As previously shown [7], a first peak of values was reached 1 months after completing the primary vaccination cycle, after which the levels gradually declined until before receiving the homologous COVID-19 vaccine booster dose. Similarly, the rate of subjects with anti-SARS-CoV-2 spike trimeric IgG values >264 kBAU/L reached 100% after 1 months from completing the primary vaccination cycle, but then decreased to 77.4% and 47.2% after 6 months and immediately before receiving the vaccine booster dose, respectively (Figure 2). A second peak of anti-SARS-CoV-2 spike trimeric IgG concentration could then be observed 1 month after receiving the vaccine booster dose (8700 kBAU/L; IQR 5463-15733 kBAU/L), which was 39-fold higher than before receiving the vaccine booster (221 kBAU/L; IQR, 123-345 kBAU/L;  $p < 0.001$ ), but was also 3-fold higher compared to values seen at the first peak, i.e., 1 months after completing the primary vaccination cycle (2990 kBAU/L; IQR, 1758-4158 kBAU/L;  $p < 0.001$ ). Accordingly, the rate of subjects with anti-SARS-CoV-2 spike trimeric IgG values >264 kBAU/L increased from 47.2% to 100% after 1 month from receiving the

vaccine booster dose. In univariate analysis, the delta increment expressed as ratio of anti-SARS-CoV-2 spike trimeric IgG values measured after and immediately before the booster dose was inversely associated with pre-booster antibodies levels (Spearman's  $r=-0.67$ ; 95%CI, -0.80 to -0.49;  $p<0.001$ ), age (Spearman's  $r=0.49$ ; 95%CI, 0.25 to 0.67;  $p<0.001$ ), but not with sex (Spearman's  $r=0.09$ ; 95%CI -0.18 to 0.35;  $p=0.512$ ). Nonetheless, only pre-booster antibodies levels ( $\beta$  coefficient, -0.27; 95%CI, -0.51 to -0.02;  $p=0.037$ ) remained independently associated with booster-elicited variation in multivariable linear regression analysis (Supplementary Figure 1).

## **Discussion**

At least two important aspects have emerged from the results of this updated analysis of an ongoing serosurveillance study in healthcare workers receiving COVID-19 BNT162b2 primary vaccination followed by a homologous booster dose. As predicted, we first showed that the third vaccine dose was effective to boost anti-SARS-CoV-2 IgG antibodies values by nearly 40-fold compared to the period immediately before, concomitantly increasing the number of subjects with “protective” values from <50% to 100%. Moreover, we also showed that the vaccine booster dose is effective to increase anti-SARS-CoV-2 spike trimeric IgG antibodies levels by nearly 3-fold compared to immediately after completing the primary vaccination cycle. Similar evidence has been previously published using other immunoassays in different populations. Specifically, Falsey et al. reported a 5.5-15.0 folds increase of SARS-CoV-2 neutralization after the third BNT162b2 vaccine dose compared to the second dose in a general population of people aged 18-55 years [10], whilst a 3.8-10.2 folds increase of neutralization activity has also been observed by Choi et al. after the third Moderna mRNA-1273 vaccine dose in healthy volunteers aged 38-76 years [11]. This information appears of utmost importance, since increasing further the serum values of

neutralizing antibodies would enable major protection against any type of SARS-CoV-2 infection (even asymptomatic), thus contributing decrease virus circulation [12], and may also provide better protection against highly mutated SARS-CoV-2 lineages (e.g., the Omicron variant), which have strong propensity to escape the humoral immunity [13]. The inverse correlation that we observed between humoral response after the vaccine booster and pre-booster anti-SARS-CoV-2 IgG antibodies levels also underpins that vaccine recipients displaying the sharpest decline of humoral protection are those who would probably benefit more from receiving vaccine boosters.

In conclusion, the results of this ad interim analysis of the effect of BNT162b2 booster dose on anti-SARS-CoV-2 spike trimeric IgG antibodies in seronegative healthcare workers support current policies fostering COVID-19 vaccine boosters to reinforce humoral immunity, especially in people at higher risk of developing severe illness, in those with sharper decline of humoral immunity (both natural and vaccine-elicited), as well as when escape SARS-CoV-2 variants are widely circulating [14].

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**Competing interests:** Authors state no conflict of interest.

**Informed consent:** Informed consent was obtained from all subjects included in this study.

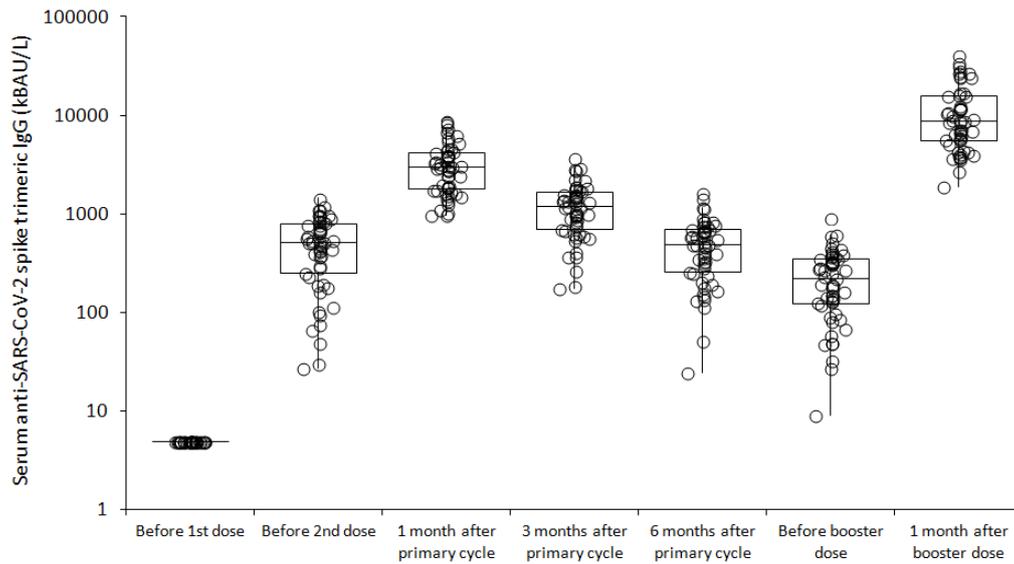
**Ethical approval:** The study protocol (59COVIDCESC; November 3, 2021) was cleared by the Ethics Committee of the Provinces of Verona and Rovigo. All subjects were informed of the study and voluntarily agreed to participate, providing a written consent.

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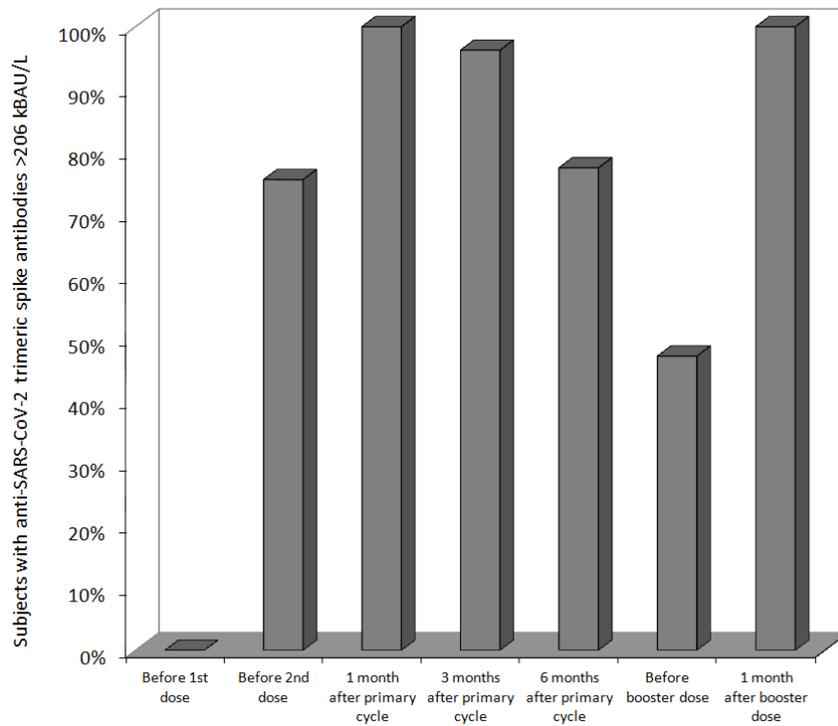
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**Figure 1.** Kinetics of serum anti-SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) spike trimeric RBD IgG antibodies in baseline seronegative recipients of BNT162b2 mRNA-based primary vaccination and booster. Results are shown as median and interquartile range (IQR).



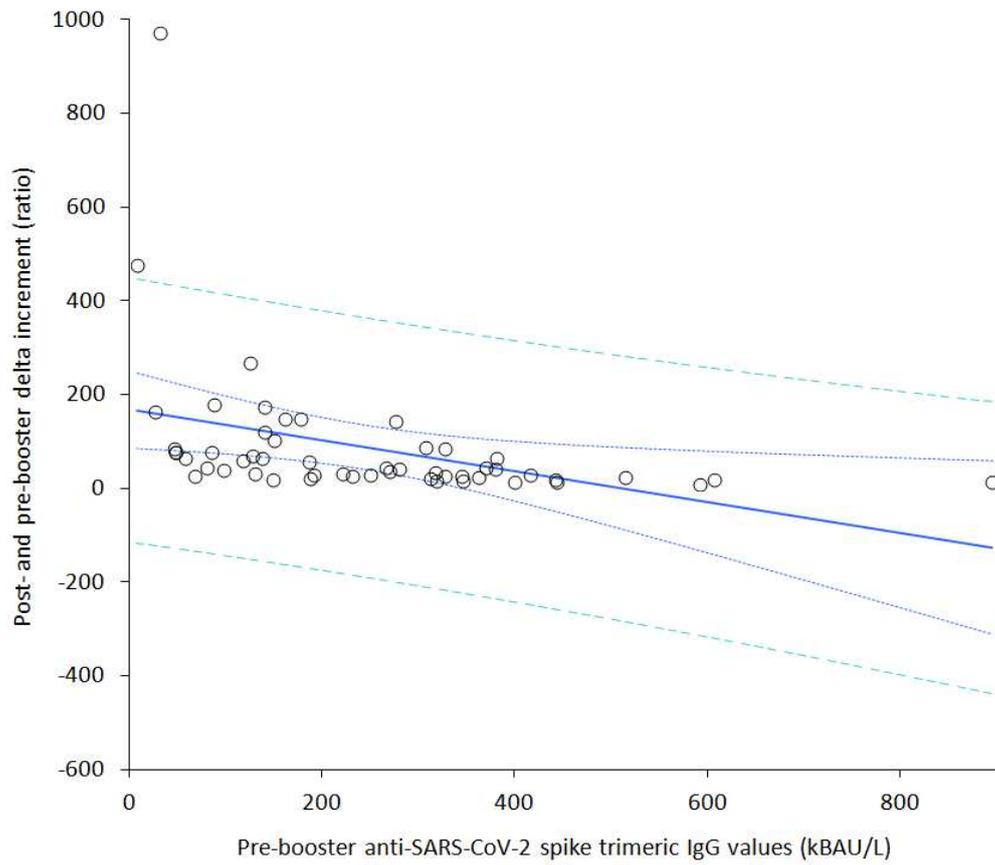
*SARS-CoV-2; severe acute respiratory syndrome coronavirus 2*

**Figure 2.** Rate of baseline seronegative recipients of BNT162b2 mRNA-based primary vaccination and booster displaying serum anti-SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) spike trimeric RBD IgG antibodies >264 kBAU/L.



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

**Supplementary figure 1.** Spearman's correlation of anti-SARS-CoV-2 spike trimeric IgG values after and immediately before the booster vaccine dose (delta variation) with pre-booster levels.



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