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Variation of total anti-SARS-CoV-2 antibodies after primary BNT162b2 vaccination and homologous booster

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ABSTRACT

Background

In this serosurveillance study, we investigated the variation of total anti-SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) antibodies in healthcare workers receiving primary BNT162b2 vaccination and homologous booster.

Methods

A total number of 524 subjects (median age, 46 years; 65.3% females), were studied. All received primary BNT162b2 vaccination (two doses) and homologous booster (one dose) >8 months after completing the primary cycle. Blood samples were collected before the first and second vaccine doses, at 1, 3 and 6 months after the second dose, as well as before and 1 month after booster. Total anti-SARS-CoV-2 neutralizing anti-

bodies were assayed with Roche Elecsys Anti-SARS-CoV-2 S chemiluminescent immunoassay.

Results

Overall, 65.1% subjects were baseline (i.e., pre-vaccination) SARS-CoV-2 seronegative and always tested SARS-CoV-2 negative ("N/N"), 16.2% were baseline SARS-CoV-2 seronegative but tested SARS-CoV-2 positive after receiving the vaccine booster dose ("N/P"), whilst 18.7% were baseline SARS-CoV-2 seropositive and always tested SARS-CoV-2 negative afterwards ("P/N"). All groups displayed a similar trend of total anti-SARS-CoV-2 S antibodies throughout the study period, though the P/N cohort exhibited higher values compared to the other two groups until receiving the booster, after which the levels become similar in all cohorts. Significant differences in total anti-SARS-CoV-2 S antibodies values were not found between N/N and N/P groups, neither 1 month after booster. The rate of subjects with protective antibodies values become 100% in all groups after booster.

Conclusions

Although baseline seropositivity is associated with more pronounced humoral immune response following primary vaccination compared to never infected subjects, SARS-CoV-2 infection after booster does not significantly foster antibody titers.

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INTRODUCTION

Several lines of evidence now attest that no existing vaccine would be completely effective against an infectious diseases, thus including those that have been developed against coronavirus disease 2019 (COVID-19) [1]. For viruses like SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), global herd immunity is unlikely to be achieved for a variety of reasons that include lower than advisable compliance to vaccination [2], progressive accumulation of non-synonymous genomic mutations that promote escape from vaccine-elicited immunity [3], as well as waning vaccine efficacy and protection over time, which is mostly attributable to a decline in the titer of anti-SARS-CoV-2 neutralizing antibodies [4,5]. To this end, epidemiological and laboratory investigations are needed to precisely recognize and measure the impact of the many determinants that may contribute to impair vaccine efficacy over time, thus allowing for the establishment of timely and appropriate measures that could be effective to limit SARS-CoV-2 circulation, prevent or reduce the risk of developing severe COVID-19 illness, and limit emergence of novel variants [6].

In a world with limited resources, where vaccine coverage remains extremely heterogeneous across different countries and populations mostly for insufficient supply [7], prioritization of primary vaccination and booster doses administration to those parts of the population that may be more exposed to complications and adverse consequences of SARS-CoV-2 infection is crucial [8]. Among the potentially more vulnerable subjects, those with blunted immunogenic response and sharper and/or faster decay of anti-SARS-CoV-2 neutralizing antibodies were found to have magnified risk of breakthrough infections and unfavorable progression of COVID-19, including increased rates of hospital admission, need of mechanical ventilation or intensive care, and a greater risk of death [9,10]. Personalized vaccine administration would also be effective to concomitantly avert the risk of rare side effects in those who could safely delay primary cycle or boosters [11].

Since the extent of vaccine-elicited protection varies considerably when combined with SARS-CoV-2 infections [12], this serosurveillance study was aimed to explore the variation of total anti-

SARS-CoV-2 antibodies in healthcare workers receiving primary vaccination with BNT162b2 and homologous booster, with or without SARS-CoV-2 infection before primary vaccination or after vaccine booster.

MATERIALS AND METHODS

The initial study population consisted of 925 ostensibly healthy individuals recruited from the healthcare staff of the Pederzoli Hospital in Peschiera del Garda (Italy), who received a primary vaccination cycle with BNT162b2 COVID-19 vaccine (Pfizer Inc., New York, NY; two doses of 30 µg, separated by 3 weeks), and an additional homologous booster (a single dose of 30 µg) more than 8 months after completing the primary vaccination. BNT162b2 is a lipid nanoparticle-formulated, nucleoside-modified RNA vaccine encoding a pre-fusion, stabilized, membrane-anchored SARS-CoV-2 full-length spike protein. A nucleic acid amplification test (NAAT) for diagnosing incident SARS-CoV-2 infection was performed every 2-4 weeks throughout the study period, using Altona Diagnostics RealStar SARS-CoV-2 RT-PCR Kit (Altona Diagnostics GmbH, Hamburg, Germany) or Seegene Allplex SARS-CoV-2 Assay (Seegene Inc., South Korea). Venous blood was collected before administration of the first (baseline) and second BNT162b2 doses, then 1, 3 and 6 months after the second BNT162b2 dose, and finally immediately before and 1 month after the homologous BNT162b2 booster dose. Subjects who became SARS-CoV-2 positive between the first dose of vaccine and the booster were actually excluded from our analysis. This is due to the fact that the time passed between these vaccine doses was so long that the influence of a SARS-CoV-2 infection on humoral immunity throughout nearly 8 months could not be standardized.

Total anti-SARS-CoV-2 neutralizing antibodies were assayed using the Roche Elecsys Anti-SARS-CoV-2 S chemiluminescent immunoassay, on a Roche Cobas 6000 immunochemistry platform (Roche Diagnostics, Basel, Switzerland). This double-antigen sandwich method encompasses a recombinant form of the receptor binding domain (RBD) of the SARS-CoV-2 spike (S) protein, displays total imprecision <4% [13], as well as optimal agreement with plaque reduction neutralization test (PRNT) in vaccinated subjects (area under the curve, 0.990; sensitivity, 0.98; specificity, 0.95) [14]. According to manufacturer's specifications, results are positive when the serum total anti-SARS-CoV-2 antibodies concentration is ≥0.8 kBAU/L (kilo binding antibody units/L). A total anti-SARS-CoV-2 antibodies titer of 656 kBAU/L was considered as predictive of ≥80% protection against modest or severe COVID-19 illness, in keeping with previously published data [15,16].

Results of testing were finally reported as median and interquartile range (IQR), and the statistical analysis was performed using Analyse-it (Analyse-it Software Ltd, Leeds, UK). Between-group comparisons were carried out with Mann-Whitney test. All participants gave informed consents for vaccination and undergoing serial anti-SARS-CoV-2 antibodies testing. This observational study was reviewed and cleared by the Ethics Committee of Verona and Rovigo provinces (3246CESC), and was conducted in accordance with the Declaration of Helsinki, under the terms of relevant local legislation.

RESULTS

The final study population consisted of 524 subjects (median age, 46 years and IQR, 34-53 years; 65.3% females; 56.6% of the original sample), as 401 subjects were lost on follow-up (for either not completing vaccination, failing to provide blood samples at one or more time point throughout the study). Eleven additional subjects tested positive for SARS-CoV-2 mRNA

after the first and before the third vaccine dose, but were also excluded due to the insufficient sample size to enable analysis of this subgroup. Of all subjects finally included, 341/524 (65.1%) were baseline (i.e. pre-vaccination) SARS-CoV-2 seronegative (i.e., <0.8 kBAU/L) and then always tested SARS-CoV-2 negative ("N/N"; median age, 47 years and IQR, 36-54 years; 63.9% females); 85/524 (16.2%) were baseline SARS-CoV-2 seronegative (i.e., <0.8 kBAU/L) and then tested SARS-CoV-2 positive after receiving the vaccine booster dose ("N/P"; median age, 43 years and IQR 31-50 years; 68.2% females); whilst 98/524 (18.7%) were baseline SARS-CoV-2 seropositive (i.e., >0.8 kBAU/L) and then always tested SARS-CoV-2 negative ("P/N"; median age, 44 years and IQR, 33-52 years; 67.3% females), respectively. We excluded the cohort of subjects who were baseline SARS-CoV-2 seropositive (i.e., >0.8 kBAU/L) and then tested SARS-CoV-2 positive after receiving the vaccine booster dose always tested (i.e., P/P) because the final sample size of this cohort was considerably low (n=9), so that inclusion in the statistical analysis may be misleading. Subjects in the N/N cohort were slightly younger than those in the two other groups (i.e., p=0.035 vs. N/P and p=0.039 vs. P/N, respectively), whilst the sex distribution was similar across the three groups (all p>0.05).

The main variations of total anti-SARS-CoV-2 S antibodies in the three groups are summarized in table 1 and figure 1. As predicted, although all groups displayed a rather similar trend throughout the study period, the P/N cohort exhibited significantly higher values compared to the other two groups until these subjects received the vaccine booster dose, after which the serum antibodies levels become comparable to those of the other two cohorts. No significant differences were observed between the N/N and N/P groups, including at the 1 month time point after receiving the vaccine booster dose (Table 1).

The rate of subjects with protective total serum anti-SARS-CoV-2 S antibodies values (i.e., >656 kBAU/L) displayed rather consistent trend across groups. Specifically, in N/N and N/P the rate of positive subjects increased from 0% to 82-84% 1 months after the second vaccine dose, but then gradually declined to 32-35% before receiving the vaccine booster, after which such rate increased in both groups to 100% (Figure 2). Unlike these subjects though, the rate of P/N subjects with protective total anti-SARS-CoV-2 S antibodies values was 7% at baseline assessment, then remained always >88% throughout the subsequent time points, and increased further to 100% after receiving the vaccine booster dose.

DISCUSSION

Some important conclusions can be made from the results of this serosurvillance study in a group of healthcare workers who received a primary BNT162b2 vaccination followed by a homologous booster.

The first important aspect, which supports previously published evidence [17-19], is that a prior SARS-CoV-2 infection before primary vaccination provides a rather efficient priming to COVID-19 vaccination, in that the total anti-SARS-CoV-2 S antibodies values appear to be consistently higher in baseline seropositive individuals (P/N) compared to seronegative ones (both N/N and N/P groups in our study). The protection against the risk of developing moderate/severe COVID-19 illness (i.e., the subjects with predictably protective antibodies values) seems also to persist for longer after primary vaccination in baseline seropositive compared to baseline seronegative subjects (i.e., 88% in P/N vs. 32-35% in N/N and N/P, respectively), which would allow to safely prioritize the administration of vaccine boosters to seronegative individuals.

The second important aspect that emerged from this serosurvillance study, is that the vaccine

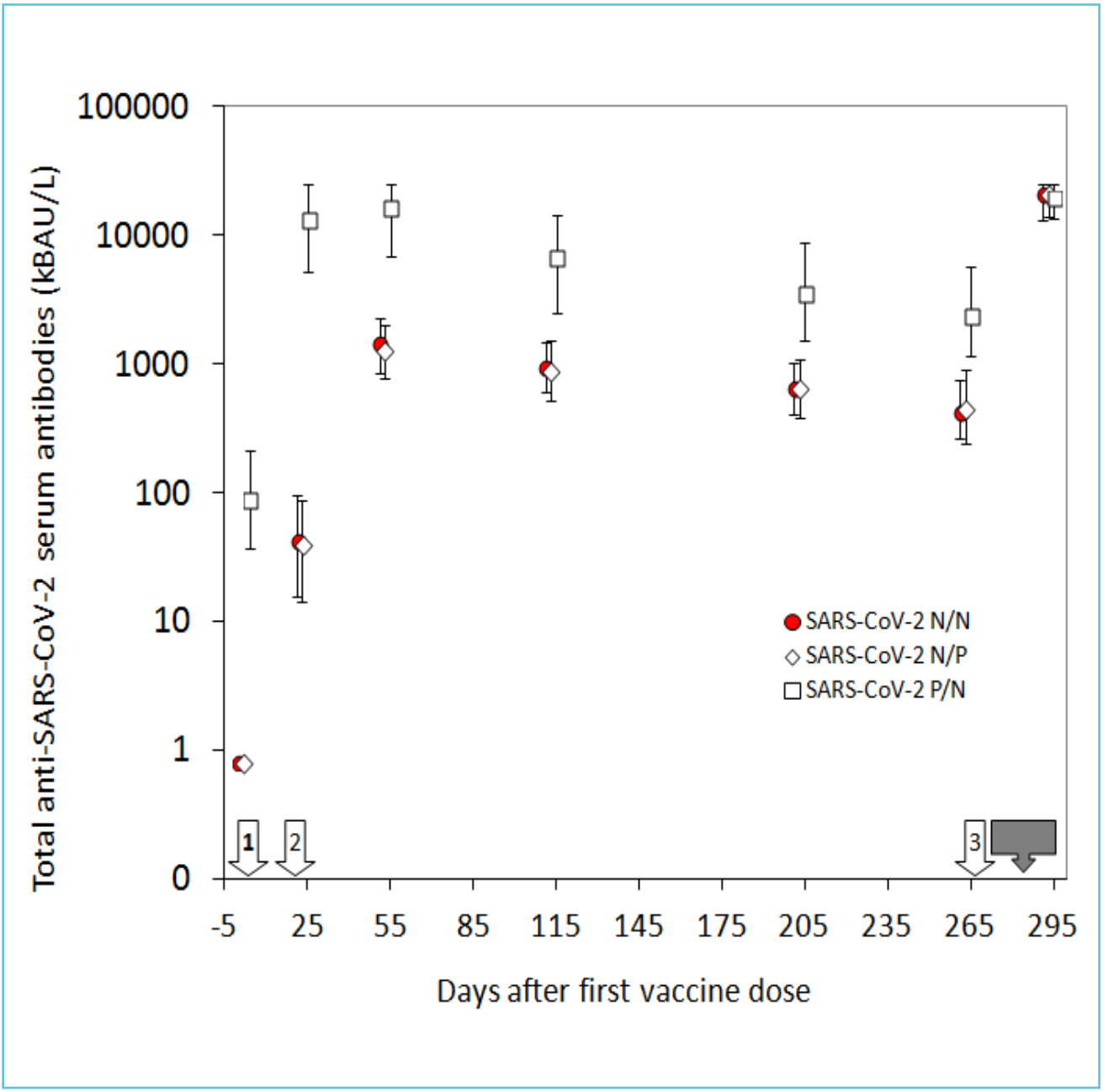
booster dose generates a considerable impact on total anti-SARS-CoV-2 S antibodies, increasing their concentration by around 50- and 8-fold in seronegative and seropositive subjects, respectively, though the final values reached do not significantly differ among all groups (Table 1). This is basically consistent with two, non-mutually exclusive, hypotheses. It can be first conjectured that the priming effect of a pre-vaccination SARS-CoV-2 infection has progressively declined over time, being almost completely lost before receiving the vaccine booster dose, such that baseline SARS-CoV-2 seronegative and seropositive subjects would become a more homogenous population. This is not really surprising, since total anti-SARS-CoV-2 antibodies display a half-life between 50-110 days [20], with a seropositive rate decreasing to less than 36% after 12 months [21].

Table 1 Serum concentration (median and interquartile range) of total anti-SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) antibodies in a cohort of healthcare workers receiving primary BNT162b2 vaccination and homologous booster

Population	Baseline	Pre-2nd dose	1 M after 2nd dose	3 M after 2nd dose	6 M after 2nd dose	Pre-booster	1 M post-booster
SARS-CoV-2 N/N							
Values (kBAU/L)	<0.8	42.8 (15.4-96.1)	1440.0 (854.5-2269.0)	936.4 (601.2-1464.0)	661.6 (407.7-1023.0)	429.8 (265.8-744.0)	20848.0 (13218.0-25000.0)
SARS-CoV-2 N/P							
Values (kBAU/L)	<0.8	39.6 (14.1-87.4)	1289.0 (770.8-2011.0)	902.9 (517.4-1538.0)	656.0 (380.5-1088.0)	452.5 (244.0-911.8)	20891.0 (14028.0-25000.0)
p vs. N/N	1.000	0.258	0.510	0.321	0.180	0.110	0.392
SARS-CoV-2 P/N							
Values (kBAU/L)	80.8 (31.1-209.8)	13312.0 (5198.3-25000.0)	16358.0 (6898.8-25000.0)	6673.5 (2525.8-14395.0)	3529.5 (1523.0-8664.3)	2366.0 (1150.5-5777.8)	19290.0 (13720.5-25000.0)
p vs. N/N	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.364
p vs. N/P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.306

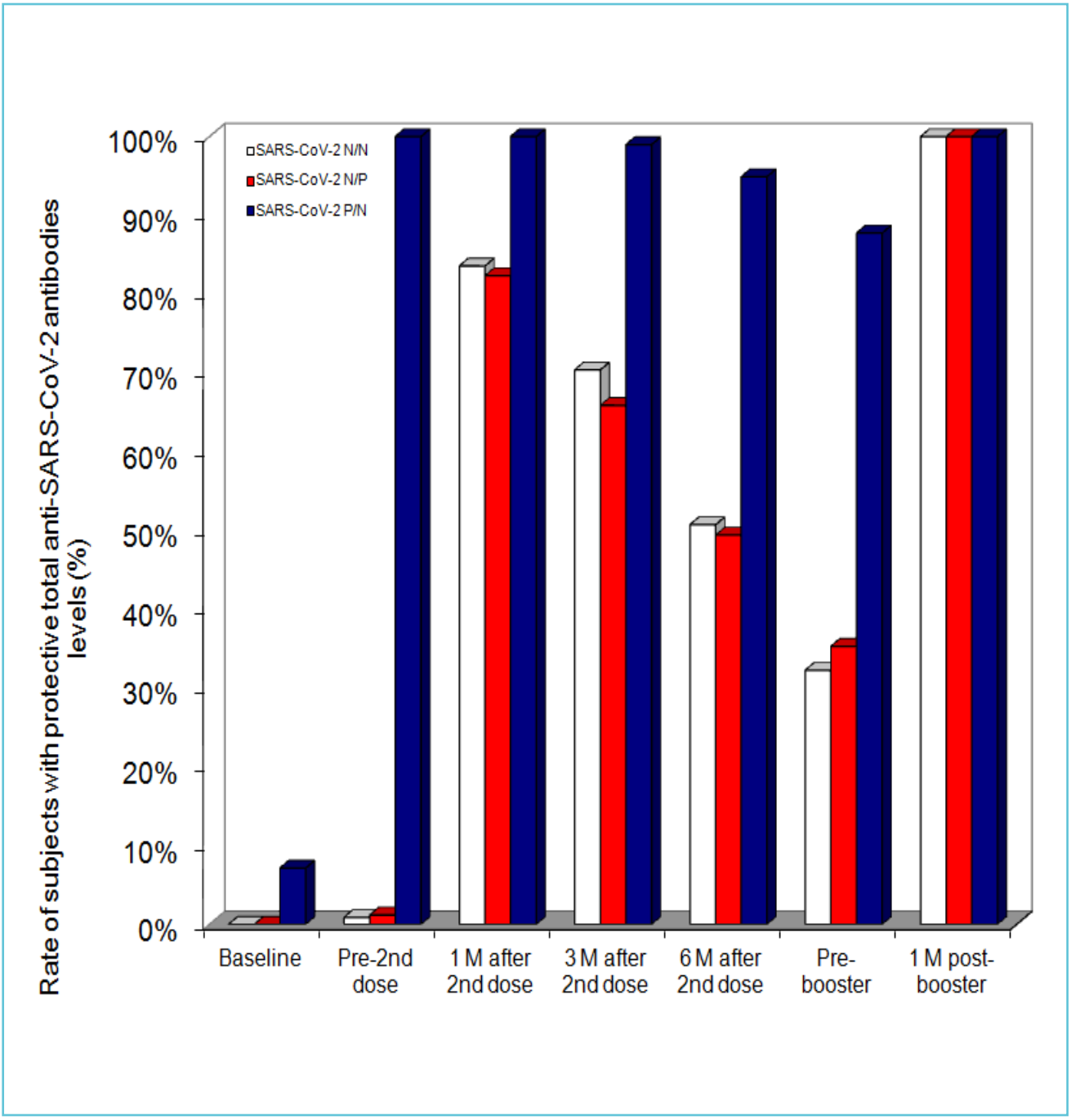
N/N, baseline SARS-CoV-2 seronegative and then always testing SARS-CoV-2 negative; N/P, baseline SARS-CoV-2 seronegative and testing SARS-CoV-2 positive after booster; P/N; baseline SARS-CoV-2 seropositive and then always testing SARS-CoV-2 negative; M, months; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Figure 1 **Serum concentration (median and interquartile range) of total anti-SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) antibodies in a cohort of healthcare workers receiving primary BNT162b2 vaccination and homologous booster. The white arrows indicate the timing of BNT162b2 vaccine doses, whilst the gray arrow indicates the SARS-CoV infection in the N/P group.**



N/N, baseline SARS-CoV-2 seronegative and then always testing SARS-CoV-2 negative; N/P, baseline SARS-CoV-2 seronegative and testing SARS-CoV-2 positive after booster; P/N; baseline SARS-CoV-2 seropositive and then always testing SARS-CoV-2 negative; M, months; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Figure 2 **Rate of subjects with protective levels of total anti-SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) antibodies (i.e., >656 kBAU/L) in a cohort of healthcare workers receiving primary BNT162b2 vaccination and homologous booster.**



N/N, baseline SARS-CoV-2 seronegative and then always testing SARS-CoV-2 negative; N/P, baseline SARS-CoV-2 seronegative and testing SARS-CoV-2 positive after booster; P/N; baseline SARS-CoV-2 seropositive and then always testing SARS-CoV-2 negative; M, months; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

On the other hand, it is also conceivable that the administration of a BNT162b2 booster dose has provided such a strong stimulus to an already primed immunological memory (i.e., memory B cells) [22], such that another potent immunogenic trigger like an incident SARS-CoV-2 infection occurred after vaccine booster would be incapable to produce further significant increases of total anti-SARS-CoV-2 S antibodies levels over the threshold achieved with vaccine boosters.

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Author contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Informed consent

Informed consent was obtained from all individuals included in this study.

Ethical approval

This observational study was reviewed and cleared by the Ethics Committee of Verona and Rovigo provinces (3246CESC).

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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