Humoral response post-BNT162b2 single booster in pre-vaccination baseline SARS-CoV-2 seronegative and seropositive subjects

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ABSTRACT

Background: we report here data on humoral immune response post-BNT162b2 primary vaccination and booster in pre-vaccination baseline severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) seronegative and seropositive subjects.
Methods: the study population consisted in 51 baseline SARS-CoV-2 seronegative and 11 baseline SARS-CoV-2 seropositive subjects, who underwent primary mRNA-based BNT162b2 vaccination (two doses) followed by homologous booster administration (third dose). Venous blood was sequentially collected up to 1 months after vaccine booster administration, and humoral response was monitored by measuring anti-SARS-CoV-2 spike trimeric IgG antibodies.
Results: the humoral response after the three doses of BNT162b2 displayed an overlapping trend in the two groups, although the baseline and post-primary vaccination concentration of anti-SARS-CoV-2 spike trimeric IgG were constantly higher in baseline SARS-CoV-2 seropositive than in baseline SARS-CoV-2 seronegative subjects (all $p<0.001$). Unlike before vaccine booster administration, the levels of anti-SARS-CoV-2 spike trimeric IgG, 1 month after receiving the third BNT162b2 dose were not significantly different between pre-vaccination baseline SARS-CoV-2 seropositive and seronegative subjects (7 430 versus 9 020 kBAU/L; $p=0.232$). In both cohorts, all recipients of vaccine booster displayed antibodies levels >264 kBAU/L.
Conclusion: the results of this study demonstrate that although baseline SARS-CoV-2 seropositive subjects have magnified humoral response to primary BNT162b2 vaccination, vaccine booster generates anti-SARS-CoV-2 spike trimeric IgG values not different from those found in baseline SARS-CoV-2 seronegative subjects. Thus, this study provides evidence that a prior SARS-CoV-2 infection does not mitigate the need for additional vaccine boosters.

Keywords: *COVID-19, SARS-CoV-2, Antibodies*

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a life-threatening viral infection sustained by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Several lines of evidence now attest that COVID-19 vaccination is the most efficient strategy for preventing clinically unfavourable consequences of infection, as well as for limiting virus circulation and preventing the constant emergence of new and highly mutated strains (1). One fact that has clearly emerged after one year from the initiation of the worldwide COVID-19 vaccination campaign, is that the immune response varies widely in recipients, both in terms of magnitude and duration. More specifically, there are several aspects that can influence vaccine reactogenicity and post-vaccination immune response. These typically include vaccine factors (e.g., interval, number and volume of doses), viral factors (e.g., antigenic phenotype, transmissibility and virulence), along with host factors like age, comorbidities and immune status (2). Laboratory monitoring and surveillance seem now feasible and reliable strategies to optimize vaccine administration, by prioritizing the usage of primary vaccination and boosters in those with blunted immune response, rapid decline, and/or at risk of developing more severe forms of COVID-19 illness (3). Several previous studies, recently reviewed by Notarte et al. (4), concluded that pre-vaccination baseline SARS-CoV-2 serostatus (i.e., presence or absence of an immune response developed after a SARS-CoV-2 infection) may strongly influence the immune response after primary COVID-19 vaccination, though lesser is known regarding the impact on serum anti-SARS-CoV-2 antibodies levels

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after receiving vaccine boosters. Therefore, this study aimed to compare the humoral response post-COVID-19 vaccination plus single booster in baseline SARS-CoV-2 seronegative and seropositive subjects.

METHODS

The leading aspects of this serosurveillance study have been earlier reported (5,6). In brief, the original cohort consisted of over 900 healthcare workers of the Hospital Pederzoli in Peschiera del Garda (Italy), who voluntarily underwent primary vaccination with the mRNA-based Pfizer/BioNTech BNT162b2 vaccine (Pfizer Inc., New York, NY, US; two 30 µg doses, separated by 3 weeks), followed by administration of a homologous vaccine booster (single dose, 30 µg) >8 months after primary vaccination. Molecular testing for diagnosing asymptomatic or symptomatic SARS-CoV-2 infection was carried out at 2-4 weeks intervals, throughout the study period, with either 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 samples, collected by venipuncture immediately before receiving the primary vaccination cycle, were then recollected after 1, 3 and 6 months, and finally before and 1 month after the administration of the homologous vaccine booster. The humoral response was monitored by measuring the serum levels of anti-SARS-CoV-2 spike trimeric IgG, using the DiaSorin Trimeric spike IgG immunoassay on Liaison XL (DiaSorin, Saluggia, Italy). The main analytical and clinical characteristics of this test are comprehensively reported elsewhere (7). In summary, this assay displays excellent agreement (i.e., 87%, with diagnostic 96% sensitivity and 85% specificity) with the reference plaque reduction neutralization test (PRNT). The limit of quantitation for defining the seropositive status is 4.81 kBAU/L. Results of all measurements were presented as median and interquartile range (IQR). The significance of difference between the two cohorts of baseline SARS-CoV-2 seronegative and seropositive subjects was estimated with Mann-Whitney and chi square test, when appropriate, using Analyse-it (Analyse-it Software Ltd, Leeds, UK). All subjects provided written consent for both receiving BNT162b2 primary vaccination and booster, as well as for having their blood collected for serosurveillance monitoring. 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

The final study population consisted of 62 subjects (6.7% of the original sample), who received all the three BNT162b2 vaccine doses, had their blood collected for specifically measuring anti-SARS-CoV-2 spike trimeric IgG and did not develop incident SARS-CoV-2 infection throughout the study period (all other participants did not receive all three vaccines doses, missed one or more time points, or developed an incident SARS-CoV-2 infection up to 1 months after receiving the vaccine booster). Eleven of these subjects (median age, 45 years; IQR, 26-48 years; 55% females) were pre-vaccination baseline SARS-CoV-2 seropositive (all with serum antibodies values >4.81 kBAU/L), whilst the remaining 51 (median age, 44 years; IQR, 34-54 years; 47% females) were baseline SARS-CoV-2 seronegative (all with serum antibodies values <4.81 kBAU/L). Age (p=0.093) and sex (p=0.203) did not significantly differ between these two cohorts.

The humoral response after the three doses of BNT162b2 is shown in Figure 1. Immediately before vaccination, the serum values of anti-SARS-CoV-2 spike trimeric IgG were obviously higher in baseline SARS-CoV-2 seropositive compared to seronegative subjects (<4.81 *versus* 56 kBAU/L; p<0.001). A rather similar kinetics was then noted after primary BNT162b2

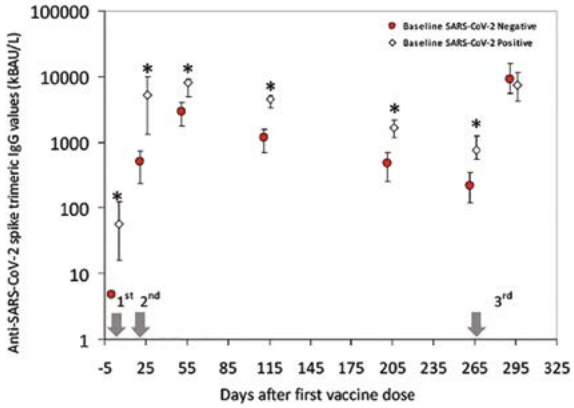

Figure 1
Variation of serum anti-SARS-CoV-2 spike trimeric RBD IgG antibodies levels (median and interquartile range) in baseline SARS-CoV-2 seronegative (n=51) and seropositive (n=11) recipients of BNT162b2 mRNA-based primary vaccination and booster. The gray arrows indicate the time of administration of the three mRNA-based BNT162b2 vaccine doses.
** p<0.001.*
SARS-CoV-2, seve re acute respiratory syndrome coronavirus 2; RBD, receptor binding domain

vaccination between the two cohorts, although serum antibodies concentrations remained constantly higher in baseline SARS-CoV-2 seropositive than in seronegative subjects (all p<0.001) (Figure 1). Unlike at previous time points, the levels of anti-SARS-CoV-2 spike trimeric IgG, 1 month after receiving the BNT162b2 booster were not significantly different between pre-vaccination baseline SARS-CoV-2 seropositive and seronegative subjects (7 430 *versus* 9 020 kBAU/L; p=0,232). In both cohorts, all subjects receiving the BNT162b2 booster dose displayed post-boost serum levels of anti-SARS-CoV-2 spike trimeric IgG >264 kBAU/L, which corresponds to the 80% threshold of vaccine efficacy against symptomatic disease (Figure 2) (8).

DISCUSSION

The results of this serosurveillance study aimed at comparing the humoral response post-BNT162b2 booster in baseline SARS-CoV-2 seronegative and seropositive subjects, confirm previous evidence, but also provide interesting new aspects.

First, despite exhibiting a globally comparable trend over time, the humoral response recorded after completing a primary vaccination cycle with the mRNA-based BNT162b2 vaccine was confirmed to be considerably higher in baseline SARS-CoV-2 seropositive subjects, with values up to 3-fold higher 1 month after the second primary dose compared to baseline SARS-CoV-2 seronegative subjects (8 060 *versus* 2 938 kBAU/L; p<0.001). Then, the serum values of anti-SARS-CoV-2 spike trimeric IgG remained constantly higher in the baseline SARS-CoV-2 seropositive cohort up to over 8 months (serum values before vaccine booster: 769 versus 221 kBAU/L; p<0.001), thus mirroring a likely higher protection against any type of SARS-CoV-2 infection, but especially against symptomatic disease. Nonetheless, a virtually unexpected trend has emerged after administration of the BNT162b2 single booster dose, wherein the serum levels of anti-SARS-CoV-2 spike trimeric IgG measured 1 month after the booster become similar between baseline SARS-CoV-2 seropositive and seronegative individuals (p=0.232). This can be basically interpreted in one of two ways. First, it is likely that the earlier amplifying effect of SARS-CoV-2 infection on primary vaccination has gradually waned over time, being nearly completely lost after 8 months, after which baseline SARS-CoV-2 seropositive and seronegative people should then be considered a more homogenous population. A second possible explanation is that the immune system has probably achieved a maximum degree of stimulation, after which additional immunogenic triggers (either natural, like an infection, or artificial, such as COVID-19 vaccination), would not be effective to generate additional increases in the serum values of anti-SARS-CoV-2 spike trimeric IgG. This hypothesis is consistent with the fact that the humoral response elicited by excess of mRNA-based vaccines seems not proportional to the overdose, achieving a maximum level of anti-SARS-CoV-2 IgG values after which no additional enhancement is predictable (9).

Irrespective of the specific mechanism(s) underlying this biological evidence, the fact that a BNT162b2 booster dose elicits similar anti-SARS-CoV-2 antibodies response in baseline SARS-CoV-2 seropositive and seronegative individuals carries important clinical implications. First, the administration of booster vaccine doses would seem advisable also in baseline SARS-CoV-2 seropositive subjects, in that the immunological memory developed after an earlier SARS-CoV-2 infection may not last enough for allowing to safely avoid a vaccine single booster. This holds especially true considering that antibody-mediated neutralization of highly mutated SARS-CoV-2 lineages (such as Omicron) is considerably impaired (10) and, therefore, additional boosters would be highly recommended to prevent clinically unfavourable infections with emerging lineages. The second important evidence garnered from current and previous data (9), is that the booster dose seems actually effective to

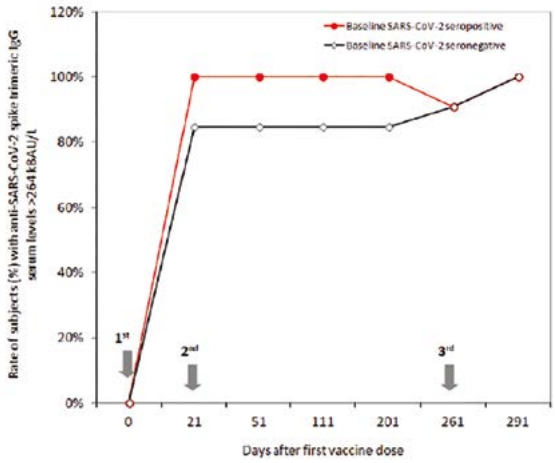

Figure 2
Rate of baseline SARS-CoV-2 seronegative (n=51) and seropositive (n=11) subjects with serum anti-SARS-CoV-2 spike trimeric RBD IgG antibodies levels >264 kBAU/L. The gray arrows indicate the time of administration of the three mRNA-based BNT162b2 vaccine doses.

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

trigger a maximum humoral response, with little further improvement expected by administering higher or additional doses in the short-term period. This calls for a major role of strict monitoring of anti-SARS-CoV-2 antibodies throughout and after vaccination, in that timely and accurate identification of subjects with more rapid and sharper decline of anti-SARS-CoV-2 antibodies titres would enable personalized administration of additional vaccine doses and proper rationalization of worldwide vaccine usage (11,12).

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CONFLICT OF INTEREST

None

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