Letter to the Editor

Gian Luca Salvagno, Brandon M. Henry, Laura Pighi, Simone De Nitto, Gianluca Gianfilippi and Giuseppe Lippi*

Three-month *ad interim* analysis of total anti-SARS-CoV-2 antibodies in healthy recipient of a single BNT162b2 vaccine booster

https://doi.org/10.1515/cclm-2022-0385
Received April 21, 2022; accepted May 2, 2022;
published online May 16, 2022

Keywords: antibodies; COVID-19; immune response; SARS-CoV-2; vaccination.

To the Editor,

Although widespread vaccination for preventing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and coronavirus disease 2019 (COVID-19) complications is now universally considered a highly effective and safe anti-pandemic strategy, vaccine efficacy has been clearly shown to wane over time, thus paving the way to administration of an additional (so-called "booster") dose after completing the primary vaccination cycle [1].

Nonetheless, recent evidence suggests that even the vaccine booster-elicited humoral immunity against SARS-CoV-2 wanes over time, such that the administration of additional booster doses has already been initiated in certain countries, with preliminary evidence of enhanced protection compared to people who have only received a single vaccine booster [2]. In keeping with this data, both the US Centers for Disease Control and Prevention (CDC) [3] and the European Centre for Disease Prevention and Control (ECDC) [4] recently recommended the administration of additional vaccine boosters for certain individuals, namely immunocompromised patients aged 50 years or older. The ECDC also suggests that consideration could be made to administer an additional (i.e., second) booster in people aged 80 years or older due to their presumably lower immune response to vaccination and higher risk of developing severe COVID-19 illness [4].

At this point in time, however, no indications have been issued to extend the administration of additional boosters to the entire general population as well as in healthcare workers. In a recent article published in this journal, Padoan et al. reported a gradual decline of anti-SARS-CoV-2 neutralizing IgG antibodies in baseline seronegative subjects 3–4 months after receiving a single BNT162b2 vaccine booster [5]. To provide further insights on this matter, this 3-month *ad interim* analysis of the effect of a single BNT162b2 booster on anti-SARS-CoV-antibodies was planned to confirm the data published by Padoan et al. [5], but also for establishing as to whether a second vaccine booster may be avoided in healthy immunocompetent individuals, thus evaluating with biological data the current healthcare strategies.

The main characteristics of this serosurveillance study based on a cohort of healthcare workers of the Pederzoli Hospital in Peschiera del Garda (Verona, Italy) have been comprehensively described elsewhere [6]. Briefly, after excluding subjects lost to follow-up or diagnosed with incident SARS-CoV-2 infection (n=763 out of the 1,003 originally enrolled, 371 testing positive for SARS-CoV-2 throughout the study and 392 for missing one or more sampling points, respectively), the current cohort comprises 240 SARS-CoV-2 baseline seronegative subjects (median age 48 years, range 23–72 years; 151 females), who underwent primary COVID-19 vaccination with Comirnaty (Pfizer/BioNTech BNT162b2; Pfizer Inc., New York, NY, US; two 30 μg doses, at 3-week interval), and subsequent administration of single homologous booster dose (30 μg) more than 8 months afterwards. Throughout the study

***Corresponding author: Prof. Giuseppe Lippi**, Section of Clinical Biochemistry, University Hospital of Verona, Piazzale L.A. Scuro 10, 37134 Verona, Italy, Phone: +0039 045 8122970, Fax: +0039 045 8124308, E-mail: giuseppe.lippi@univr.it. https://orcid.org/0000-0001-9523-9054

Gian Luca Salvagno, Laura Pighi and Simone De Nitto, Section of Clinical Biochemistry, University of Verona, Verona, Italy; and Service of Laboratory Medicine, Pederzoli Hospital, Peschiera del Garda, Italy

Brandon M. Henry, Clinical Laboratory, Division of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Gianluca Gianfilippi, Medical Direction, Pederzoli Hospital, Peschiera del Garda, Italy

molecular testing was carried out at 2–4 weeks intervals (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) for detecting incident SARS-CoV-2 infections. Blood was drawn before the first and second vaccine doses, then 1, 3, and 6 months thereafter, and finally before and 1 and 3 months after receiving a 30 µg BNT162b2 vaccine booster. Serum was employed for measuring total anti-SARS-CoV-2 antibodies with Roche Elecsys Anti-SARS-CoV-2 S on Roche Cobas 6000 (Roche Diagnostics, Basel, Switzerland; samples were diluted to reach values up to 25,000 kBAU/L). Written informed consent for participating to this serosurveillance study was obtained from all participants. Statistical analysis was carried out with Analyse-it software (Analyse-it Software Ltd, Leeds, UK), using the Mann-Whitney test. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Verona and Rovigo Provinces (59COVID-CESC; November 3, 2021).

The longitudinal change of total anti-SARS-CoV-2 antibodies is shown in Figure 1. Without specifically addressing earlier changes, which have been thoughtfully described before [6], a decline in the serum levels of total anti-SARS-CoV-2 antibodies could be observed from 1 to 3 months after receiving the BNT162b2 single booster dose. More specifically, the median serum values of total anti-SARS-CoV-2 antibodies significantly decreased from 22,094 (interquartile range [IQR], 13,742–25,000) kBAU at 1 month post-booster to 11,612 (IQR, 6,553–20,767) kBAU (mean decrease: 35 ± 27%) at 3 months post-booster (p<0.001). The percentage decrease of total anti-SARS-CoV-2 antibodies between 1 and 3 months after the vaccine booster displayed a significant inverse Spearman's correlation with 1-month post-booster antibodies values (r= −0.53; 95% CI, −0.62−−0.43); and age (r= −0.22; 95% CI, −0.34−−0.10; p=0.001), but not with sex (r= −0.06; 95% CI, −0.19–0.06; p=0.319). Accordingly, the mean percentage decrease of total anti-SARS-CoV-2 antibodies over such period was higher in subjects with 1-month post-booster total anti-SARS-CoV-2 antibodies values <12,000 kBAU/L than in those with higher levels (mean decline: 54 vs. 38%; p<0.001), as well as in subjects <50 years than in those aged 50 years or older (mean decline: 46 vs. 31%; p=0.001). In multiple linear regression analysis both 1-month post-booster antibodies values (p<0.001) and age (p=0.004) remained negatively associated with the percentage decrease of total anti-SARS-CoV-2 antibodies measured 1 and 3 months after the booster. Cumulatively, these two parameters contributed to explain 44% of the total variance in the decline of humoral immunity observed between 1 and 3 months after the vaccine booster.

In conclusion, our data obtained with a different immunoassay for measuring total anti-SARS-CoV-2 antibodies support those earlier published by Padoan et al. [5] and also those more recently published by Favresse et al. using our same method [7], thus confirming that the serum levels of anti-SARS-CoV-2 antibodies gradually wane even after the first BNT162b2 booster dose, with the amplitude of such reduction more accentuated in people having lower 1-month post-booster values and younger age. Although we cumulatively observed that such reduction was significant (i.e., 35%), all the subjects included in this study still had considerably high values 3 month after the single vaccine booster. Therefore, the evidence emerged in this *ad interim* analysis is seemingly supportive of current healthcare strategies based on

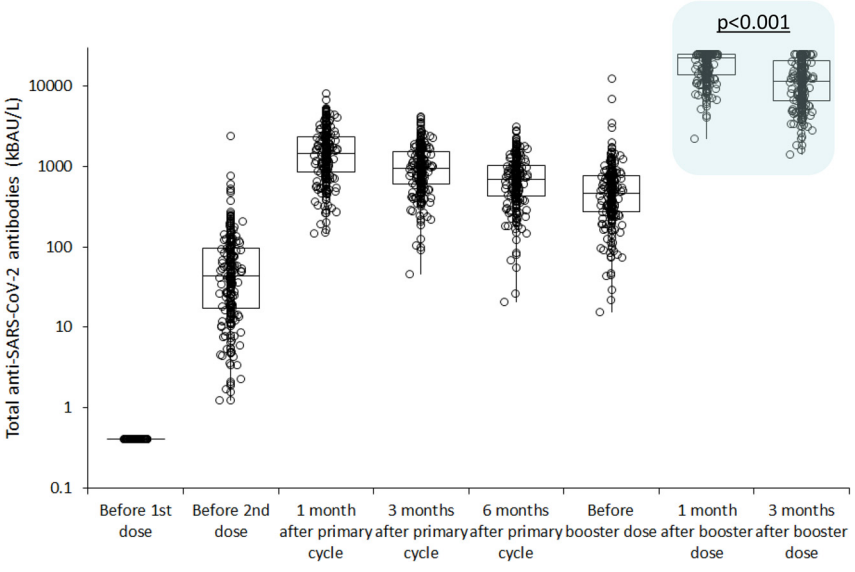

Figure 1: Longitudinal variation of serum anti-SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) total antibodies in baseline seronegative recipients of BNT162b2 mRNA-based primary vaccination and single homologous booster. Results are shown as median and interquartile range (IQR). SARS-CoV-2; severe acute respiratory syndrome coronavirus 2.

postponing the administration of additional COVID-19 vaccine booster doses in the general immunocompetent and non-elderly population (thus including healthcare workers) for a longer period compared to immunocompromised and older people.

Acknowledgments: The authors are thankful to the entire staff of the Pederzoli Hospital of Peschiera del Garda (Verona, Italy) for accepting to participate to this study.
Research funding: The authors received no funding for this work.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
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.

References

1. Wald A. Booster vaccination to reduce SARS-CoV-2 transmission and infection. JAMA 2022;327:327–8.
2. Offit PA. Covid-19 boosters – where from here? N Engl J Med 2022. https://doi.org/10.1056/NEJMe2203329 [Epub ahead of print].
3. Centers for Disease Control and Prevention. CDC recommends additional boosters for certain individuals. Available from: https://www.cdc.gov/media/releases/2022/s0328-covid-19-boosters.html [Accessed 14 April 2022].
4. European Centre for Disease Prevention and Control. COVID-19: joint statement from ECDC and EMA on the administration of a fourth dose of mRNA vaccines. Available from: https://www.ecdc.europa.eu/en/news-events/ema-ecdc-statement-fourth-covid-vaccine-dose [Accessed 14 April 2022].
5. Padoan A, Cosma C, della Rocca F, Barbaro F, Santarossa C, Dall'Olmo L, et al. Clin Chem Lab Med 2022;60:1110–5.
6. Salvagno GL, Henry BM, Pighi L, De Nitto S, Gianfilippi GL, Lippi G. Three-month analysis of total humoral response to Pfizer BNT162b2 mRNA COVID-19 vaccination in healthcare workers. J Infect 2021;83:e4–5.
7. Favresse J, Dogné JM, Douxfils J. Assessment of the humoral response in Omicron breakthrough cases in healthcare workers who received the BNT162b2 booster. Clin Chem Lab Med 2022;60:e153–6.