Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Three-month analysis of total humoral response to Pfizer BNT162b2 mRNA COVID-19 vaccination in healthcare workers

Dear Editor,

We read with interest the article by Tré-Hardy et al.,[1] who monitored the anti-SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) spike antibodies levels using the LIAISON® SARS-CoV-2 IgG kit in naïve recipients of mRNA-1273 vaccine. Although it was found that these antibodies persisted up to 3 months after vaccination, a significant decrease of their serum levels could be observed in some seronegative participants, thus supporting the opportunity to administer an additional vaccine dose in specific subgroups of recipients. To provide further elements on this important public health matter, we report here an ad-interim analysis of data obtained after a 3-month follow-up in a cohort of healthcare workers who received the Pfizer BNT162b2 mRNA vaccine.

The study population consisted of healthcare workers undergoing voluntary vaccination with Pfizer BNT162b2 mRNA COVID-19 vaccine (Comirnaty; Pfizer Inc, NY, USA) at the hospital of Peschiera del Garda (Italy). All subjects received two 30 μg BNT162b2 vaccine doses, 3 weeks apart. Blood was collected immediately before receiving the first vaccine dose, immediately before receiving the second vaccine dose (i.e., 21 days after the first dose), as well as 50 days and 3 months after the first vaccine dose, via a previously specified protocol.[2] Serum was separated by centrifugation for 15 min at 1500 $\times$ g at room temperature, divided in aliquots and stored at -70 °C until measurement. Total anti-SARS-CoV-2 antibodies were assayed using the Roche Elecsys Anti-SARS-CoV-2 S chemiluminescent immunoassay, on a Roche Cobas 6000 (Roche Diagnostics, Basel, Switzerland). This method was found to have good correlation and agreement with a pseudovirus neutralizing test ($r = 0.58$; Cohen's kappa, 0.60)[3].

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). Comparisons between groups were carried out with Mann-Whitney test or Chi square test (with Yates' correction), when appropriate. All participants provided written informed consents for receiving BNT162b2 mRNA COVID-19 vaccination and undergoing total anti-SARS-CoV-2 antibodies follow-up. 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 871 healthcare workers (median age 45 years, IQR 34–53 years; 65.7% females), 192 (22.0%) of which had positive total anti-SARS-CoV-2 S at baseline (i.e., >0.8 kU/L). No significant differences in age ($p = 0.191$) or sex ($p = 0.101$) were observed between baseline seronegative and seropositive subjects. The kinetics of total anti-SARS-CoV-2 S antibodies is shown in Fig. 1. The antibodies levels were consistently higher in baseline total anti-SARS-CoV-2 antibodies seropositive subjects throughout the study period (i.e., $p < 0.001$), and reached a peak after the second vaccine dose, though a significant decline was then observed in both cohorts at 3 months. Compared to the value measured at 30 days after the second vaccine dose, the decline was nearly 2-fold more accentuated in the seropositive than in the seronegative cohort (i.e., -57% vs. -33%; $p < 0.001$). Although no subject in either cohort displayed total anti-SARS-CoV-2 S antibodies values below the method-dependant cut-off (i.e., 0.8 kU/L), a reduction in serum levels was observed in 568/679 (83.7%) baseline seronegative subjects and in 173/192 (90.1%) baseline seropositive subjects ($p = 0.018$). Interestingly, in baseline anti-SARS-CoV-2 seronegative subjects, the total anti-SARS-CoV-2 S antibodies values were significantly lower (i.e., $p<0.001$) at 30 days and at 3 months after the first vaccine dose in those aged >65 years compared to younger patients (Fig. 1). No statistically significant differences were instead observed between sexes in total anti-SARS-CoV-2 S antibodies levels as well as in the 3-month variation.

In conclusion, the results of our study extend the evidence provided by Tré-Hardy et al.[1] that total anti-SARS-CoV-2 S antibodies levels tend to decline at 3 months after the first vaccine dose, though we also showed that such decline occurs in both baseline seropositive and seronegative subjects, and with serum levels that tend to be significantly lower in older subjects. These findings not only confirm the importance of serological testing for monitoring the immunogenic response after vaccination,[4] but provide further support to the conclusion that personalization of COVID-19 vaccine administration, with use of further vaccine boosters, may be advisable in selected categories of the population.

Ethical approval

The study protocol (number 3246CESC) was cleared by the Ethics Committee of the Provinces of Verona and Rovigo. All sub-

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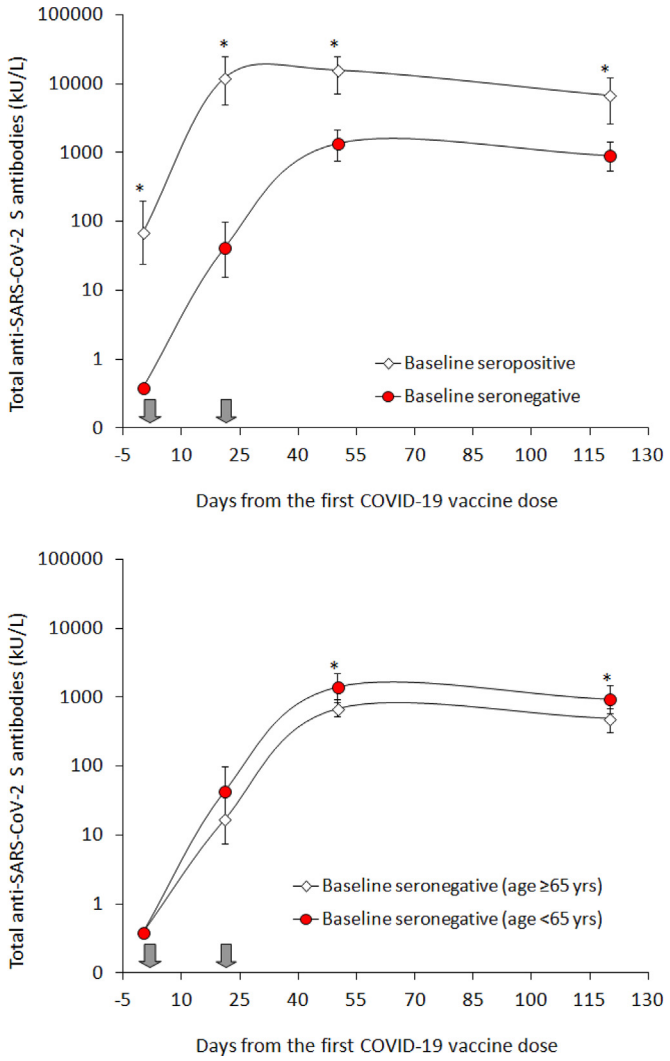

Fig. 1.. Total anti-SARS-CoV-2 S antibodies response in healthcare workers undergoing Pfizer BNT162b2 mRNA vaccination. Serum samples were drown at baseline, 21 days after the first vaccine dose, 30 days after the second vaccine dose (50 days after the first vaccine dose) and 3 months after the first vaccine dose in both anti-SARS-CoV-2 seronegative and seropositive subjects. Results are shown as median and interquartile range. The green arrows indicate the two vaccine doses administration.

* $p<0.001$

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

jects were informed of the study and voluntarily agreed to participate, providing a written consent).

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Declaration of Competing Interest

The authors have no relevant competing interest to disclose in relation to this work.

CRediT authorship contribution statement

Gian Luca Salvagno: Conceptualization, Visualization, Investigation, Funding acquisition, Formal analysis, Data curation. **Brandon M. Henry:** Investigation, Funding acquisition, Formal analysis, Data curation, Visualization, Writing – review & editing. **Laura Pighi:** Investigation, Funding acquisition, Formal analysis, Data curation, Visualization. **Simone De Nitto:** Investigation, Funding acquisition, Formal analysis, Data curation, Visualization. **Gian Luca Gianfilippi:** Conceptualization, Visualization, Investigation, Funding acquisition, Formal analysis, Data curation. **Giuseppe Lippi:** Conceptualization, Visualization, Investigation, Funding acquisition, Formal analysis, Data curation, Supervision, Writing – original draft.

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Gian Luca Salvagno
Section of Clinical Biochemistry, University Hospital of Verona, Piazzale L.A. Scuro, 10, Verona 37134, Italy
Service of Laboratory Medicine, Pederzoli Hospital, Peschiera del Garda, Italy

Brandon M. Henry
The Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States

Laura Pighi, Simone De Nitto
Section of Clinical Biochemistry, University Hospital of Verona, Piazzale L.A. Scuro, 10, Verona 37134, Italy
Service of Laboratory Medicine, Pederzoli Hospital, Peschiera del Garda, Italy

Gian Luca Gianfilippi
Medical Direction, Pederzoli Hospital, Peschiera del Garda, Italy

Giuseppe Lippi*
Section of Clinical Biochemistry, University Hospital of Verona, Piazzale L.A. Scuro, 10, Verona 37134, Italy

*Corresponding author.
E-mail addresses: giuseppe.lippi@univr.it, ulippi@tin.it (G. Lippi)