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Letter to the editor

Induction of immune response after SARS-CoV-2 mRNA BNT162b2 vaccination in healthcare workers

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A two-dose regimen of the BNT162b2 mRNA COVID-19 vaccine (30 μg per dose, given 21 days apart) was found to be safe and 95% effective against COVID-19, with mild to moderate side-effects gone within a few days in recently published clinical trials.[1,2] Due to the high level of protection against COVID-19 needed in the current pandemic, the European Medicines Agency (EMA) authorised its use in the EU on December 21, 2020.

Recent real-world data have shown a 30–50% reduction of COVID-19 cases after the first vaccine dose.[3,4] This increased to 75% after the second dose with an 85% drop in COVID-19 hospitalizations.[4,5]

In Italy, the COVID-19 vaccination campaign started on December 27, 2020, having as primary target healthcare workers (HCWs). Here, we describe the immunogenicity data induced by two injections of BNT162b2, 30 μg 21 days apart, in a subset of 965 HCWs vaccinated at the Bambino-Gesù Children Hospital, Rome, Italy (292 [30.2%] males, median age: 46 years [IQR:36–56]).

Serum samples were available before the first dose and at 7, 21, and 28 days afterwards. All HCWs had a negative SARS-CoV-2 status by molecular (Allplex2019-nCov, Seegene) and antibody assays (Elecsys®Anti-SARS-CoV-2, Roche). Presence and titers of anti-spike (S) antibodies in serum samples were evaluated by Elecsys®Anti-SARS-CoV-2-S (Roche, cut-off:0.8U/mL) detecting antibody against the S1-receptor-binding-domain (RBD), and the LIAISON® SARS-CoV-2 TrimericS IgG assay (DiaSorin, cut-off: 13 AU/mL), detecting IgG against the trimeric S protein. Recipients were stratified according to age (23–55 or ≥56 years) and sex. The ability of the BNT162b2-vaccine in eliciting SARS-CoV-2 specific cellular responses was assessed by trimeric spike-specific memory B and T cells in a subset of 28 HCWs, randomly selected from the enrolled population. Among them, 11 were males (39.3%) and 10 aged ≥56 years (36%; median age [IQR]: 55 [26–67] years). Refer to Supplementary Information for memory B and T cells methodology.[6-9]

At day 7, while only few individuals had low positive titers (2 to anti-RBD:3.31 and 6.95 U/mL, and 5 to the S protein: median 26 [IQR:21–31]) (Fig .1A–B), a primary cellular response was already present. Indeed, a significant increase of B cells specific for the trimeric spike protein was found in the peripheral blood of 84% of vaccinees by flow cytometry in unstimulated peripheral blood mononuclear cells ($P = 0.0002$), and by ELISpot after polyclonal stimulation ($P < 0.0001$) (Fig . 1C–D). At this time-point, all B cells specific for the trimeric spike were IgM memory B cells.[6] In line with B cell data, we observed a significant expansion of SARS-CoV-2 specific CD4+ T cells[7] ($P = 0.0202$), with 64% of vaccinees showing elevated proportion of $CD4^{+}CD40L+$ T cells (Fig. 1E). Thus, our data confirms the ability of this vaccine to induce strong T cell responses, as reported by others.[8]

At day 21 (before the second administration), 97.5% of individuals showed positive anti-RBD antibodies, though with modest titers (median:54[IQR:26–109], Fig. 1A). Similar results were observed for anti-trimeric S antibodies (Fig. 1B). A further increase of trimeric spike-specific IgM B cells by ELISpot was observed, thus suggesting that this vaccine rapidly activates the germline repertoire and favors the adaptability of the human memory B cell pool to the SARS-CoV-2 challenge.[10–12]

The highest anti-S titers were detected in samples at day 28, when 99.8% of subjects showed a median anti-RBD titer of 2003(IQR:1089-2,500, Fig. 1A), values far higher than those reported to have neutralizing activity.[2] Similar results were observed for the anti-trimeric S titers (Fig. 1B). Noteworthy, in the 28 HCWs in whom the trimeric spike-specific memory B and T cells were explored, the magnitude of anti-S antibody production detected at day 28 significantly correlated ($P = 0.018$; $rho = 0.44$) with the increase of SARS-CoV-2 specific CD4+ T cells detected at day 7. When stratified for age, the correlation was still significant only in the group of individuals aged 22–55 years ($rho = 0.53$, $p = 0.02$), but not in the ≥56 year-old group, probably due to the small and not representative sample size. The role of SARS-CoV-2 CD4+ T cell expansion in predicting the antibody titer as described here as a preliminary finding requires further investigation and a larger sample size to be confirmed.

Of note, the anti-RBD titer was significantly higher in 23–55 year-old participants compared to ≥56 year-old ones at both 21 and 28 days (Day 21: 39[IQR:15–98] vs. 14[IQR:4–45], $P = 0.000003$; Day 28: 2197 [IQR:1260-2557] vs. 1525[IQR:555-2500], $P = 0.0000005$) (Fig. 1A). Similar results were observed for anti-trimeric S titers (Fig. 1B). These results were also confirmed in both male and female HCWs. The lower

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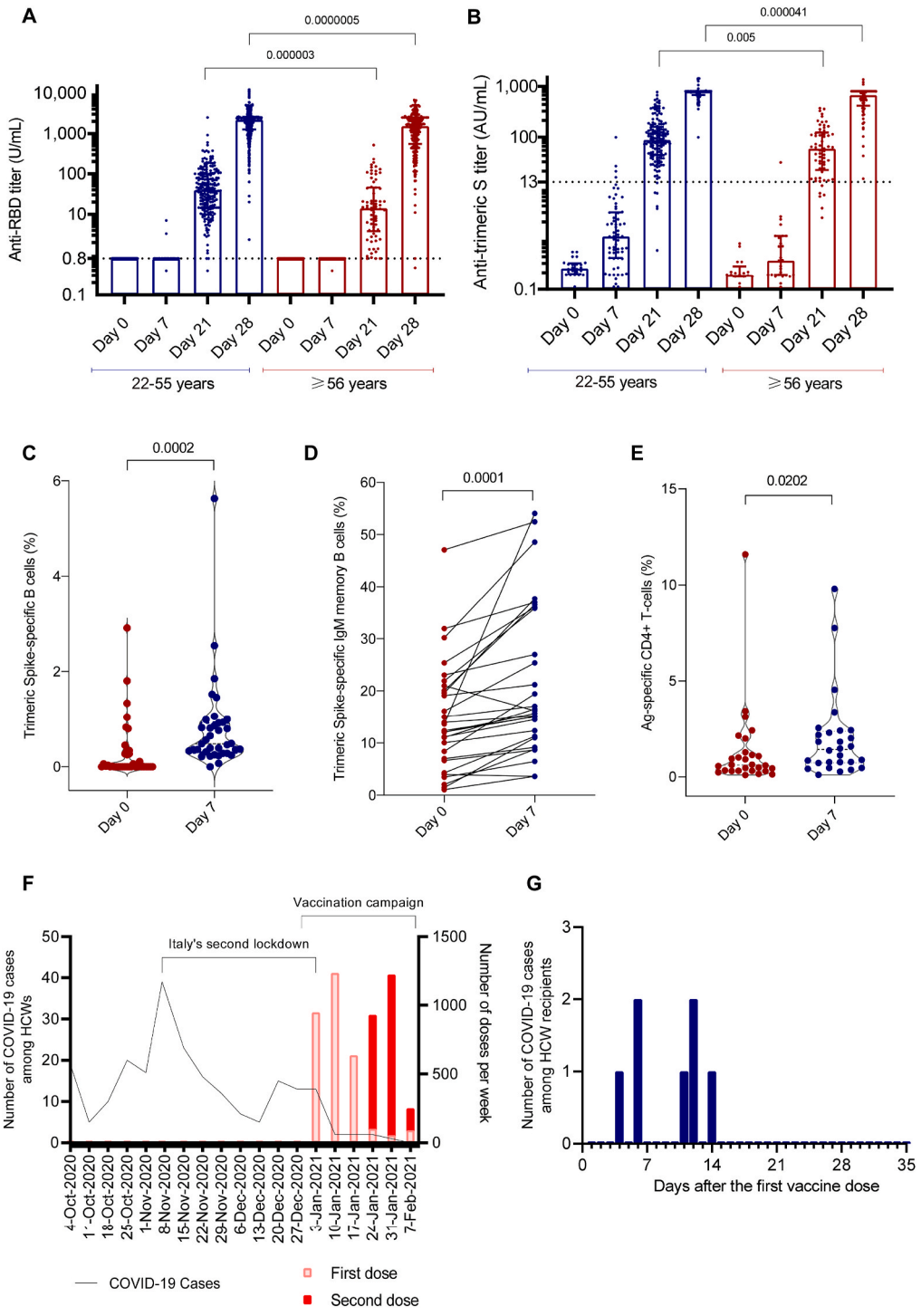

Fig. 1. SARS-CoV-2 antibody responses (Panels A-B). Anti-RBD and anti-trimeric S titers assessed by Elecsys® Anti-SARS-CoV-2 S assay (Roche, cut-off 0.8 U/mL) (Panel A) LIAISON® SARS-CoV-2 TrimericS IgG assay (Diasorin, cut-off 13 AU/mL) (Panel B) before vaccine administration (Day 0), at 7 (Day 7), 21 (Day 21) and 28 (Day 28) days after the first dose, against participants' age. Anti-S titers at different time-points were compared according to age by the Mann-Whitney test. Scatter dot plots denote interquartile ranges and horizontal bars median end-point titers. Whisker end-points denote the maximum and minimum values below or above the median at 1.5 times the interquartile range. The box indicates the width of titers' distribution proportionate to the number of points at that Y value. Blue and red dots defined 18–55 and $\geq$ 56 years ranges, respectively. Each dot represents a single patient. **Frequency of trimeric spike-specific B and T cells 7 days after the first vaccine dose (Panels C, D, E).** The frequency of trimeric spike-specific B cells before and 7 days after the first dose was showed by violin plot (Panel C). Connected scatter plot depicts the percentage in ELISpot of trimeric spike-specific IgM memory B cells (calculated within the total IgM memory B cells) (Panel D). Antigen-specific CD4+T cells expansion at 7 days following the first dose of vaccination is shown by violin plot (Panel E). Blue and red dots defined values at day 0 and day 7, respectively. Each dot represents a single patient. Statistical significance was calculated using the paired-Wilcoxon test and a P-value <0.05 was considered significant. Ag = antigen. **Number of COVID-19 cases among HCWs and number of vaccinations against weeks (Panel F).** Black line indicates the number of COVID-19 among HCWs. Red bars indicate the number of second doses against date. Pink bars indicate the number of first doses against date. **SARS-CoV-2 infections occurring among HCWs after the first vaccine dose against days after the first vaccine dose (Panel G).** Black bars indicate the number of infection cases. No infection occurred after day 14. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

titer observed in our aged HCWs was confirmed by other recently published reports,[13,14] and, even if at day 28 the anti-S titers reached levels higher than those reported to have neutralizing activity, monitoring of neutralizing antibodies over time is needed to detect potential drop as soon as possible, especially in the older population, known to be at higher risk of serious disease manifestation.[15]

The weekly tracing of SARS-CoV-2 infections among HCWs in our hospital (see Supplementary Information for detailed description of tracing) showed a consistent number of new infections during the whole of the second wave, from October 2020 until the beginning of the vaccination campaign (Fig. 1F), generally characterized by high SARS-CoV-2 load (median:$>10^6$ copies/mL). During the vaccination period, 7 HCWs developed SARS-CoV-2 infection (as confirmed by molecular positive swabbing), all within 14 days of the first administration, and all with a SARS-CoV-2 load by ddPCR) <1000 copies/mL (Fig. 1G).[16] Three were submitted to molecular testing after a contact with infected cohabiting family members, while four as a routine control. Six were women (85.7%) and 2 aged >55 years. The departments they worked in had a similar Covid-19 risk. No information about anti-S titers before the SARS-CoV-2 infection was available.

Overall, the immunological results combined with the decrease of new COVID-19 cases among HCWs in the observed time frame demonstrate an excellent performance of the COVID-19 mRNA BNT162b2 in daily practice. This data is fully consistent with recent published reports from front-running countries describing high rates of immune responses to vaccination in HCWs following just a single dose of BNT162b2 vaccine,[14] low incidence of SARS-CoV-2 infections among vaccinated HCWs,[17] and low viral load among post-vaccinated infections.[18] Prolonged structured follow-up and monitoring are required to confirm the long-term efficacy of COVID-19 vaccination strategy.

Declaration of interest

We declare no competing interest.

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Ethical approval

Ethical approval was obtained from the Bambino Gesù Children’s Hospital IRCCS Ethical Committee. Written informed consent was provided by all participants.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jve.2021.100046.

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