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MAJOR ARTICLE

Early Immunogenicity and Safety of the Third Dose of BNT162b2 Messenger RNA Coronavirus Disease 2019 Vaccine Among Adults Older Than 60 Years: Real-World Experience

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Background. Despite high vaccine coverage, an increase in breakthrough coronavirus disease 2019 (COVID-19) infections, prompted administration of a third BNT162b2 dose to people aged >60 years in Israel since July 2021. Here, we report real-world immunogenicity following third dose.

Methods. Overall, 208 healthcare workers aged >60 years were included. Paired pre– and post–second and/or third dose immunoglobulin G (IgG) and neutralizing antibody titers were compared. A subpopulation of low responders to the second dose was also tested for T-cell activation. For 25 paired serum samples, we tested neutralization of wild-type vs neutralization of Delta and Lambda variants, pre– and post–third dose. Active surveillance of vaccine adverse events was conducted through surveys.

Results. A pronounced immune response was observed following the third dose, including a 33-fold and 51-fold increase in IgG and neutralizing antibody, respectively. The neutralizing antibody levels post–third dose were 9.34 times higher than post–second dose (geometric mean titer, 2598 [95% confidence interval {CI}, 2085–3237] vs 207 [95% CI, 126–339]). Nine previously low responders had a significant antibody increase post–third dose, and 7 of 9 showed increase in T-cell activation. Additionally, sera obtained post–third dose highly and comparably neutralized the wild-type and Delta and Lambda variants. Of 1056 responders to the adverse-event survey, none had serious events.

Conclusions. We demonstrate a rapid and broad immune response to the third BNT162b2 dose in individuals >60 years of age.

Keywords. BNT162b2; COVID-19; boosting effect; third dose; elderly.

The introduction of the BNT162b2 messenger RNA (mRNA) coronavirus disease 2019 (COVID-19) vaccine has been a tipping point in harnessing the COVID-19 pandemic. Earlier attempts to control pandemic surges with lockdowns and social distancing were not as effective, with 203 million cases worldwide and >4 million deaths [1]. In Israel, where the vaccine rollout was early and efficient, an impressive decline in new cases was achieved a little over 2 months since the initiation of the vaccination campaign, with a marked decrease in severe cases and hospitalizations [2]. Facing the emergence of the Delta variant 6 months later, with evidence of waning of vaccine immunity [3] and decreasing vaccine effectiveness, with increasing severe disease among the elderly [4], Israel introduced a third booster dose of BNT162b2, initially to adults over the age of 60 years, since 29 July 2021 and eventually to all those who were vaccinated with a second dose at least 5 months earlier.

The initially recommended BNT162b2 vaccine schedule included 2 doses with a suggested 3-week interval between doses [5]. This schedule was decided upon with the assumption that it would improve immune response [6]. Many vaccines, such as the pertussis vaccine, the hepatitis B virus vaccine, and others, have established schedules that include 3 doses for increased immunogenicity [7]. As data are still accumulating, it might be too early to determine the best schedule for the BNT162b2 vaccine, and perhaps the waning immunity that is currently apparent is a consequence of suboptimal scheduling.

Following many discussions and accumulating data showing a decay in humoral immunity among vaccinated population within 6 months [8], decreased effectiveness of the vaccine with time [4, 9], and the recently reported correlation between

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humoral immunity and breakthrough infections [10], a third dose of BNT162b2 was approved by the Israeli Ministry of Health for individuals >60 years of age. Here, we present the early immune response (within 14 days) as well as initial safety data following the third dose.

MATERIALS AND METHODS

Study Setting

Sheba Medical Center is the largest tertiary medical center in Israel, with 14 519 healthcare workers (HCWs) including 10 747 employees, 539 students, 1167 volunteers, and 2066 retired HCWs. Between December 2020 and April 2021, a total of 91% of the center personnel received 2 doses of the BNT162b2 vaccine. Of these, 3829 were older than 60 years. On 28 July 2021, the Israeli Ministry of Health decided to administer a third vaccine dose to individuals aged 60 years or older. Of all 3829 Sheba HCWs aged >60 years, 3522 received 2 COVID-19 vaccine doses by March 2021 and were eligible to receive the third dose, which was offered at the medical center. Of these, 974 HCWs aged >60 years participated in the Sheba HCW serology study [11] with monthly serology follow-up.

Study Design and Population

On 29 July 2021, the first HCWs over 60 years old received the third vaccine dose. HCWs included in this study were those who fulfilled the following criteria: (1) age >60 years; (2) were recruited to the Sheba HCW serology study since receiving the second dose; (3) had available paired sera from before/after second dose; and/or (4) had available paired sera from before/after third dose. Those who did not have a serology test from the previous month had a test obtained prior to receiving the third vaccine dose. One week to 2 weeks after receiving the third dose, an additional serology test was collected (Figure 1). Of study participants, 72 had serology tests before and after the second dose; of these, 67 had both immunoglobulin G (IgG) and neutralizing antibody assays. One hundred fifty-nine had serology tests before and after the third dose. For 29 HCWs, tests from all 4 time-points were available. Tests were conducted for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) receptor-binding domain (RBD) IgG testing, and for a select sample, SARS-CoV-2 pseudoneutralization assay was conducted as well. The study population is described in Supplementary Table 1.

A subpopulation of 9 participants was selected from those who had no or low-level humoral immune response detected following the first and second doses of BNT162b2 (IgG and neutralizing antibodies were below or close to the cutoff), defined as low responders. These participants had no known immunosuppression and did not receive any medication that could impair immune response. For these low responders, both humoral and T-cell activation was determined before and after the third dose.

SARS-CoV-2 RBD IgG Testing

Samples were centrifuged at 4000*g* for 4 minutes in room temperature. Serum was tested for IgG antibodies against SARS-CoV-2 RBD using the commercial automatic chemiluminescent microparticle immunoassay SARS-CoV-2 IgG II Quant (Abbott Laboratories, Abbott Park, Illinois) according to the manufacturer's instructions. Antibody levels were measured in binding antibody units (BAU) per the World Health Organization standard measurements.

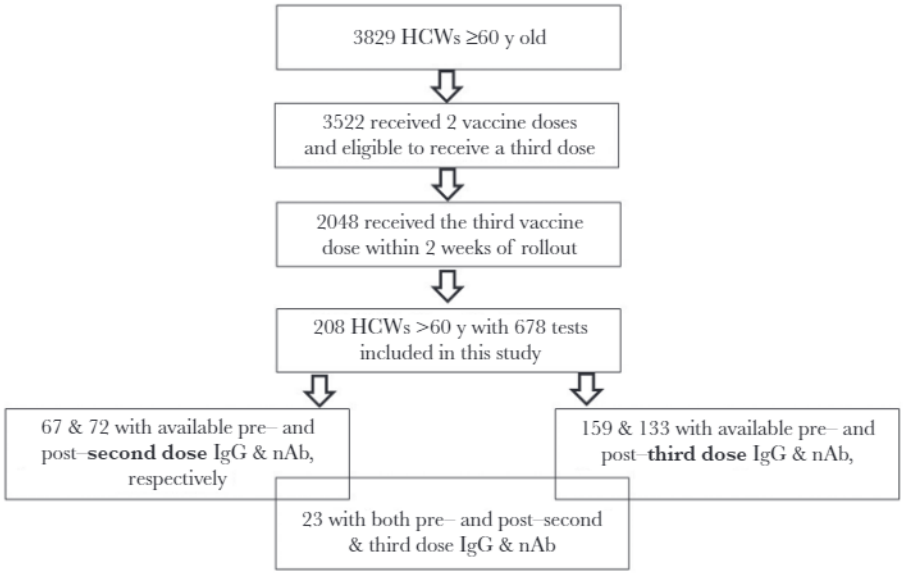

Figure 1. Flowchart of the study population and inclusion criteria. Abbreviations: IgG, immunoglobulin G; HCWs, healthcare workers; nAb, neutralizing antibody.

SARS-CoV-2 Pseudoneutralization Assay

A SARS-CoV-2 pseudovirus neutralization assay was performed as previously described [11] to detect SARS-CoV-2 neutralizing antibodies using a green fluorescent protein reporter–based pseudotyped virus with a vesicular stomatitis virus backbone coated with the SARS-CoV-2 spike (S) protein, which was generously provided by Dr Gert Zimmer (Institute of Virology and Immunology, Mittelhäusern, Switzerland). A ≥4-fold increase in neutralization titers between 2 consecutive samples was considered a significant increase.

SARS-CoV-2 Microneutralization Assay

To further determine neutralization of different strains by serum of vaccinated individuals, before and after receiving the third booster shot, a SARS-CoV-2 microneutralization assay was performed as previously described [12] on a convenience sample of 25. In brief, Vero-E6 cells at a concentration of 20×10^3 cells/well were seeded in sterile 96-well plates with 10% fetal calf serum minimum essential–Eagle medium, and incubated at 37°C for 24 hours. One hundred $TCID_{50}$ (median tissue culture infective dose) of wild-type (hCoV19/Israel/CVL-45526-ngs/2020), B.1.617.2 (Delta, hCoV-19/Israel/CVL-12804/2021), and C.37 (Lambda, hCoV-19/Israel/CVL-13489-ngs/2020) SARS-CoV-2 isolates were incubated with heat-inactivated samples diluted 1:8 to 1:16 384 in 96-well plates for 60 minutes at 33°C. Virus serum mixtures were added to the Vero-E6 cells and incubated for 5 days at 33°C, after which Gentian violet staining (1%) was used to stain and fix the cell culture layer. Neutralizing dilution of each serum sample was determined by identifying the well with serum dilutions without observable cytopathic effect. A dilution equal to ≥1:10 was considered neutralizing.

Isolation of Peripheral Blood Mononuclear Cells

To assess a T-cell response in a subpopulation with a low humoral response, peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using UNI-SEP+ (Novamed). Plasma was collected and spun at 1000*g* for 20 minutes to remove platelets before collection of PBMCs. Following 1 wash with phosphate-buffered saline and 1 wash with 4Cell Nutri-T-Medium (Sartorius), cells were resuspended in 4Cell Nutri-T-Medium and counted using the Countess II Cell counter (Invitrogen).

Interferon-γ Enzyme-Linked Immunosorbent Spot Assay

PBMCs collected by Ficoll were either frozen with CTL-Cryo ABC Media Kit (CTL, Germany) according to the manufacturer's protocol and kept at −70°C or used fresh. Frozen PBMCs were thawed 1 day prior to analysis, washed, and left for overnight rest in CTL test medium. Interferon gamma (IFN-γ)–secreting cells were enumerated using enzyme-linked immunosorbent spot assay (ELIspot) IFN-γ kits (IFN-γ kit, AID Autoimmun Diagnostika GmbH, Strassberg, Germany or IFN-γ FluoroSpot kit, CTL) according to the manufacturer's instructions. For antigen stimulation, 50 µL of SARS-CoV-2 peptide pools (S-complete, Miltenyi Biotec, or PM-WCPV-S, JPT Peptide Technologies GmbH, Berlin, Germany) covering the complete protein coding sequence of the original Wuhan-like strain spike glycoprotein of SARS-CoV-2 were used. Test medium was used as negative control and phytohemagglutinin was used as positive control. IFN-γ–secreting cell frequency was quantified using the ImmunoSpot S6 Ultimate reader (CTL) or the AID ELISpot Reader (Strassberg, Germany). The unspecific background (mean spot forming unit from negative control wells) was subtracted from experimental readings.

Adverse Event Active Surveillance

One week after receiving the third booster, all vaccinated HCWs (n = 2048) received a text message or a phone call with a short questionnaire survey regarding side effects. They were asked about various localized and systemic side effects, the duration of these symptoms, and whether they required medical care or hospitalization. Additionally, HCWs and their treating physicians were encouraged to report any serious adverse event or hospitalization.

Statistical Analysis

Log transformation was calculated for IgG and neutralizing antibody results. To compare paired pre– and post–second or third dose antibody levels, as well as post–second to post–third dose levels, log of antibody levels were compared using Wilcoxon signed-rank test. Data analysis was performed using SAS for UNIX 9.4 software.

Ethical Considerations

All HCWs who received the vaccine in the medical center were offered to participate in this study, and provided written informed consent. The study was approved by the institutional review board at Sheba Medical Center.

RESULTS

Sheba Medical Center initiated the third dose BNT162b2 vaccine rollout to all workers, volunteers, and retired personnel aged >60 years on 30 July 2021. For this study, 208 HCWs were eligible and had either pre– and post–second dose paired sera (n = 72), or pre– and post–third dose paired sera (n = 159) or all 4 samples available (n = 23) (Figure 1). The study population is described in Supplementary Table 1.

Anti-S IgG Antibody Levels

The IgG geometric mean titer (GMT) of the pre–second dose was 29.3 BAU (95% confidence interval [CI], 20.2–42.6) (n = 67), and the GMT post–second dose was 733.8 BAU (95% CI, 472.3–1140) (Table 1). The pre– and post–third dose IgG titers were 65.8 BAU (95% CI, 57.0–76.0) vs 2189 BAU (95% CI, 1834–2613) (Figure 2A and 2B). The vast majority of participants (89%) had a >10-fold increase in their IgG levels, and 7 (4%) had no substantial change (<4-fold increase). Among 23 participants who had

Table 1. Summary of Immune Responses Before and After Second and Third Doses of BNT162b2

Characteristic	Titer	Pre–Second Dose	Post–Second Dose	Pre–Third Dose	Post–Third Dose
Time before/after vaccine, d, mean (IQR)		4.7 (0–7)	7.7 (7–7)	3.2 (0–5.5)	9 (7–11)
IgG (second dose, n = 67; third dose, n = 159)	Geometric mean	29.3	733.8	65.8	2189
	Upper and lower 95% CI	20.2–42.6	472.3–1140	57.0–76.0	1834–2613
nAbs (second dose, n = 72; third dose, n = 133)	Geometric mean	3.2	207	50.9	2598
	Upper and lower 95% CI	2.3–4.5	126.4–339.5	41.8–61.9	2085–3237
Subcohort of participants with all 4 time-points available (n = 23)					
IgG	Geometric mean	25.8	803.1	58.6	1787
	Upper and lower 95% CI	13.4–49.7	402–1602	39.6–86.6	1049–3045
nAbs	Geometric mean	3.8	226.9	51.8	2120
	Upper and lower 95% CI	2.1–6.8	92.9–554.3	35.9–74.8	1220–3686
Low responders (n = 9)					
IgG	Geometric mean	…	16.1	11.2	1041
	Upper and lower 95% CI	…	3.8–67.9	6.0–21.2	476–2274
nAbs	Geometric mean	…	5.4	21.7	4476
	Upper and lower 95% CI	…	1.4–20.7	5.6–84.9	2300–8708
T cells	IFN-γ–secreting cells/10^6 PBMCs	…	…	16.9	57.8
	Upper and lower 95% CI	…	…	7.2–40.2	30.9–108
Microneutralization assay (n = 25)					
Wild-type	Geometric mean	…	…	24.25	843.4
	Upper and lower 95% CI	…	…	17.25–34.09	489.1–1454
Delta variant	Geometric mean	…	…	21.71	604.7
	Upper and lower 95% CI	…	…	15.13–31.14	360.3–1015
Lambda variant	Geometric mean	…	…	17.39	675.6
	Upper and lower 95% CI	…	…	10.79–28.01	381.2–1197

Abbreviations: CI, confidence interval; IFN-γ, interferon gamma; IgG, immunoglobulin G; IQR, interquartile range; nAbs, neutralizing antibodies; PBMCs, peripheral blood mononuclear cells.

samples from all 4 time-points, the geometric mean of IgG titers was higher after the third dose than after the second dose (1787 IU [95% CI, 1049–3045] vs 803.1 IU [95% CI, 402–1602]), with a mean fold difference of 2.23 (95% CI, 1.24–3.99) (Figure 2C).

Pseudoneutralization Assay

Neutralizing antibodies obtained from 72 HCWs aged >60 years increased from 3.2 (95% CI, 2.3–4.5) to 207 (95% CI, 126.4–339.5) before and after the second dose, respectively. Following the third dose, neutralizing antibody results were available for 133 participants; all of them had an increase in their titers, with GMT of 50.9 (95% CI, 41.8–61.9) and 2598 (95% CI, 2085–3237) in the pre– and post–third dose assays, respectively (Table 1, Figure 2D and 2E). For 23 participants, neutralization assay results were available in all 4 periods: pre– and post–second dose as well as pre– and post–third dose. The GMT after the third dose was significantly higher than after the second dose (2120 [95% CI, 1220–3686] vs 226.9 [95% CI, 92.9–554.3]) (GMT fold difference of 9.34 [95% CI, 4.13–21.13]) (Figure 2F).

Neutralization of Wild-Type vs Delta and Lambda Strains

To test whether administration of the third dose results in improved neutralization of WT as well as Delta and Lambda strains, we obtained paired serum samples from 25 vaccinated individuals before and after the third vaccine dose (Table 1). The third dose led to a significant increase in neutralization capacity of sera derived from all individuals (Figure 3). Overall, the following fold changes in neutralizing titers were observed: 34-fold (95% CI, 21.3–56.8) (GMT fold difference of 34.7 [95% CI, 21.3–56.8]) for the wild-type virus; 28-fold (95% CI, 18.1–42.8) (GMT fold difference of 27.8 [95% CI, 18.1–42.7]) for the Delta variant; and 38.85-fold (95% CI, 22.82–66.16) (GMT fold difference of 38.5 [95% CI, 66.7–228.3]) for the Lambda variant (Figure 3).

Low Responders and T-Cell Function

IgG antibodies against the RBD in 9 low responders increased from 16.1 (95% CI, 3.8–67.9) after the second dose to 1041 (95% CI, 47.6–2274) after the third dose (Figure 3A). All low responders had an increase in neutralizing antibodies, from GMT of 5.4 (95% CI, 1.4–20.7) after the second dose to 4476 (95% CI, 2300–8708) after the third dose (Figure 3B).

Seven of 9 HCWs had an increase in T-cell activation as presented by secretion of IFN-γ after exposure to peptides of the spike protein. The average number of IFN-γ secreting T cells was 16.9 (95% CI, 7.2–40.2) and 57.8 (95% CI, 30.9–108) before and after the third vaccine dose, respectively (GMT difference, 3.45 [95% CI, 1.05–11.1]) (Figure 4C).

Active Surveillance of Adverse Effects

In total, 1059 HCWs responded to an adverse event survey, 950 to an electronic survey, and an additional 100 to an identical telephone survey. The most common adverse event was localized pain

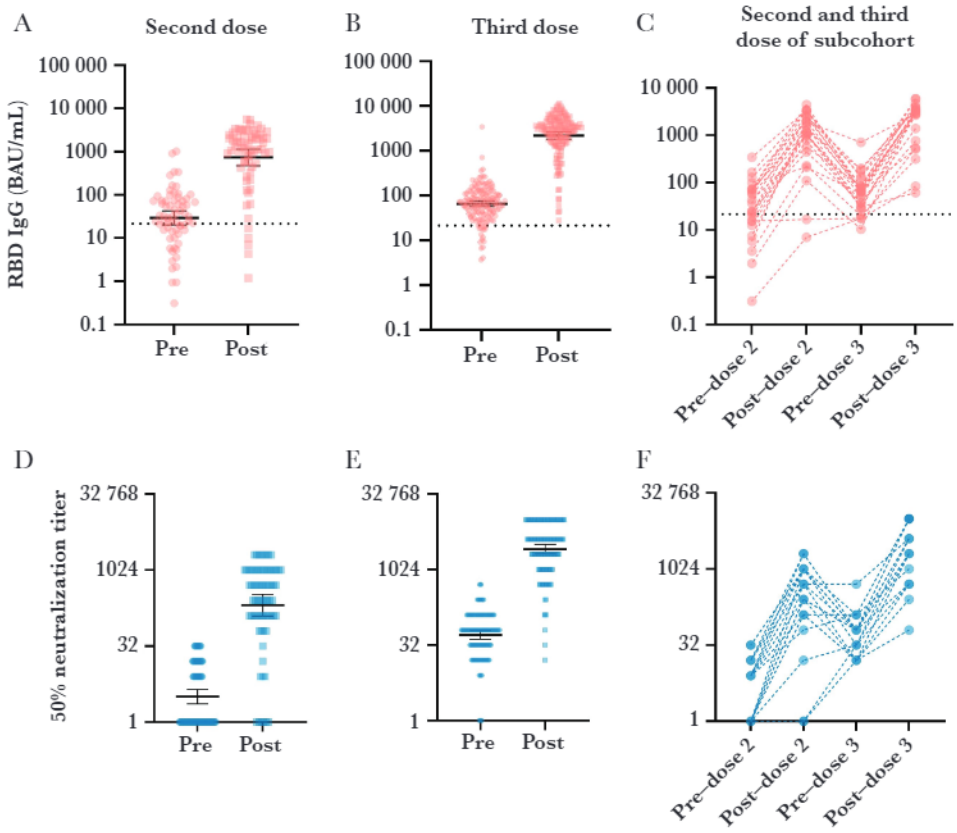

Figure 2. Paired receptor-binding domain immunoglobulin G (IgG) and 50% neutralization titer prior to and 1–2 weeks after receiving second and third dose of BNT162b2 messenger RNA coronavirus disease 2019 vaccine. *A*, IgG pre– and post–second dose of vaccine. *B*, IgG pre– and post–third dose of vaccine. *C*, IgG levels before and after second and third dose of a cohort of 23 participants. *D*, Neutralization titer pre– and post–second dose of vaccine. *E*, Neutralization titer pre– and post–third dose of vaccine. *F*, Neutralization titers before and after second and third dose of a cohort of 23 participants. Abbreviations: BAU, binding antibody units; IgG, immunoglobulin G; RBD, receptor-binding domain.

in the site of injection, experienced by 805 of 1079 (76%) respondents; overall, 849 of 1059 (80%) experienced a local adverse event, most commonly local pain (76%) (Supplementary Table 2). The mean duration of these symptoms was 2.1 days (standard deviation [SD], 1.3). Systemic symptoms were not as common, experienced by 436 of 1059 participants (41%), the most common being fatigue, reported by 353 of 1059 (33%). Fever (defined as a temperature >37.5°C) was reported by 84 of 1059 participants (8%), with a mean duration of 1.44 (SD, 1.35) days. The mean length of systemic symptoms reported was 2.2 (SD, 1.6) days. None of the participants had an adverse event requiring hospitalization, and 15 of 1059 (1.4%) required some medical attention, mostly antipyretic and anti-inflammatory medications provided for pain and fever. In addition to the active surveillance, HCWs were encouraged to report serious adverse events to a designated hotline or through the treating physician, and no such reports were received of hospitalizations or of other serious adverse events.

DISCUSSION

In this real-life study of adults older than 60 years, we demonstrate that within 1 week after receiving the third dose of the BNT162b2 mRNA COVID-19 vaccine, a significant response was observed in all participants, even among those who did not respond to previous doses. Moreover, the response to the third dose was greater than the response to the second dose, given approximately 5–6 months earlier. Furthermore, we detected a significant increase in the humoral and T-cell responses following the third vaccine dose in a subgroup of low responders.

By comparing the neutralization of wild-type vs the Delta and Lambda variants, we demonstrate effective and comparable neutralization of all 3 strains following the third dose. Finally, we present a very good safety profile of the third dose in this age group in real life, with respect to the more common local and systemic adverse events.

Since neutralizing antibody levels have been shown to be correlated with breakthrough infections [10, 13], these early results are very promising and reassuring, raising the expectation for a rapid clinical response with a decrease in severe and critical cases once a high enough third-dose coverage of the older population will be achieved.

One of the concerns about a third dose was that boosting with existing vaccines (that all currently incorporate only the

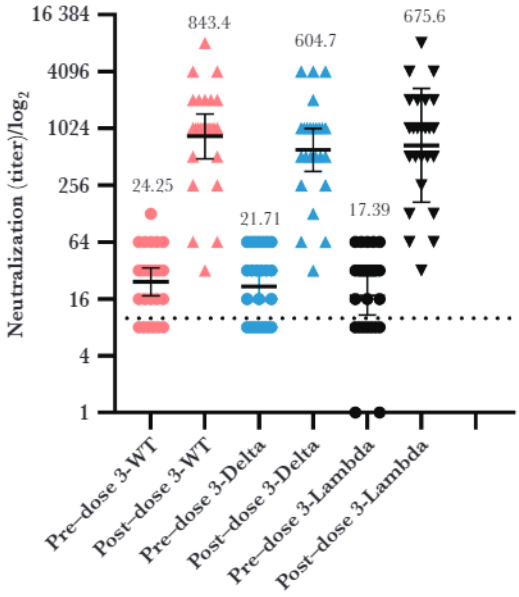

Figure 3. Microneutralization assay of sera of vaccinated individuals against wild-type strain, Delta variant, and Lambda variant viruses prior to and after receiving the third dose of BNT162b2 messenger RNA coronavirus disease 2019 vaccine. Abbreviation: WT, wild-type.

ancestral spike) might not be effective in providing protection against variant strains. Yet, here we demonstrated that the effect of the current vaccine against the Delta variant, as well as the recently emerging Lambda variant, was comparable to that of the original strain, as has been suggested in the model by Cromer et al [14].

The development and production of the BNT162b2 vaccine was unprecedented in the short time since SARS-CoV-2 emerged until the launching of large-scale vaccination campaigns [15]. As this agility was crucial when facing a deadly pandemic, it seems that the decision to administer the vaccine in 2 doses only 3 weeks apart might not have been biologically optimal for long-term immunity, yet may have been necessary to effectively prevent infections. A study from England, where extension of the interval between vaccine doses was introduced to accelerate population coverage, suggested that delaying the second dose of the vaccine may increase long-term T-cell response [16].

The creation of an optimal schedule for vaccine administration is complicated, and lessons learned from vaccines against other viruses might assist in determining the best schedule for the BNT162b2 vaccine. For hepatitis B vaccine, it is long known that there is a correlation between the peak titer of anti-Hepatitis B surface antibodies 1 month after vaccination and the duration of the presence of antibodies [17, 18]. The reduction in vaccine effectiveness in people vaccinated in January compared to March [4], in addition to the accumulative data of antibody response serving as correlation of protection [10], suggests that a still-unknown antibody level is required for protection against infection. It is therefore plausible that waning antibody levels gradually after vaccination is responsible for the increase in breakthrough infections and decrease in vaccine effectiveness. Our observation that the GMT of neutralizing antibodies was 10-fold higher after the third dose, compared to that after the second dose, might suggest that this dose produced better booster effect and that its protection might last longer.

A third dose has been administered to immunocompromised patients, and in these patients significant humoral and cellular responses were noted [19–21]; nonetheless, these patients also experienced a higher frequency of systemic adverse events [21]. Among our participants the frequency of serious adverse events was negligible, and mild adverse events were of short duration.

This study has a few limitations. First, we describe a very early immunogenic effect that might not represent the peak

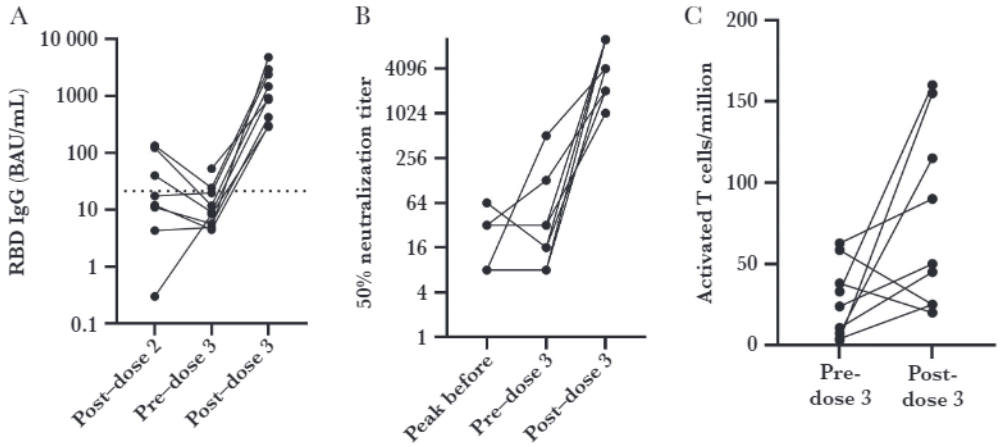

Figure 4. Results of receptor-binding domain (RBD) immunoglobulin G (IgG), 50% neutralization titers, and T-cell activity of 9 participants who responded poorly to the first 2 doses of the BNT162b2 vaccine. *A*, RBD IgG including the levels after the second dose of vaccine, levels prior to third dose, and levels 1 week after third dose. *B*, 50% neutralization titer of these participants after second dose, before third dose, and 1 week after third dose. *C*, Interferon-γ secretion frequencies following T-cell activation with spike protein before and after third dose. Abbreviations: BAU, binding antibody units; IgG, immunoglobulin G; RBD, receptor-binding domain.

response, which may be higher at 2–4 weeks after vaccination [11]. Furthermore, as this study was performed <4 weeks since beginning of rollout of the third vaccine dose, it was too early to gather clinical data regarding the effectiveness in preventing SARS-CoV-2 infection and disease. Yet, since then the third vaccine dose has been reported to be highly effective [22]. A third potential limitation is the generalizability of the study, since the study population was HCWs (including retired, employees, and volunteers) who might be somewhat healthier than the general population above the age of 60.

The initial decision to vaccinate with a third dose all adults older than 60 years in Israel was taken after a significant rise in breakthrough infections among vaccinated patients [23]. This was rapidly followed by a decision to vaccinate all individuals who received the second dose at least 5 months earlier, regardless of age [24]. The introduction of the highly virulent Delta variant [25] combined with the decay in the humoral response 6 months following the second dose of the BNT162b2 vaccine, an effect that was more pronounced with increasing age [3]. Since the initiation of this vaccination campaign, several studies have reported reduced rates of transmission and severe disease [22, 26, 27].

In conclusion, here we show that in a real-life setting the third dose of the BNT162b2 vaccine produces high immunogenicity against wild-type strain as well as against the Delta and Lambda SARS-CoV-2 variants with a favorable safety profile; this, combined with early reports regarding decrease in infectivity, suggests that the benefits of this intervention might outweigh its risks.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. G. R. Y. has received institutional research grants from Pfizer on an unrelated topic (pneumococcal infections) and from Nanoscent LTD on COVID-19 volatile organic chemicals, and has received honoraria from MSD and Teva, unrelated to the topic of this study. All other authors report no potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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