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ORIGINAL CLINICAL SCIENCE

Kinetics of cellular and humoral responses to third BNT162B2 COVID-19 vaccine over six months in heart transplant recipients – implications for the omicron variant



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KEYWORDS:

heart transplantation;
BNT162b2 vaccine;
SARS-CoV-2-
specific T-cell;
neutralization;
omicron

BACKGROUND: The durability of the immune response following the 3-dose BNT162b2 vaccination is unknown. The complexity of the situation is enhanced by the threat that highly transmissible variants may further accelerate the decline in the protection afforded by mRNA vaccines.

METHODS: One hundred and three 3-dose-vaccinated heart transplant recipients were longitudinally assessed for the kinetics of variant-specific neutralization (Cohort 1, $n = 60$) and SARS-CoV-2-specific-T-cell response (Cohort 2, $n = 54$) over 6 months. Neutralization and T-cell responses were compared between paired samples at 2 time points, using the Kruskal-Wallis test followed by Dunn's multiple comparison test for continuous variables and McNemar's test for dichotomous variables. The Bonferroni method of p values adjustment for multiple comparison was applied.

RESULTS: The third dose induced high neutralization of the wild-type virus and delta variant (geometric mean titer [GMT], 137.2 [95% CI, 84.8-221.9] and 80.6, [95% CI, 49.3-132.0], respectively), and to a lesser degree of the omicron variant (GMT, 10.3 [95% CI, 5.9-17.9]). At 6 months, serum neutralizing activity declined but was still high for the wild-type virus and for the delta variant (GMTs 38.1 [95% CI, 21.2-69.4], $p = 0.011$; and 28.9 [95% CI, 16.6-52.3], $p = 0.022$, respectively), but not for the omicron variant (GMT 5.9 [95% CI, 3.4-9.8], $p = 0.463$). The percentages of neutralizing sera against the wild-type virus, delta and omicron variants increased from 70%, 65%, and 38%, before the third dose, to 93% ($p < 0.001$), 88% ($p < 0.001$), and 48% ($p = 0.021$) at 3 weeks after, respectively; and remained high through the 6 months for the wild-type (80%, $p = 0.06$) and delta (77%, $p = 0.102$). The third dose induced the development of a sustained SARS-CoV-2-specific-T-cell population, which persisted through 6 months.

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CONCLUSIONS: The third BNT162b2 dose elicited a durable SARS-CoV-2-specific T-cell response and induced effective and durable neutralization of the wild-type virus and the delta variant, and to a lesser degree of the omicron variant.

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In July 2021, Israel took the pioneering decision to administer a third dose of the BNT162b2 vaccine. The first population to receive the third dose consisted of immunocompromised patients, including heart transplant (HT) recipients, who had demonstrated an inadequate immune response to the 2-dose BNT162b2 vaccine regimen and remained at high risk of developing severe complications from COVID-19 infection.¹ Our group showed that the third dose of the BNT162b2 vaccine, given 6 months after the second dose, induced enhanced humoral and cellular immune responses in HT recipients with a good safety profile.² This positive immune response laid the ground for the administration of a third dose for the general population, driven by observations of waning immunity in the 6 months following the second dose and a lowered protection afforded by the vaccine.^{3,4} The third dose of the BNT162b2 vaccine did indeed reduce the rates of confirmed infection and severe COVID-19 illness in the general population.⁴ However, the durability of the immune response following the 3-dose vaccination regimen still remains unclear, and the complexity of the situation is enhanced by the ever-present threat that highly transmissible variants may further accelerate the decline in the protection afforded by mRNA vaccines.^{5,6} These concerns take on added urgency when governments are faced with making decisions in light of the emergence of variants of concern (VOCs), particularly the current rapid emergence of the SARS-CoV-2 omicron variant responsible for the dramatic rise in cases of COVID-19 cases worldwide.

Third doses of some vaccines have been shown to increase neutralization efficiency against variants. For example, in non-immunocompromised individuals, at 5 months following the second BNT162b2 dose, only a low neutralization efficiency against the wild-type virus was observed. The third dose of the BNT162b2 vaccine induced effective neutralization of the wild-type virus and the omicron variant at 1 month after administration, but the durability of this effect remains unknown.^{2,7} Being the first individuals to receive the third dose, around mid-2021, our HT population constitutes an important cohort for delineating the durability of the immune responses induced by the third vaccine dose, particularly at this time in the ongoing pandemic.

Currently, for immunocompromised patients, there are no data on the effectiveness and duration of booster-induced immune responses against emerging VOCs. Specifically, the omicron variant, presenting with numerous spike protein mutations, raises serious concerns of reduced vaccine effectiveness. Nonetheless, acknowledging the importance of ensuring effective and durable immunization for solid organ transplant recipients, the Centers for Disease Control and Prevention has recently suggested that

moderately and severely immunocompromised individuals who have completed an mRNA COVID-19 vaccine primary series plus an additional mRNA vaccine dose may receive a fourth COVID-19 booster dose at least 3 months after receiving the third mRNA vaccine dose.⁸

Here, we report the results of a longitudinal study of HT patients that was conducted to assess the kinetics of immune responses over the 6 months after administration of the third dose of the BNT162b2 vaccine. We examined T cell responses and neutralizing activity against the wild-type virus (first identified in Wuhan, China), the B.1.617.2 (delta) variant, and the B.1.1.529 (omicron) variant.

Methods

Study population and design

The cohort comprised 103 adult (≥ 18 years) stable HT patients vaccinated with 3 doses of the Pfizer BNT162b2 COVID-19 vaccine (Pfizer, New York and BioNTech, Mainz, Germany). The study was conducted from July 12, 2021 to December 22, 2021. Exclusion criteria included vaccination before transplant and SARS-CoV-2 infection (presence of a positive polymerase-chain reaction assay result for SARS-CoV-2, and a history of suspected clinical SARS-CoV-2 infection). None of the patients in the cohort was treated for rejection or with T-cell depleting agents or specific B-cell depleting agents during the 9 months prior to vaccination or during the study period.

Serum samples from all 103 HT patients vaccinated with the third dose of the BNT162b2 vaccine were tested immediately before (T0) and at 3 weeks after (T1) the third dose for neutralizing antibodies against sublineage B.1 of the wild-type virus, the B.1.617.2 (delta) variant, and the B.1.1.529 (omicron) variant (Figure 1). Patients who had exhibited third-dose induction of neutralizing antibodies against the wild-type virus or the variants were prospectively assessed for the durability of neutralization at 5 to 6 months after the third dose (T2) (Cohort 1, $n = 60$, Figure 1).

The SARS-CoV-2-specific T-cell response was assessed at 5 to 6 months after the third dose in a subset of patients (Cohort 2, $n = 54$, Figure 1). Based on our previous findings demonstrating cellular responses in the absence of antibody responses,² we non-randomly selected from patients not exhibiting neutralizing activity after the third dose ($n = 43$, Figure 1), those with risk factors of interest, such as patients with a history of recurrent symptomatic rejections, allosensitization or established allograft vasculopathy ($n = 19$, Figure 1). Limited by the availability of the assay we also systematically sampled patients from Cohort 1 in a 2:1 ratio ($n = 35$, 5 tests were disqualified technically; Figure 1).

The institutional protocol for post-transplant immunosuppression comprises a calcineurin inhibitor, a mycophenolate-based drug, and a corticosteroid. Conversion to everolimus is instituted per the patient's risk profile, as is corticosteroid withdrawal. The study was approved by the Institutional review board of the Sheba Medical Center (8314-21-SMC). Written informed consent was obtained from all participants.

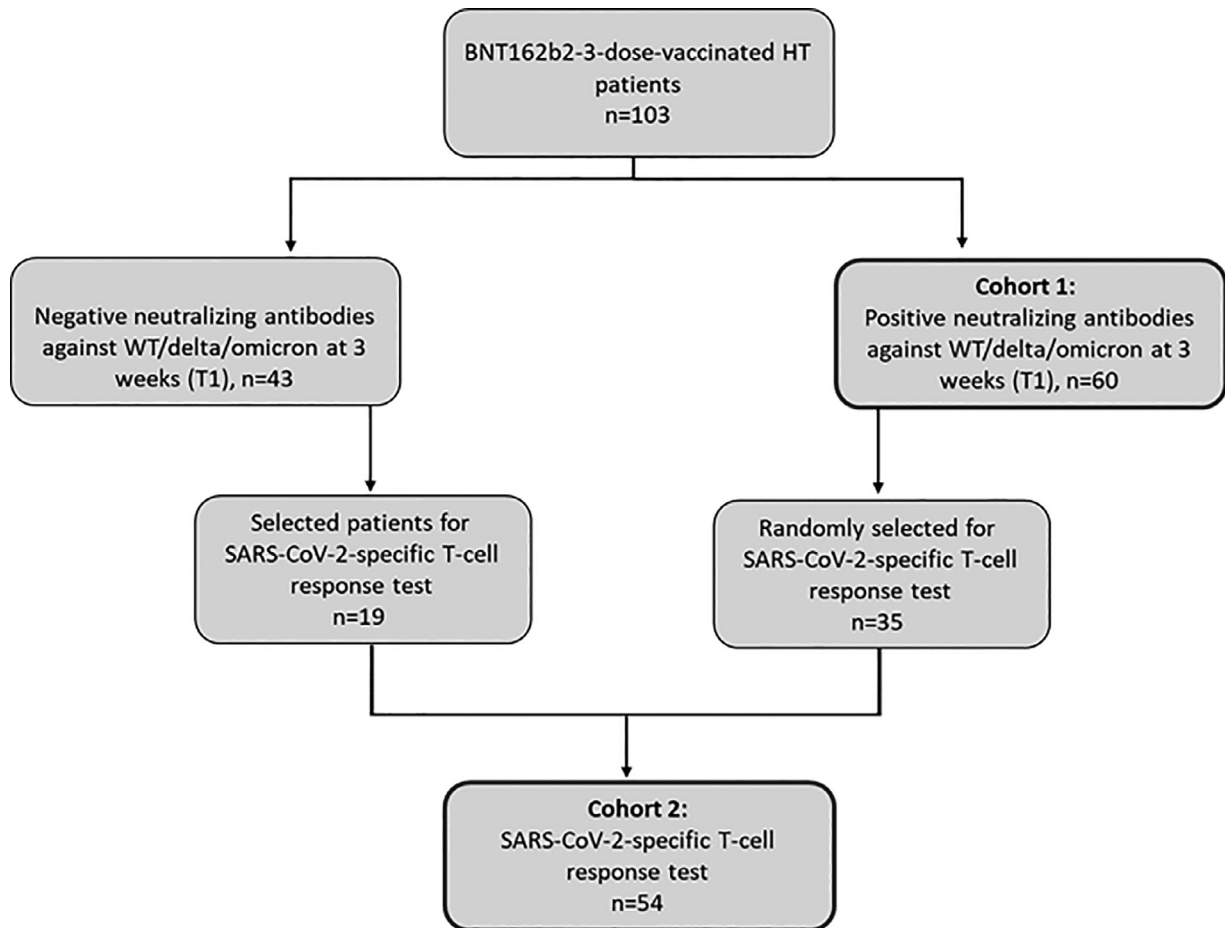


Figure 1 Recruitment of participants, testing, and follow-up. The study population comprised adult HT patients vaccinated with 3 doses of the Pfizer BNT162b2 COVID-19 vaccine. Patients were longitudinally assessed for the kinetics of variant-specific neutralization (Cohort 1) and for SARS-CoV-2-specific T-cell response (Cohort 2) over 6 months.

Viral isolation of the wild-type virus and the delta and omicron variants

Nasopharyngeal samples from 3 SARS-CoV-2 positive individuals were identified by sequencing, one with the wild-type sub-lineage B.1.1.50 (hCoV-19/Israel/CVL-45526-ngs/2020), one with the B.1.617.2 (hCoV-19/Israel/CVL-12804-ngs/2021) variant, and one with the B.1.1.529 (hCoV-19/Israel/CVL-49814-ngs/2021) variant. Confluent Vero-E6 cells were incubated for 1 hour at 33°C with 300 μ L of the nasopharyngeal sample containing the relevant variant, followed by the addition of 5 mL of 2% FCS MEM-Eagle medium. Upon CPE detection, supernatants were aliquoted and stored at -80°C .

Viral titration

To calibrate and determine the 50% tissue culture infectious dose (TCID₅₀) for each variant, Vero-E6 cells at a concentration of 20×10^3 /well were seeded in 3 sterile 96-well plates with 10% FCS MEM-Eagle medium and stored at 37°C for 24 hours. Tenfold serial dilutions of each variant were prepared using 2% FCS MEM-Eagle medium and incubated for 5 days with the Vero-E6 cells. Following gentian violet staining, the TCID₅₀ of each variant was calculated using the Spearman-Kärber method.

SARS-COV-2 micro-neutralization assay

Vero-E6 cells were seeded at 20×10^3 cells/well in sterile 96-wells plates with 10% FCS MEM-Eagle medium and stored at 37°C for 24 hours. For the wild-type, delta, or omicron isolates, 100 TCID₅₀ were incubated with inactivated serum diluted 1:8 to 1:16,384 in 96-well plates for 60 min at 33°C. Virus-serum mixtures were added to the Vero E-6 cells and incubated for five days at 33°C, after which gentian violet (1%) was used to stain and fix the cell culture layer. The neutralizing dilution of each serum sample was determined by identifying the well with the highest serum dilution without an observable cytopathic effect. A dilution equal to 1:10 or above was considered neutralizing.

Isolation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using UNI-SEP+(Novamed). Plasma was collected and spun at $1,000 \times g$ for 20 min to remove platelets before collection of PBMCs. Following one wash with phosphate-buffered saline and one wash with 4Cell® Nutri-T Medium (Sartorius), cells were resuspended in 4Cell Nutri-T Medium and counted using a Countess II Cell counter (Invitrogen).

IFN- γ ELISPOT assay

Fresh PBMCs were used in the ELISpot assay, performed with the ELISpot IFN- γ kit (Autoimmun Diagnostika GmbH) according to the manufacturer's instructions. Briefly, fresh PBMCs were added to duplicate wells at 2×10^5 cells in $50 \mu\text{L}$ per well and stimulated with $50 \mu\text{L}$ of SARS-CoV-2 peptide pools (S-complete, Miltenyi Biotech) ($2 \mu\text{g}/\text{mL}$ per peptide). 4Cell Nutri-T Medium was used as the negative control, and phytohemagglutinin, as the positive control. After 16 to 20 hours at 37°C , 5% CO_2 , 95% humidity, cells were removed, and secreted IFN- γ was detected by adding an alkaline-phosphatase-conjugated secondary antibody for 2 hours. The plates were developed using the BCIP/NBT substrate. ELISpot plates were scanned on an AID ELISpot Reader. The unspecific background (mean spot forming units from negative control wells) was subtracted from the experimental readings.

Statistical analysis

Continuous variables were tested for distribution by using the Shapiro-Wilk test, and results are presented as means \pm standard deviation if normally distributed, and as median (interquartile range) if non-normally distributed. T-cell response and neutralizing activity were compared between paired samples at 2 time points (T0 vs T1, T1 vs T2, T0 vs T2). For the continuous variables, a logarithmic transformation was performed, and each 2 time points were compared by non-parametric Kruskal-Wallis followed by Dunn's multiple comparison test, for wild-type and variants; and for the T-cell response. For dichotomous variables McNemar's test was used. The Bonferroni method of P values adjustment for multiple comparison was applied. Statistical significance was

inferred if P values were below 0.05. Statistical analyses were conducted using R (version 4.0.3). Plots of log-transformed neutralizing antibodies and geometric mean titers (GMTs) with a 95% confidence interval (CI) were obtained using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA).

Results

Third dose induction and durability of neutralization against variants

Of the 103 HT patients assessed for third dose-induced neutralization at 3 weeks, 60 patients demonstrated neutralizing antibodies against the wild-type virus or the variants (Figure 1, Cohort 1; Figure 2A, T1, $n = 60$), and were followed up at 5 to 6 months for the durability of neutralization. For 4 of the 60 patients, neutralization data was not available at the second time period (Figure 2A, T2, $n = 56$). Mean age of the 60 patients was $59.3 (\pm 15.4)$ years, 45 (75%) were male, and median time from transplant to third dose was 8.1 (4.5-13.3) years. Comorbidities were frequent, with hypertension (67%) and diabetes mellitus (45%) being the most common. Immunosuppression with a calcineurin inhibitor and mycophenolate was the most frequently followed protocol (72%); 16 (27%) patients were treated with everolimus and a low dose of a calcineurin inhibitor, and 17 (27%) had been weaned off chronic steroids (Table 1).

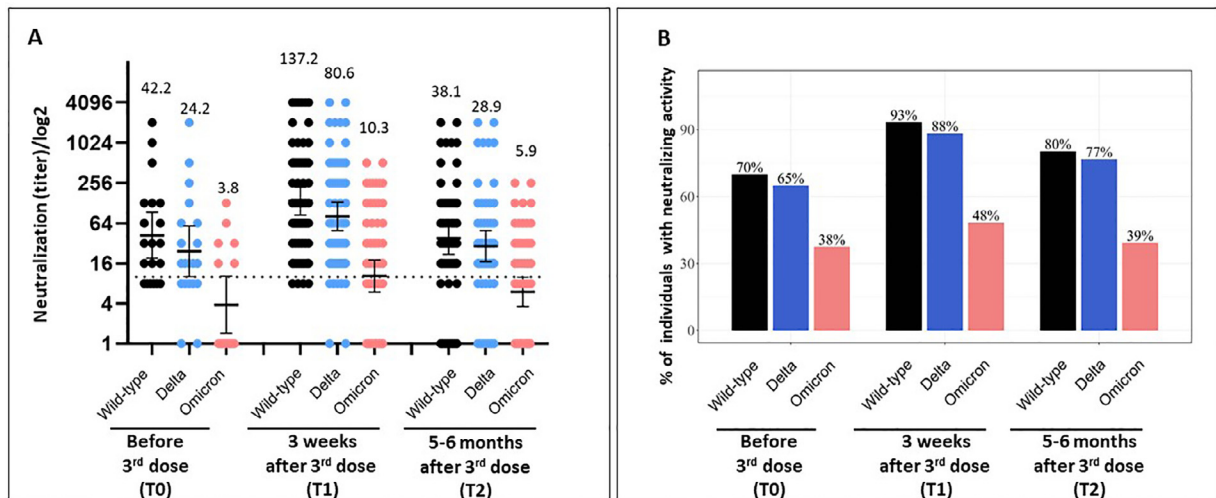


Figure 2 Third dose induction and durability of neutralization against variants over 6 months. (A) 103 BNT162b2-3-dose-vaccinated heart transplant recipients were longitudinally assessed for the kinetics and durability of neutralization against the wild-type virus (black), delta (blue), and omicron (red) variants over 6 months. Sixty patients demonstrated neutralizing antibodies against the wild-type virus or variants at 3 weeks after the third dose (T1, $n = 60$), and were followed up at 5 to 6 months for the durability of neutralization. For 4 patients neutralization data was not available at the follow-up period at 5 to 6 months (T2, $n = 56$). Neutralizing antibodies from sera samples obtained from all 103 patients at the time of third vaccination are also shown (T0, $n = 20$; all patients for whom neutralizing activity was evident at time of vaccination also demonstrated neutralizing activity at 3 weeks after the third vaccine dose). Dashed lines indicate the cut-off titer. Solid lines and numbers indicate the geometric mean titer, and error bars show the 95% confidence interval. Wild-type: T1 vs T0, $p = 0.038$; T2 vs T1, $p = 0.011$. Delta: T1 vs T0, $p = 0.027$; T2 vs T1, $p = 0.022$. Omicron: T1 vs T0, $p = 0.294$; T2 vs T1, $p = 0.463$. (B) Fraction of individuals showing neutralization above the threshold at each time point. Wild-type: T1 vs T0, $p < 0.001$; T2 vs T1, $p = 0.060$. Delta: T1 vs T0, $p < 0.001$; T2 vs T1, $p = 0.102$. Omicron: T1 vs T0, $p = 0.021$; T2 vs T1, $p = 0.618$.

Table 1 Baseline Characteristics and Vaccination Timetable for the Neutralization Study Cohort

Variable	Total cohort <i>n</i> = 60
Recipient characteristics	
Age, years, (mean ± SD)	59.3 ± 15.4
Male sex, <i>n</i> (%)	45 (75)
Body mass index, kg/m ² (mean ± SD)	27 ± 4.6
Diabetes mellitus, <i>n</i> (%)	27 (45.0)
Hypertension, <i>n</i> (%)	40 (66.7)
Cardiac allograft vasculopathy, <i>n</i> (%)	16 (26.7)
Immunosuppression data	
Mycophenolic acid therapy, <i>n</i> (%)	44 (74.6)
Everolimus therapy, <i>n</i> (%)	16 (27.1)
Chronic prednisone, <i>n</i> (%)	43 (72.9)
Immunosuppression protocol	
Tacrolimus + mycophenolate + prednisone, <i>n</i> (%)	26 (44.1)
Cyclosporine + mycophenolate + prednisone <i>n</i> (%)	5 (8.5)
Tacrolimus + mycophenolate, <i>n</i> (%)	10 (16.9)
Cyclosporine + mycophenolate, <i>n</i> (%)	1 (1.7)
Cyclosporine + everolimus + prednisone, <i>n</i> (%)	2 (3.4)
Tacrolimus + everolimus + prednisone, <i>n</i> (%)	9 (15.3)
Everolimus + cyclosporine, <i>n</i> (%)	2 (3.4)
Everolimus + mycophenolate, <i>n</i> (%)	1 (1.7)
Everolimus + tacrolimus, <i>n</i> (%)	1 (1.7)
Tacrolimus + prednisone, <i>n</i> (%)	1 (1.7)
Tacrolimus + everolimus + mycophenolate + prednisone, <i>n</i> (%)	1 (1.7)
Laboratory data (on day of 3rd vaccine)	
Lymphocyte absolute, K/ μ L, median (IQR)	1.7 [1.1-2.2]
White blood cell, K/ μ L, (mean ± SD)	7.2 ± 2.4
Neutrophil absolute, K/ μ L, median (IQR)	4.7 [3.8-5.9]
Neutrophil/lymphocyte ratio, median (IQR)	2.9 [2.3-4.1]
Estimated glomerular filtration rate, mL/min/1.73 m ² , median (IQR)	85.6 [60.8-107.9]
C-reactive protein, mg/L (mean ± SD)	5.4 ± 6.2
Timetable	
Heart transplantation to 3rd vaccine, years, median (IQR)	8.1 [4.5-13.3]
Time of 2nd vaccine from 1st vaccine, days (mean±SD)	21.3 ± 3.1
Time of 3rd vaccine from 2nd vaccine, days (mean±SD)	167.5 ± 18.0
Time of neutralization assay from 3rd vaccine, days (mean ± SD)	154.7 ± 4.4

SD, standard deviation.

Three vaccine doses led to better neutralization of the wild-type virus and the two variants. Samples obtained at the time of third vaccination exhibited neutralizing activity against the wild-type virus and the delta variant (GMT, 42.2 [95% CI, 19.0-93.7] and 24.2 [95% CI, 10.1-58.3], respectively, **Figure 2A**, T0), but no neutralizing activity against the omicron variant [GMT, 3.8 (95% CI, 1.5-10.3), **Figure 2A**, T0]. At 3 weeks after the third dose, serum samples exhibited high neutralizing activity against the wild-type virus (GMT, 137.2 [95% CI, 84.8-221.9]; $p = 0.038$, T1 vs T0) and the delta variant [GMT, 80.6 [95% CI, 49.3-132.0]; $p = 0.027$, T1 vs T0), and lower neutralizing activity against the omicron variant (GMT, 10.3 [95% CI, 5.9-17.9]; $p = 0.294$, T1 vs T0). At 6 months following the third dose (**Figure 2A**), serum neutralizing activity declined but was still high for the wild-type virus (GMT, 38.1 [95% CI, 21.2-69.4]; $p = 0.011$, T2 vs T1) and for the delta variant (GMT,

28.9 [95% CI, 16.6-52.3]; $p = 0.022$, T2 vs T1) but not for the omicron variant (GMT, 5.9 [95% CI, 3.4-9.8]; $p = 0.463$, T2 vs T1). These titers were lower than the peak titers by a factor of 3.6, 2.8, and 1.7 for the wild-type virus and the delta and omicron variants, respectively, through the 6 months of follow-up.

The percentages of sera demonstrating neutralizing activity (i.e., above the threshold) against the wild-type virus, delta and omicron variants increased from 70%, 65%, and 38%, before the third dose (T0), to 93% ($p < 0.001$), 88% ($p < 0.001$), and 48% ($p = 0.021$) at 3 weeks after the third dose (T1), respectively; and remained high through the 6 months for the wild-type (80%; $p = 0.060$, T2 vs T1) and delta (77%; $p = 0.102$, T2 vs T1; **Figure 2B**). The percentage of neutralizing sera against the omicron variant remained low (39%; $p = 0.618$, T2 vs T1).

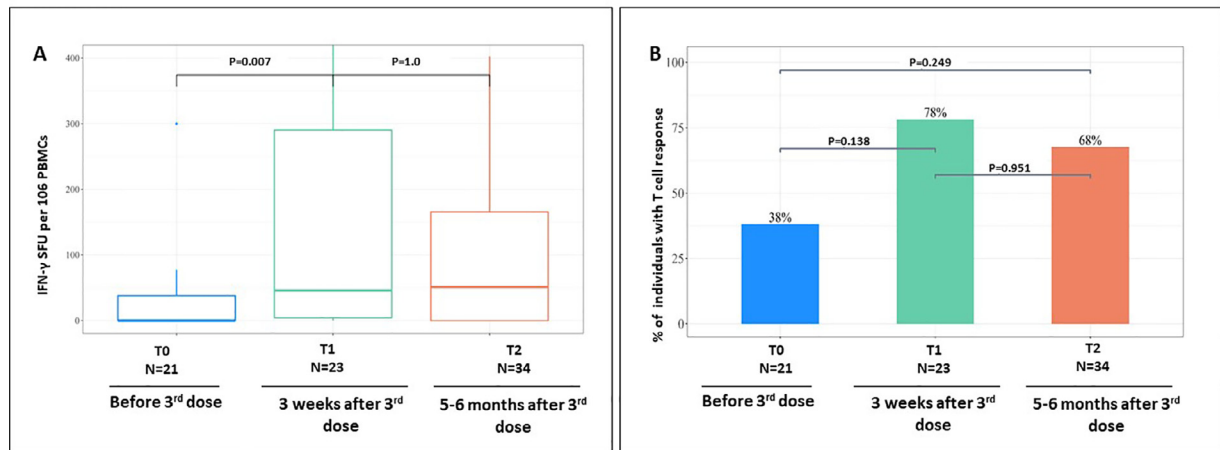


Figure 3 Third dose induction and durability of SARS-CoV-2-specific T-cell response. (A) IFN- γ responses (spot numbers) were compared between the first time point (T0, before the third dose), the second time point, T1 (3 weeks after the third dose), and the third time point, T2 (5-6 months after the third dose). Data are presented as median and interquartile range (IQR). (B) Fraction of individuals showing positive SARS-CoV-2-specific T-cell response at baseline (T0, before the third dose), 3 weeks after the third dose (T1), and 5 to 6 months after the third dose (T2).

Third dose induction and durability of SARS-CoV-2-specific T-cell response

Of a total 103 BNT162b2-3-dose-vaccinated patients, whole blood samples were obtained for 54 patients (Cohort 2; mean age 56.3 [\pm 15.0] years, and 39 [72%] male; Figure 1 and Supplementary Table 1). The lymphocyte count on the day of third vaccine dose was 1.5 K/ μ L (1.1-2.2), with a neutrophil/lymphocyte ratio of 3.2 (2.3-4.5). There was a significant increase in IFN- γ spot numbers from the time of the third dose to 3 weeks after the dose ($p = 0.007$). The IFN- γ spot response was maintained through the 6 months after the third dose ($p = 1.0$; T2 vs

T1, Figure 3A). Notably, over 70% of individuals in the cohort showed an inducible SARS-CoV-2-specific T-cell response at 3 weeks after the third dose (T1), and this proportion was maintained during the 6 months after the booster dose ($p = 0.951$; Figure 3B). In 12 of the patients, the SARS-CoV-2-specific T-cell response was measured in 2 time periods, namely, 3 weeks (T1) and 6 months (T2) following the third dose (Figure 4). Comparison of paired samples from the same patient at 3 weeks (T1) and 5 to 6 months after the third dose (T2) showed no significant difference in IFN- γ spot numbers (Figure 4), supporting the durability of the third dose-induced T-cell response. Importantly, an inducible SARS-CoV-2-specific T-cell response

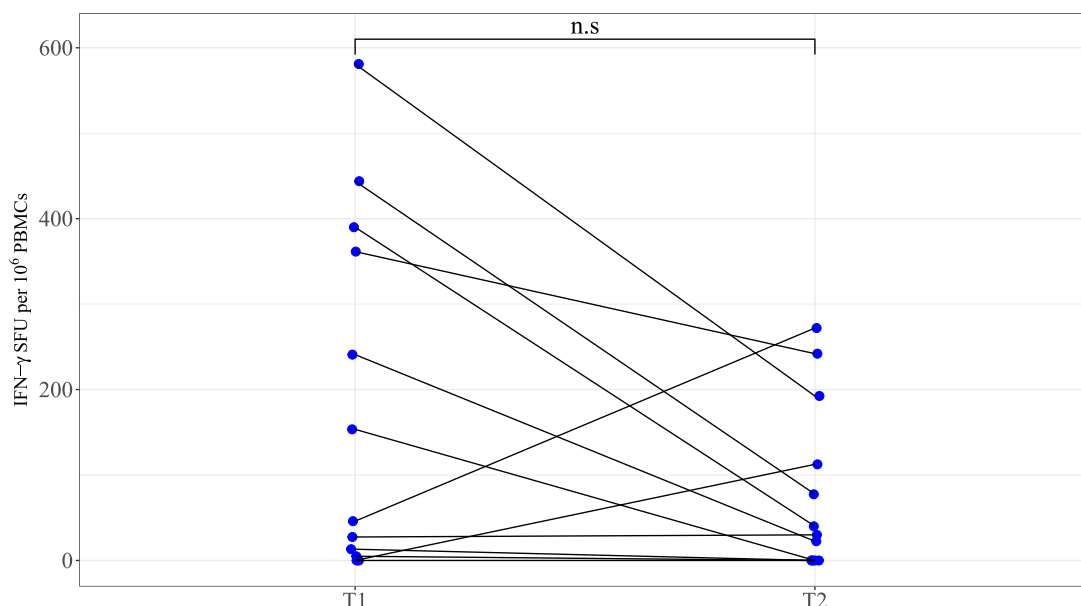


Figure 4 Spike-specific T cell responses induced by the third booster dose of the BNT162b2 vaccine. IFN- γ responses were compared between paired samples at 2 time points (T2 vs T1, $n = 12$).

but negative neutralization was observed for 3 patients. No correlation was found between the SARS-CoV-2-specific T-cell response and neutralization.

Discussion

Principal findings

Several important findings emerged from this prospective longitudinal study in severely immunocompromised but third-dose vaccinated individuals. First, the third BNT162b2 dose induced high neutralization of the wild-type virus and the delta variant and to a lesser degree of the omicron variant. Second, at 6 months following the third dose, serum neutralizing activity elicited by the third BNT162b2 vaccination was still evident for the wild-type virus and for the delta variant, albeit to a lesser degree, but not for the omicron variant. Third, the third dose induced the development of a sustained SARS-CoV-2-specific T-cell population. Fourth, cellular responses were evident in the absence of measurable neutralizing antibodies, suggesting a cellular benefit, even when there did not appear to be an antibody response.

Comparison with other studies

The BNT162b2 vaccine was previously reported to have >90% efficacy in the general population against the ancestor Wuhan virus,^{9,10} but not for HT recipients. Detectable antibodies against the receptor-binding domain were demonstrated in only 10% to 57% and a cellular response in 10% to 70% of HT recipients at different time points following 2 doses of mRNA vaccines.^{1,11-13} With the emergence of new VOCs, significant concerns continue to be raised regarding the effectiveness of the vaccines against these novel variants. We have, uniquely, longitudinally assessed the 3-dose BNT162b2-induced neutralization response to the wild-type virus and to 2 variants responsible for COVID-19 surges by using micro-neutralization assays involving cell cultures infected with live viruses (wild type and variants). In contrast to previous studies reporting the waning of vaccine (2 doses)-induced or disease-induced neutralization responses, we provide longitudinal data on the ability of the 3-dose vaccine regimen to induce initial variant-specific neutralizing responses at different time points following 2 and 3 vaccine doses.

Meaning of the study: possible explanations and implications for clinicians and policymakers

We demonstrate that the third dose elicited high neutralization of the wild-type virus and delta variant and to a lesser degree of the omicron variant. The clinical significance of this observation has yet to be determined, but it raises concerns regarding efficacy of the BNT162b2 vaccine in the immunocompromised population against this now dominant variant. Whether a fourth dose will induce higher

neutralization response against the omicron variant in this population is yet to be determined. Notably, for the general population, a third dose induced an increase in neutralization of the wild-type virus (GMT, 891.4) and, to a lesser degree, of the omicron variant (GMT, 108) at 1 month after the third dose,⁷ with the latter titer being close to that observed for the wild-type in our HT population 3 weeks after the third dose (GMT, 137). The low neutralization for the HT patients might be partially explained by the low inducible SARS-CoV-2-specific T-cell response observed in our HT population after the second dose. The high inducible SARS-CoV-2-specific T-cell response observed in our study following the third dose might indicate that a fourth dose would induce more effective neutralization against the omicron variant in severely immunocompromised populations.

Unanswered questions and future research

The balance between the adaptive immune responses, comprising both humoral and SARS-CoV-2-specific T-cell responses, that work together is an important factor in the development of protective immunity against viral infections.¹⁴ Activated CD4+ T-cells are important for B-cell activation and the generation of neutralizing antibodies to maintain a durable antibody response.¹⁴ Whether SARS-CoV-2-specific T-cells, in the absence of an effective neutralization response, induce immunity/protection is unknown, but numerous studies support the correlation between the induction of neutralization and immunity. The utility of vaccine-induced neutralizing activity as a predictive metric of protection has been demonstrated in the pre-clinical and clinical studies of SARS-CoV-2 vaccines designed to elicit robust T-cell responses based on the induction of neutralizing antibodies.¹⁵⁻¹⁷ We should, however, keep in mind that the interplay and balance between the humoral and cellular immune responses against SARS-CoV-2 is complex and much is still to be learnt, particularly as recent data suggest that exposure to SARS-CoV-2 can induce virus-specific T-cell responses without seroconversion.¹⁸

Strengths and limitations

The strength of our study lies in several directions: (1) The research is timely, deals with urgent public health concerns, is relevant to the medical community due to the exponential rise in omicron variant COVID-19 infections. (2) This is a longitudinal study of the first population vaccinated with 3 doses of the BNT162b2 vaccine with data up to 6 months after the third dose. Thus, the study population constitutes a leading cohort for delineating the durability of the immune responses induced by the third vaccine dose, particularly at this time in the ongoing pandemic. Importantly, any hint of vaccine immunological efficacy in HT patients may be magnified several-fold in the non-suppressed vaccinated population. (3) We examined BNT162b2 vaccine neutralization of emerging SARS-CoV-2 variants, including delta and rapidly spreading omicron, using micro-neutralization

assays involving cell cultures infected with the original live viruses. The importance of neutralization assays is emphasized by data demonstrating a correlation between the level of neutralizing antibodies and symptomatic disease. (4) We provide longitudinal data on the ability of the 3-dose BNT162b2 vaccine regimen to induce SARS-CoV-2-specific cellular responses and on the durability of these responses. Vaccine-induced spike-specific T cells are multi-specific and are capable of recognizing different regions of the spike protein.^{19,20} Thus, despite the ability of emerging variants to alter T-cell specificities, these variants do not escape the entire repertoire of spike-specific T cells. Indeed, it has been demonstrated that, for most vaccinated individuals, VOCs are recognized by the spike-specific T cells induced by mRNA vaccines.^{21,22} Here, we present novel data indicating a third dose-induced durable SARS-CoV-2 specific T-cell response for up to 6 months.

The limitations of the study include the relatively small number of patients (although this is the leading and largest cohort for long-term boosting data). In addition, only peripheral circulatory virus-specific T cells were analyzed (i.e., no information on other localized SARS-CoV-2-specific T cells, e.g., bone marrow), and the IFN- γ ELISpot assay can detect only peripheral T cells secreting the Th1 cytokine IFN- γ . The values for the SARS-CoV-2-specific T cell response that confer protection are unknown.²³ We did not longitudinally routinely perform polymerase-chain-reaction testing for SARS-CoV-2, which could have resulted in underdiagnosis of SARS-CoV-2 infection. Also, the study was not designed to establish predictors of vaccine-induced neutralization, since its aim was to assess the long-term kinetics of vaccine-induced neutralizing antibody. Finally, clinical correlation of these data will be needed.

Conclusions

The third BNT162b2 dose elicited a durable SARS-CoV-2-specific T-cell response and induced high and durable neutralization of the wild-type virus and the delta variant, and to a lesser degree of the omicron variant, providing an encouraging indication of vaccine neutralization against virulent variants. Any hint of vaccine immunological efficacy in HT patients may be magnified several fold in the non-suppressed vaccinated population. Our findings may inform vaccination strategies to control the future trajectory of the COVID-19 pandemic, particularly the need for scheduling booster doses into immunization protocols.

Disclosure statement

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of the presented manuscript or other conflicts of interest to disclose.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at <https://doi.org/10.1016/j.healun.2022.05.014>.

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