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The Journal of Heart and Lung Transplantation

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Fourth BNT162b2 vaccination neutralization of omicron infection after heart transplantation

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KEYWORDS:
BNT162b2 vaccine;
heart transplantation;
fourth dose;
variants of concern;
omicron

We investigated changes in receptor-binding domain IgG and neutralizing antibodies against the omicron and delta variants, vs the wild-type virus, in response to a fourth BNT162b2 dose in 90 heart transplant (HT) recipients. The fourth dose induced anti-RBD IgG antibodies and a higher neutralization efficiency against the wild-type virus and the variants; however, neutralization efficiency against the omicron variant was lower than that against the delta variant (the latter demonstrating efficacy similar to that against the wild-type virus). Notably, while IgG anti-RBD antibodies were detectable in >80% of the HT recipients, only about half demonstrated neutralization efficiency against the omicron variant. A SARS-CoV-2-specific-T-cell response following the fourth dose was evident in the majority of transplant recipients. Boosting vulnerable groups improves antibody responses (including neutralizing responses) and cellular immunity, but the incomplete immunological response, particularly for omicron, suggests continued preventive measures and optimization of vaccination strategies that elicit strong, and long-lasting immune responses, in this high-risk population, should remain a priority.

J Heart Lung Transplant 2022;41:1210−1213

The emergence of new SARS-CoV-2 variants of concern (VOCs), particularly the highly transmissible omicron variant, has highlighted the need to improve vaccine-induced immune responses.[1] Currently, the strategy of repeated booster doses is controversial, and data on the efficacy of repeated boosters is limited. This issue is of particular relevance for solid organ transplant recipients, who are vulnerable to worst effects of COVID-19,[2] and for whom ongoing COVID-19 excess deaths are reported, even after the advent of vaccinations and new therapeutics.[3] It has been shown that the vaccine immune paresis that renders transplant patients vulnerable to severe infection, even after vaccination,[4] is further impacted by waning immunity after the third dose of the BNT162b2 vaccine.[1,5,6] In addition, the higher mutation frequency in immunocompromised patients[7] poses further challenges to the management of COVID-19 in transplant, and the general, populations. On December 30, 2021, Israel began vaccinating high-risk populations with a fourth homologous BNT162b2 (Pfizer−BioNTech) dose, but its effectiveness against emerging VOCs is unknown. We investigated changes in receptor-binding domain (RBD) IgG and neutralizing antibodies against the omicron and delta variants, vs the wild-type virus, in response to a fourth BNT162b2 dose in heart transplant (HT) recipients.

Ninety stable adult HT recipients who received 4 doses of the BNT162b2 COVID-19 vaccine were followed prospectively. Exclusion criteria included SARS-CoV-2 infection (a positive polymerase-chain-reaction assay result for

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1053-2498/$ - see front matter
https://doi.org/10.1016/j.healun.2022.04.014

SARS-CoV-2 and a history of suspected clinical SARS-CoV-2 infection). The study was approved by our institutional review board (8314-21-SMC). Serum samples, collected longitudinally immediately before and 16.1 ± 4.0 days after the fourth dose, were tested for SARS-CoV-2 anti-RBD IgG antibodies (SARS-CoV-2 IgG II Quant assay, Abbott, USA) and for neutralizing antibodies (using live virus microneutralization assays) against sublineage B.1 of the wild-type virus, the B.1.617.2 (delta) variant and the B.1.1.529 (omicron) variant. The wild-type virus and VOCs were isolated by sequencing nasopharyngeal samples from 3 SARS-CoV-2 positive individuals [wild-type virus (hCoV-19/Israel/CVL-45526-ngs/2020); delta (hCoV-19/Israel/CVL-12804-ngs/2021); omicron (hCoV-19/Israel/CVL-49814-ngs/2021)]. Vero-E6 cells at a concentration of $20*10^3$ cells/well were seeded with 10% FCS MEM-Eagle medium and stored at 37˚C for 24 hour. Median tissue culture infectious doses for the wild-type virus and variants were incubated with inactivated serum diluted 1:8 to 1:16384 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 (1%) was used to stain and fix the cell culture layer. The neutralizing dilution was determined by identifying the well with the highest serum dilution without observable cytopathic effect. A dilution equal to 1:10 or above was considered neutralizing. SARS-CoV-2-specific-T-cell response was evaluated in a subset of patients by IFN-γ release of stimulated peripheral blood mononuclear cells.[5] Continuous variables were tested for distribution by using the Shapiro-Wilk test, and results are presented as means ± standard deviation if normally distributed, and as median (interquartile range) if nonnormally distributed. Neutralizing activity was compared between paired samples at 2-time points, using the Wilcoxon signed-rank test. The reduction in neutralization efficacy of variants vs the wild-type virus was calculated for each patient at each time point. 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 were obtained using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA).

The HT recipients (age 57.2 ± 13.8, 69% males, Table 1) received the fourth BNT162b2 dose 173.4 ± 4.2 days after the third dose. There were no safety concerns. Anti-RBD IgG antibodies were detected in 54 (61.4%) and 71 (80.7%) HT patients before and after the fourth dose, respectively (Figure 1A), with GMTs increasing from 12.5 to 96.9 AU/ml (Figure 1B). The fourth dose induced better neutralization of the wild-type virus and the delta and omicron variants, with GMTs increasing from 11.1, 9.4, and 2.9 to 41.9, 38.8, and 10.4, respectively (Figure 1C). The percentages of patients demonstrating neutralizing activity against the wild-type virus and the delta and omicron variants increased from 48%, 47%, and 24% to 68% ($p < 0.01$), 66% ($p < 0.01$), and 49% ($p < 0.01$), respectively (Figure 1D). Nonetheless, a lower neutralization efficiency of the vaccine against the omicron variant (but not against the delta variant) compared to the wild-type virus was observed after the fourth dose ($p < 0.001$) (Figure 1E). The T-cell response was evaluated in a subset of 20 patients; of these, 10 (50%) and 15 (75%) demonstrated COVID-19 specific T-cell immunity before and after the fourth dose, respectively.

The fourth dose induced anti-RBD IgG antibodies and a higher neutralization efficiency against wild-type viruses and variants; however, neutralization efficiency against the omicron variant was lower than that against the delta variant (the latter demonstrating efficacy similar to that against the wild-type virus). Notably, while IgG anti-RBD antibodies were detectable in >80% of the HT recipients, only

Table 1 Baseline Characteristics and Vaccination Timetable

Variable	Total cohort *n* = 90
Recipient characteristics	
Age, years, (mean ± SD)	57.2 ±13.8
Male sex, *n* (%)	62 (68.9)
Body mass index, kg/m^2 (mean ± SD)	26.6 ± 4.7
Diabetes mellitus, *n* (%)	31 (37.8)
Hypertension, *n* (%)	58 (69.9)
Cardiac allograft vasculopathy, *n* (%)	21 (25.9)
Immunosuppression regimens	
Calcineurin inhibitor + mycophenolic acid + prednisone, *n* (%)	49 (54.4)
Calcineurin inhibitor + mycophenolic acid, *n* (%)	19 (21.1)
Calcineurin inhibitor + everolimus + prednisone, *n* (%)	14 (15.7)
Mycophenolic acid + everolimus + prednisone, *n* (%)	2 (2.2)
Everolimus + calcineurin inhibitor, *n* (%)	3 (3.3)
Everolimus + mycophenolic acid, *n* (%)	1 (1.1)
Calcineurin inhibitor + prednisone, *n* (%)	2 (2.2)
Laboratory data (on day of fourth vaccine)	
Lymphocyte absolute, K/μl, median (IQR)	1.3 [1.0 - 2.0]
Neutrophil/lymphocyte ratio, median (IQR)	2.7 [2.1 - 4.1]
Estimated glomerular filtration rate, ml/min/1.73 m^2, median (IQR)	78.8 [59.4 - 98.8]
C-reactive protein, mg/l (mean ± SD)	7.3 ± 16.6
Timetable	
Heart transplantation to fourth vaccine, years, median (IQR)	6.5 [3.5 - 14.1]
Time of second vaccine from first vaccine, days (mean ± SD)	21.3 ± 3.1
Time of fourth vaccine from third vaccine, days (mean ± SD)	173.4 ± 4.2
Time of neutralization assay from fourth vaccine, days (mean ± SD)	16.1 ± 4.0

Abbreviation: SD, standard deviation.

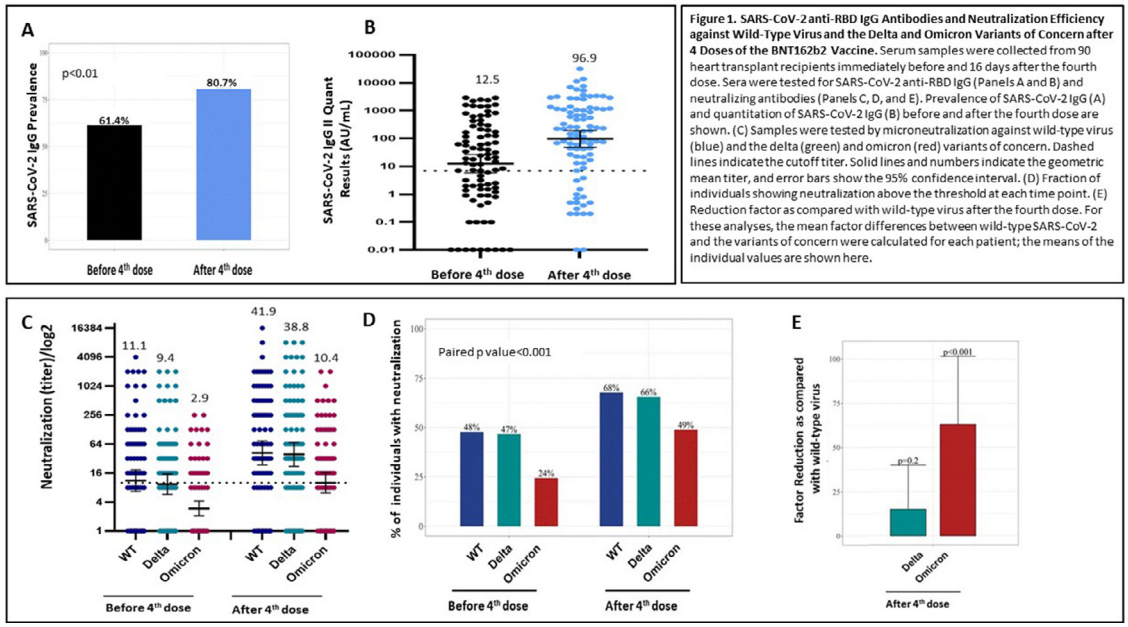

Figure 1. SARS-CoV-2 anti-RBD IgG Antibodies and Neutralization Efficiency against Wild-Type Virus and the Delta and Omicron Variants of Concern after 4 Doses of the BNT162b2 Vaccine. Serum samples were collected from 90 heart transplant recipients immediately before and 16 days after the fourth dose. Sera were tested for SARS-CoV-2 anti-RBD IgG (Panels A and B) and neutralizing antibodies (Panels C, D, and E). Prevalence of SARS-CoV-2 IgG (A) and quantitation of SARS-CoV-2 IgG (B) before and after the fourth dose are shown. (C) Samples were tested by microneutralization against wild-type virus (blue) and the delta (green) and omicron (red) variants of concern. Dashed lines indicate the cutoff titer. Solid lines and numbers indicate the geometric mean titer, and error bars show the 95% confidence interval. (D) Fraction of individuals showing neutralization above the threshold at each time point. (E) Reduction factor as compared with wild-type virus after the fourth dose. For these analyses, the mean factor differences between wild-type SARS-CoV-2 and the variants of concern were calculated for each patient; the means of the individual values are shown here.

about half demonstrated neutralization efficiency against the omicron variant. The importance of neutralization assays has previously been shown by data indicating a correlation between neutralizing antibodies and symptomatic disease, and this is the first study to report the fourth vaccination neutralization of infection with VOCs in this at-risk population.

Our novel findings have immediate implications for vaccination and therapeutic strategies during the ongoing COVID-19 pandemic. The importance of our findings is emphasized by recent concerns regarding the limited efficacy of monoclonal antibodies against the omicron variant,[8,9] as passive antibody prophylaxis is being considered as an alternative strategy in efforts to protect transplant patients. Until new vaccines, or other strategies, offering better protection against VOCs become available, our data indicate that boosting vulnerable groups improves antibody responses (including neutralizing responses) and cellular immunity, may be an acceptable strategy. Nonetheless, the incomplete immunological response, particularly against the omicron variant, suggests that continued vigilance and preventive measures in this high-risk population should remain a priority. Additional protection against omicron infection and severe disease provided by a fourth dose reported for the general population[10] is encouraging and could translate into a higher benefit for high risk-populations.

Our results should be interpreted with caution. While this study suggests a favorable safety profile, it was not designed to establish the clinical efficacy or the durability of the vaccine-induced immune responses, thus comparison with alternative strategies such as passive antibody prophylaxis cannot be determined. Importantly, continuous assessment and optimization of vaccination strategies that elicit strong, and long-lasting immune responses, aiming to prevent infection and transmission, and prevent severe disease and death, should be thought of. Clinical correlation of these data will be needed.

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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