Received: 28 January 2022 | Revised: 4 March 2022 | Accepted: 5 March 2022

DOI: 10.1111/ajt.17020

BRIEF COMMUNICATION

Neutralization against Omicron variant in transplant recipients after three doses of mRNA vaccine

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Funding information
Ajmera Transplant Centre; University Health Network; University of Toronto; Canadian Institutes of Health Research; Canadian Coronavirus Variants Rapid Response Network

The SARS-CoV-2 virus Omicron variant has now supplanted wild-type virus as the dominant circulating strain globally. Three doses of mRNA COVID-19 vaccine are recommended for transplant recipients as their primary vaccine series. However, the immunogenicity of mRNA vaccines as they specifically relate to the Omicron variant are not well studied. We analyzed Omicron-specific neutralization in transplant recipients after three-doses of mRNA-1273 vaccine. Neutralization was determined using a SARS-CoV-2 spike pseudotyped lentivirus assay with constructs for Omicron and Delta variants. A total of 60 transplant patients (kidney, kidney-pancreas, lung, heart, liver) were analyzed 1 month and 3 months after completion of three doses of mRNA-1273. At 1 month, 11/60 (18.3%) patients had detectable neutralizing antibody responses to Omicron (log_{10}ID50 of 2.38 [range 1.34–3.57]). At 3 months, 8/51 (15.7%) were positive (median log_{10}ID50 [1.68; range 1.12–3.61; approximate fivefold reduction over time]). The proportion of positive patients was lower for Omicron versus wild-type, and Omicron vs. Delta ($p < .001$). No demographic variables were found to be significantly associated with Omicron response. Many patients with a positive anti-RBD response still had undetectable Omicron-specific neutralizing antibody. In conclusion, three doses of mRNA vaccine results in poor neutralizing responses against the Omicron variant in transplant patients.

KEYWORDS
infection and infectious agents—viral: SARS-CoV-2/COVID-19, infectious disease, translational research/science, vaccine

1 | INTRODUCTION

Three doses of mRNA vaccine in transplant recipients increase antibody titers, neutralization of wild-type (WT) virus, and T-cell responses and is a standard recommendation.[1,2] However, recently the SARS-CoV-2 Omicron variant has rapidly supplanted wild-type virus as the dominant circulating strain. The Omicron variant exhibits multiple amino acid substitutions within the spike protein resulting in enhanced vaccine escape.[3] In immunocompetent persons, third dose mRNA vaccines induce neutralizing immunity against Omicron, leading to a wider population-based recommendation for boosters.[3] However, transplant patients are known to have suboptimal vaccine

Abbreviations: COVID-19, coronavirus disease 2019; ID, infectious dose; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus–2; WT, wild-type.

immunogenicity and COVID-19 infections occurring in immunocompromised hosts may be a critical factor for variant evolution.[4] Therefore, it is important to understand vaccine immunogenicity against circulating variants over time in these patients. We analyzed serum from organ transplant recipients one month and three months after three doses of mRNA-1273 vaccine for anti-RBD titers and neutralization activity against Omicron SARS-CoV-2 variant compared with Wild-type and Delta.

2 | METHODS

Patients were identified and enrolled from previous trials of mRNA-1273 vaccine.[1] Additional informed consent was obtained for long-term follow-up, blood collection and testing of serum samples against circulating variants. mRNA-1273 was administered at a 0-,1-, and 3-month dosing schedule with blood collected at months 4 and 6. Sera were analyzed for anti-RBD and neutralization against wild-type (WT), Delta and Omicron SARS-CoV-2 pseudoviruses.

2.1 | Spike-pseudotyped lentivirus neutralization assay

Neutralization assays were performed using a previously validated SARS-CoV-2 spike pseudotyped lentivirus assay with constructs for Omicron and Delta variants. Spike cDNAs encoding full-length wild-type SARS-CoV-2 bearing the D614G mutation, or the full-length Delta variant, were obtained from Twist Bioscience. The spike cDNA encoding the full-length Omicron variant was obtained from Thermo Fisher Scientific. For expression in mammalian cells, the aforementioned cDNAs were cloned downstream of the CMV promoter/enhancer in the HDM expression plasmid (kindly provided by Dr. Jesse Bloom). These spike expression plasmids are freely available through CoVaRR-Net (https://nbcc.lunenfeld.ca/resources/). The pseudovirus neutralization assay was performed as described previously,[5,6] with a minor modification of the starting dilution for Omicron to 1:40 and Delta to 1:45. Briefly, pseudotyped lentivirus particles were generated from co-transfection of the viral packaging (psPAX2, Addgene), the ZsGreen and luciferase reporter (pHAGE-CMV-Luc2-IRES-ZsGreen-W, kindly provided by Jesse Bloom) and the spike protein constructs into HEK293TN cells (System Biosciences, LV900A-1). Viral supernatants were harvested, clarified at 500g, and filtered through 0.45 μm filters prior to aliquoting and storage at −80°C. A viral titer assay was performed using HEK293T-ACE2/TMPRSS2 cells, and a virus dilution resulting in ~15% infection and >1000 relative luciferase units over the control was used in the neutralization assay (1:15 – 1:250 dilution of virus stock, ~350K relative luciferase units per pseudovirus). For the neutralization assay, diluted and heat-inactivated (56°C, 1 h) patient sera samples (1:22.5 in assay media) were first prepared and serially diluted 3-fold (2.5-fold for Omicron testing) over 7 dilutions, followed by incubation with diluted virus at a 1:1 ratio for 1 h at 37°C. The virus and serum mixture were then added to HEK293T-ACE2/TMPRSS2 cells and incubated for 48 h prior to lysis using the BrightGlo Luciferase Assay System (Promega, Madison, WI). Luminescence signals were detected using a PerkinElmer Envision instrument. The 50% neutralization titers (ID50s) were calculated in GraphPad Prism 9 (GraphPad Software, San Diego, CA) using a nonlinear regression (log[inhibitor] versus normalized response – variable slope) algorithm. A positive neutralization assay can be defined as any dilution that results in 50% viral neutralization as calculated based on the above generated curve ($\log_{10}$ID50 > 0). Both the HEK293TN and HEK293T-ACE2/TMPRSS2 cells were maintained at 85% confluency for no more than 25 passages.

2.2 | Anti-RBD and anti-nucleocapsid protein antibody

Anti-receptor binding domain (RBD) testing was performed using the Roche Elecsys anti-SARS-COV-2 S enzyme immunoassay as per manufacturer's instructions. The detection threshold for this assay is 0.4 U/ml although positive detection is defined as ≥0.8 U/ml. Absence of previous diagnosis of COVID-19 was confirmed by testing for anti-nucleocapsid protein antibody using the 1-month post-third dose sample (Abbott Laboratories) as per manufacturer's instructions. A cut-off of <1.4 was considered negative as per manufacturer's instructions.

2.3 | Statistical methods

Descriptive statistics were used to determine the proportion of patients with detectable neutralizing antibodies at one-month and 3-months post-third dose vaccine for WT, Delta, and Omicron. Comparison of proportion of patients with detectable neutralization against Omicron vs. WT and Omicron vs. Delta was done using McNemar test for paired data. Fold reduction in neutralizing ability for Omicron compared with WT, and for Delta compared with WT was calculated for each patient by dividing the absolute ID50 value for the WT virus by the absolute ID50 value for the variant (Delta or Omicron). Univariate analysis for factors associated with a positive Omicron neutralization was performed using Chi-squared or Fisher's exact test. Analysis was done using SPSS version 25 and Prism GraphPad version 9.

3 | RESULTS

3.1 | Patient characteristics

A total of 60 patients (male 37; female 23) were analyzed at one-month post-third dose, of which 51 patients also provided samples at 3-months post-third dose. Median age was 66.9 years (IQR 64.0–71.8) and type of transplant included kidney (n = 20), liver (n = 4), lung (n = 11), heart (n = 10), pancreas (including kidney/pancreas)

(n = 15). Median time from transplant was 3.57 years (IQR 1.99–6.75). Tacrolimus, prednisone, and mycophenolate were the most common immunosuppression agents. Baseline demographic data are shown in Table 1. No patient had previous COVID-19 as confirmed by negative anti-nucleocapsid antibody.

3.2 | Sars-CoV-2 neutralization

Neutralization at 1 month and 3 months post-third-dose vaccination is shown in Figure 1A for WT, Delta, and Omicron. One month post-third dose, 11/60 (18.3%) patients had detectable neutralizing antibody responses to Omicron with a median $\log_{10}$ID50 of 2.38 (range 1.34–3.57; in the subgroup of positive patients). At 3 months post-third dose, 8/51 (15.7%) patients had detectable neutralizing antibody responses with a further reduction in median $\log_{10}$ID50 (1.68; range 1.12–3.61; approximate fivefold reduction over time). The proportion of positive patients was lower for Omicron vs. WT, and Omicron vs. Delta at 1 and 3 months ($p < .001$ all comparisons; McNemar test). Neutralization titers were several fold-lower for Omicron vs. WT and Delta vs. WT both at 1 month and 3 months (Figure 1B). For example, at 3 months, the neutralization titer was a median of 18.8-fold lower for Omicron compared with WT (range 1 to 2204-fold reduction).

3.3 | Responders versus non-responders

We assessed demographic factors and immunosuppression in relation to those who developing Omicron specific neutralization response vs. those who did not. The data are shown in Table 1. Overall, we found no specific association of Omicron neutralization at 1-month with type of transplant, immunosuppression or other baseline demographic data (p = NS for all comparisons). We then assessed anti-RBD response in all patients. At the 1-month time point, 42/60 (70.0%) had detectable anti-RBD with a median titer of 1335.5 U/ml (IQR 231–3759) in the detectable patients (Figure 2). Of the patients with positive Omicron neutralization, 10/11(90.9%) also had detectable anti-RBD (median 9983 U/ml; IQR 5438–28660). Of the patients with negative Omicron neutralization, 32/49 (65.3%) still had positive anti-RBD (median 730 U/ml; IQR 45–1578). At the 3-month time point, 39/51 (76.5%) had detectable anti-RBD with a median titer of 457.6 U/ml (IQR 106–1784) (Figure 2). Of the patients with positive Omicron neutralization, 7/8 (87.5%) also had detectable anti-RBD (median 3527 U/ml; IQR 3022.5–4300). Of the patients with negative Omicron neutralization, 32/43 (74.4%) still had detectable anti-RBD (median 200 U/ml; IQR 52.3–1146.5).

TABLE 1 Factors associated with neutralization response to omicron variant 1 month after third dose of mRNA-1273

Characteristic	mRNA–1273 (n = 60)	Omicron responders (n = 11)	Omicron non-responders (n = 49)	p-value
Age (years), median (interquartile range)	66.9 (64.0–71.8)	68.4 (62.2–72.4)	66.8 (64.1–71.4)	0.60
Male sex, n (%)	37 (62%)	7 (63.6%)	30 (61.2%)	0.99
Time from transplantation to intervention (years), median (interquartile range)	3.57 (1.99–6.75)	3.26 (1.53–14.9)	3.85 (2.34–6.71)	0.77
Type of transplant (%)				
Thoracic	21 (35%)	3 (27.3%)	18 (36.7%)	0.73
Lung	11	1	10	
Heart	10	2	8	
Abdominal	39 (65%)	8 (72.7%)	31 (63.2%)	0.73
Kidney	20	5	15	
Pancreas and kidney-pancreas	15	1	14	
Liver	4	2	2	
Immunosuppression				
Prednisone (%)	50 (83%)	8 (72.7%)	42 (85.7%)	0.37
Calcineurin inhibitor (%)	59 (98%)	10 (90.1%)	49 (100%)	0.18
Tacrolimus	47 (78%)	8 (72.7%)	39 (79.6%)	
Cyclosporine	12 (20%)	2 (18.2%)	10 (20.4%)	
Mycophenolate mofetil/ mycophenolate sodium (%)	44 (73%)	7 (63.6%)	37 (75.5%)	0.46
Azathioprine (%)	8 (13%)	1 (9.1%)	7 (14.3%)	0.99
Sirolimus (%)	6 (10%)	1 (9.1%)	5 (10.2%)	0.99
Lymphocyte count at time of 3rd dose vaccine (10^3 cells/μl), median (interquartile range)	1.15 (0.90–1.60)	1.1 (0.80–1.25)	1.2 (0.90–1.60)	0.36

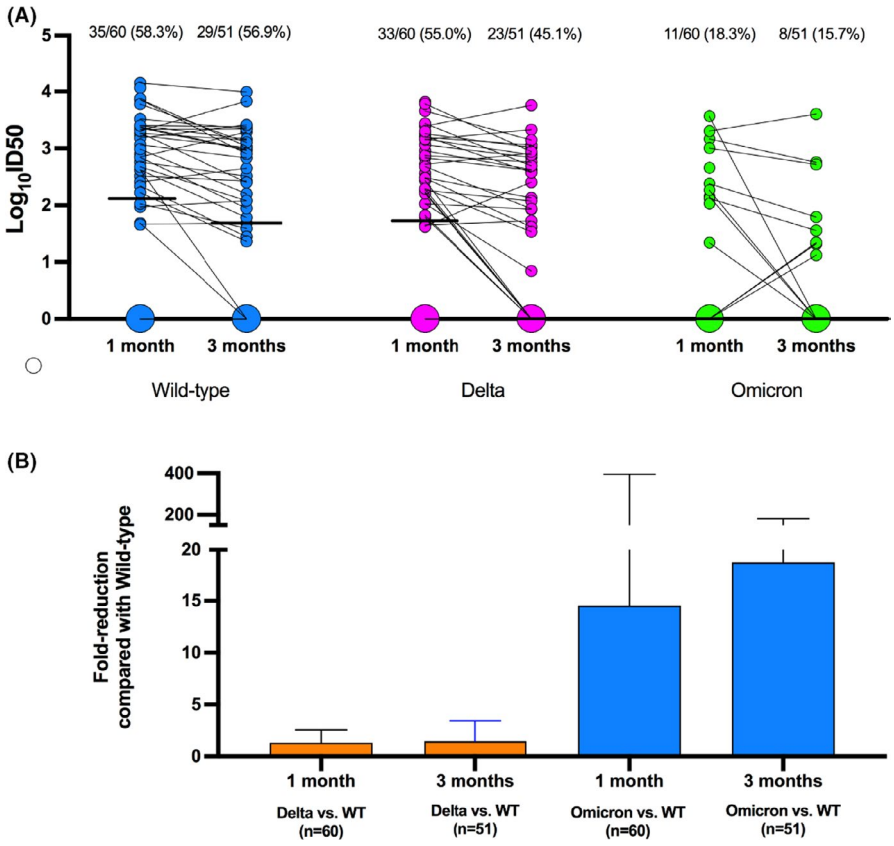

FIGURE 1 (A) Pseudotyped lentivirus neutralization at 1 month and 3 months post-third dose of mRNA-1273: Neutralization against wild-type (WT) SARS-CoV-2 pseudovirus, Delta and Omicron variants is shown. Titer is shown as the $\log_{10}$ID50 value at 1 month and 3 months after receipt of three doses of mRNA-1273 vaccine ($n = 60$ patients who provided samples at the first time point and $n = 51$ patients who provided samples at the second time point). Proportion of patients with a detectable neutralization for WT, Delta, and Omicron is shown above each graph. Horizontal lines represent median $\log_{10}$ID50 (note that median is on the x-axis for several plots). Larger dots on x-axis are representative of values for multiple patients. Proportion of patients with detectable neutralization was lower for Omicron vs. WT and Omicron vs. Delta at 1 month and 3 months ($p < .001$ for all comparisons, McNemar test for paired data). (B) Fold reduction in ID50 for Delta and Omicron compared to wild-type virus: Fold reduction was calculated for each patient by dividing their absolute ID50 value for the WT virus by the absolute ID50 value for the variant (either Delta or Omicron) virus. The height of the bar represents the median value and error bars represent the interquartile range of values. Fold-reduction is shown for 1-month post-third dose samples ($n = 60$) and 3-month post-third dose samples ($n = 51$)

4 | DISCUSSION

We show that even with three doses of mRNA vaccine, organ transplant recipients have poor neutralizing antibody responses for Omicron as compared with WT and Delta at both 1 month and 3 months after the third dose. Neutralization titers diminish over time and are consistently several fold-lower for Omicron as compared with WT and Delta. This may explain the high numbers of vaccine breakthrough infections with Omicron variant.

We also show that many patients had detectable anti-RBD titers at 1 month (70%) and 3 months after third vaccine dose. However, despite having detectable RBD antibodies, the vast majority of patients did not have neutralizing capacity against the Omicron variant. Although RBD titers were usually higher in those that were able to neutralize Omicron, the significant overlap in values between groups especially at titers >1000 U/ml suggests that standard antibody measurements require cautious interpretation.

Limitations of our study are that no clear protective correlate exists for current immunogenicity assays (including neutralization) although estimates suggest that $\log_{10}$ID5 values between 1.0 to 1.48 may be 50% protective.[7] Although we did not have a comparator group, according to the literature, the neutralization response to Omicron variant in healthy individuals (using a similar pseudovirus neutralization assay) after the third dose is 95%–100%.[3,8] T-cell responses may be better preserved across variants and could limit disease severity. Alternative strategies, such as passive antibody prophylaxis may be preferable in organ transplant recipients. In summary, organ transplant recipients appear minimally protected against Omicron infection despite three doses of vaccine based on assessment of variant-specific neutralizing antibody.

ACKNOWLEDGMENTS

The study was funded by the Ajmera Transplant Centre, University Health Network, University of Toronto. Vaccine was

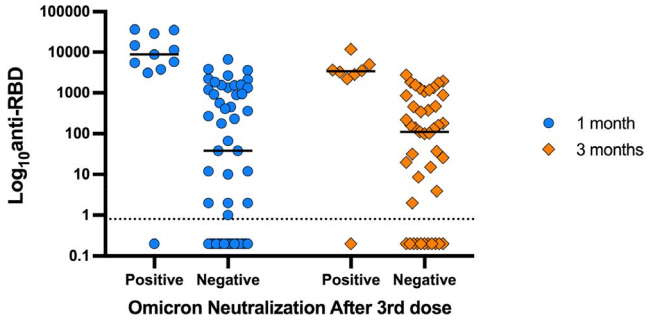

FIGURE 2 Anti-RBD titers after third dose of mRNA-1273 in patients with and without Omicron variant neutralization: Anti-RBD (receptor binding domain) titers (expressed on a log scale) in patients that demonstrated a positive and negative omicron neutralization after three doses of mRNA-1273 vaccine. Circles show values at 1 month post-third dose vaccine and diamonds indicate values at 3 months post-third dose vaccine. Horizontal line for each group represents the median value. Dotted line denotes the cut-off value for a positive anti-RBD test

provided by the University Health Network pharmacy. Funding for lentivirus variant assays was provided from the Canadian Institutes of Health Research through CoVaRR-Net, the Canadian Coronavirus Variants Rapid Response Network (to ACG). Moderna had no role in funding the study or in the design, conduct, analysis or any other aspect of the study. We are grateful to Ms. Ilona Bahinskaya, and Ms. Natalia Pinzon for their contributions to the study.

DISCLOSURE

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. D.K. has received research grant from Roche, GSK and advisory fees from Roche, GSK, Sanofi, Merck and Exevir. A.H. has received research grants from Roche and Merck and advisory fees from Merck and Takeda. A.-C.G. has received research funds from a research contract with Providence Therapeutics Holdings, Inc for other projects. The other authors have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Kumar D, Hu Q, Samson R, et al. Neutralization against Omicron variant in transplant recipients after three doses of mRNA vaccine. *Am J Transplant*. 2022;00:1–5. doi:10.1111/ajt.17020