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BRIEF COMMUNICATION

A third dose of SARS-CoV-2 vaccine increases neutralizing antibodies against variants of concern in solid organ transplant recipients

Andrew H. Karaba[1] | Xianming Zhu[2] | Tao Liang[1] | Kristy H. Wang[1] | Alex G. Rittenhouse[1] | Olivia Akinde[2] | Yolanda Eby[2] | Jessica E. Ruff[2] | Joel N. Blankson[1] | Aura T. Abedon[3] | Jennifer L. Alejo[3] | Andrea L. Cox[1,4,5] | Justin R. Bailey[1] | Elizabeth A. Thompson[1,5] | Sabra L. Klein[1,4] | Daniel S. Warren[3] | Jacqueline M. Garonzik-Wang[6] | Brian J. Boyarsky[3] | Ioannis Sitaras[4] | Andrew Pekosz[1,4] | Dorry L. Segev[3] | Aaron A.R. Tobian[2] | William A. Werbel[1]

[1]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland

[2]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland

[3]Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, Maryland

[4]W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland

[5]Bloomberg Kimmel Institute for Cancer Immunotherapy, Johns Hopkins University School of Medicine, Baltimore, Maryland

[6]Department of Surgery, University of Wisconsin School of Medicine and Health, Madison, Wisconsin

Correspondence
William A. Werbel, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA.
Email: wwerbel1@jhmi.edu

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Abstract

Vaccine-induced SARS-CoV-2 antibody responses are attenuated in solid organ transplant recipients (SOTRs) and breakthrough infections are more common. Additional SARS-CoV-2 vaccine doses increase anti-spike IgG in some SOTRs, but it is uncertain whether neutralization of variants of concern (VOCs) is enhanced. We tested 47 SOTRs for clinical and research anti-spike IgG, pseudoneutralization (ACE2 blocking), and live-virus neutralization (nAb) against VOCs before and after a third SARS-CoV-2 vaccine dose (70% mRNA, 30% Ad26.COV2.S) with comparison to 15 healthy controls after two mRNA vaccine doses. We used correlation analysis to compare anti-spike IgG assays and focused on thresholds associated with neutralization. A third SARS-CoV-2 vaccine dose increased median total anti-spike (1.6-fold), pseudoneutralization against VOCs (2.5-fold vs. Delta), and neutralizing antibodies (1.4-fold against Delta). However, neutralization activity was significantly lower than healthy controls ($p < .001$); 32% of SOTRs had zero detectable nAb against Delta after third

Abbreviations: Anti-N, anti-nucleocapsid antibody; Anti-RBD, anti-receptor binding domain antibody; Anti-S, anti-spike antibody; AU, arbitrary unit; AUC, area under the curve; CFR, case fatality rate; CM, complete media; ELISA, enzyme-linked immunosorbent assay; HC, healthy control; IM, infection media; KTR, kidney transplant recipient; MSD, Meso Scale Diagnostics; nAb, neutralizing antibody; NT50, 50% neutralization titer; OD, optical density; SOTR, solid organ transplant recipient; $TCID_{50}$, 50% tissue culture infectious dose; VOC, variant of concern.

Aaron A.R. Tobian and William A. Werbel contributed equally.

vaccination compared to 100% for controls. Correlation with nAb was seen at anti-spike IgG >4 Log_{10}(AU/ml) on the Euroimmun ELISA and >4 Log_{10}(AU/ml) on the MSD research assay. These findings highlight benefits of a third vaccine dose for some SOTRs and the need for alternative strategies to improve protection in a significant subset of this population.

KEYWORDS
basic (laboratory) research/science, immunobiology, infection and infectious agents – viral, infectious disease, SARS-CoV-2/COVID-19, solid organ transplantation, translational research/science, vaccine

1 | INTRODUCTION

Solid organ transplant recipients (SOTRs) are at increased risk for severe COVID-19.[1,2] Therefore, effective and optimized vaccines that prevent COVID-19 disease in this group are critical. Unfortunately, these patients were excluded from the phase III SARS-CoV-2 vaccines trials[3,4]; recent publications suggest that breakthrough disease is more common among fully-vaccinated SOTRs than the general population.[5,6] Furthermore, given many SOTRs develop weak SARS-CoV-2 antibody responses after two doses of an mRNA-based vaccine,[7-10] third doses have been authorized for immunocompromised persons in multiple countries. Yet, published data on neutralizing capacity of SOTR plasma after additional vaccine doses are limited.[11-13] In particular, it is unknown if a third vaccine dose would result in protection against more transmissible variants of concern (VOCs) that exhibit immune escape, including the Delta variant which currently comprises >99% of new cases in the United States.[14] To assess whether a third dose of SARS-CoV-2 vaccine in SOTRs would improve the SARS-CoV-2-specific neutralizing response, we measured total SARS-CoV-2-specific IgG and neutralizing activity using pseudoneutralization and live-virus assays against the vaccine strain and VOCs before and after a third dose of SARS-CoV-2 vaccine and compared this to vaccinated healthy controls (HC).

2 | MATERIALS AND METHODS

2.1 | Cohorts

SOTR participants were enrolled in a national prospective, observational cohort, Johns Hopkins IRB00248540, as previously described.[8,15] The full cohort began in December 2020. All members were contacted in May 2021 to identify persons who planned to receive third vaccine doses and were willing to undergo a large blood draw (~30 ml) before and after a third dose. This series describes the participants who were consented and able to donate samples. Specifically, SOTRs submitted blood 0–4 weeks before and 2 weeks after third vaccine doses that were independently obtained in the community. Participants were contacted by digital surveys to report any incident suspected or confirmed COVID-19 diagnoses after the third vaccine dose. Since this study focused on vaccine immunogenicity in persons without known prior infection, all individuals were evaluated for anti-nucleocapsid. One potential participant with a positive pre-third dose response was excluded from this study. HC participants were enrolled under Johns Hopkins IRB00027183.[16] Blood was collected in Acid Citrate Dextrose tubes and plasma was isolated by Ficoll centrifugation and stored at −80°C.

2.2 | IgG measurement

Plasma was tested using the clinically available EUROIMMUN anti-SARS-CoV-2 IgG enzyme-linked immunosorbent assay (ELISA) versus the S1 domain of spike protein, performed per the manufacturer's protocols. Optical density (OD) of the sample was divided by calibrator provided arbitrary unit (AU) ratio, for which ≥1.1 was considered positive and ≥0.8–1.1 were considered indeterminate.[17,18] Plasma was thawed and anti-N, anti-RBD, and anti-S IgG was measured using the multiplex chemiluminescent Meso Scale Diagnostics (MSD) V-PLEX COVID-19 Respiratory Panel 3 Kit according to the manufacture's protocol at a dilution of 1:5000.

2.3 | Pseudoneutralization/ACE2 inhibition measurement

The MSD pseudoneutralization/ACE2 inhibition assay measures the ability of participant plasma to inhibit ACE2 binding to spike protein. Plasma was thawed and ACE2 blocking was measured using the ACE2 MSD V-PLEX SARS-CoV-2 ACE2 kits according to the manufacturer's protocol at a dilution of 1:100. Plates come precoated with spike proteins corresponding to variants of interest. They were washed and incubated with plasma for one hour, human ACE2 protein conjugated with a SULFO-TAG (light-emitting label) added for another hour, washed, read buffer added, and read with a MESO QuickPlex SQ 120 instrument. If the plasma fully bound the coated spike protein and blocked binding of the added ACE2, then no light is emitted during the read phase of the assay, corresponding to 100% ACE2 inhibition. If there was no binding of spike by participant plasma, then the added ACE2 fully binds the coated

spike protein and illuminates during reading, corresponding to 0% inhibition. An eight-point calibration curve was included in each plate. The last point only contained assay diluent. Results were reported as percent ACE2 inhibition based on the equation provided by the manufacturer ([1 – Average sample ECL/Average ECL signal of blank well] x100).

2.4 | Viruses and cells

VeroE6-TMPRSS2 cells[19] were cultured in complete media (CM) as described.[17] The SARS-CoV-2/USA-WA1/2020 virus was obtained from BEI Resources. The Delta variant of SARS-CoV-2 (hCoV19/USA/MD-HP05660/2021, EPI_ISL_2331507) was isolated on VeroE6-TMPRSS2 cells plated in 24-well dishes and grown to 75% confluence. CM was removed and replaced with 150 μl of infection medium (IM), which is identical to CM but contains only 2.5% fetal bovine serum, and 150 μl of the viral transport media containing a swab from a patient with SARS-CoV-2 positive. The cultures were incubated at 37°C for 2 h, the inoculum was aspirated and replaced with 0.5 mL of IM and the cells cultured at 37°C for 5 days. IM was harvested when cytopathic effect was visible and stored at −70°C. SARS-CoV-2 was verified by extracting RNA using a viral RNA extraction kit (Qiagen), and detected using quantitative RT-PCR.[20] The consensus sequence of the virus isolate did not differ from the sequence derived from the clinical specimen.

Viral titer was determined on VeroE6-TMPRSS2 cells using a 50% tissue culture infectious dose (TCID50) assay as previously described.[21,22]

2.5 | Neutralization assay

Neutralizing antibody (nAb) levels were determined as described using twofold dilutions of plasma (starting at 1:20).[23] Infectious virus was added to the dilutions at a concentration of 1 × 10^4 TCID50/ml (100 TCID50 per 100 μl). Samples were incubated for 1 hour then 100 μl of each dilution was added to 1 well of a 96-well plate of VeroE6-TMPRSS2 cells in sextuplet for 6 hours at 37˚C. The inocula were removed, fresh IM was added, and the plates were incubated at 37˚C for 2 days or until complete cytopathic effect was visible in wells exposed to only virus. The cells were fixed with 4% formaldehyde, incubated for 4 hours, and then stained with Napthol Blue Black (MilliporeSigma). The nAb titer was calculated as the highest serum dilution that eliminated the cytopathic effect in 50% of the wells and area under the curve (AUC) was calculated using GraphPad Prism.

2.6 | Statistical analysis

SOTRs with available demographic and immunological data on pre and post third dose of SARS-CoV-2 vaccine were included in the analysis. Wilcoxon signed rank test was used to compare the median of SARS-CoV-2 anti-Spike and anti-RBD IgG level and percent ACE2 inhibition before and after third dose of vaccine among SOTRs. The median of IgG level and ACE2 inhibition between SOTRs and HCs were compared using Wilcoxon rank sum test. Pearson correlation was used to evaluate the linear association between Spike IgG and percent ACE2 inhibition among SOTRs. A spline knot was added at 4 $\log_{10}$(AU) MSD IgG. Bonferroni correction was conducted to control multiple comparison when analyzing variants ($p < .01$ was considered statistically significant). The analysis was also stratified by type of third dose vaccine, age, sex, and graft transplanted to evaluate effect measure modification. Missing values were treated using available case strategy in subgroup analysis.

3 | RESULTS

Pre- and post-third dose samples were available for 47 SOTRs followed in our ongoing longitudinal observational cohort studying immunogenicity and safety of SARS-CoV-2 vaccination. Most participants had previously undergone anti-spike antibody testing using clinically available assays.[15] The median age was 63 (interquartile range 49–70) years and 55% were female. Sixty-four percent of SOTRs were kidney transplant recipients (KTR) and all initially received two doses of an mRNA-based vaccine (23 Moderna mRNA-1273, 24 Pfizer BNT162b2). Most were taking a calcineurin inhibitor-based maintenance immunosuppression regimen (77%) and 30% were on “triple immunosuppression” with a calcineurin inhibitor, an antimetabolite, and corticosteroids. Seventy percent of SOTRs received a third mRNA vaccine dose and 30% received the Janssen Ad26.COV2.S vaccine. None reported a known history of COVID-19. The included analytic cohort had similar clinical and demographic characteristics as the larger parent cohort (Table S1). Relevant differences included receipt of a third dose closer in proximity to their second dose and more frequent receipt of Ad26.COV2 as a third dose. Among mRNA-vaccinated HCs ($N = 15$), none had known medical conditions, and all received two doses of BNT162b2. Table 1 presents full demographic and clinical data.

Anti-S1-receptor binding domain (RBD), anti-spike (S), and anti-nucleocapsid (N) total IgG were measured in plasma before and after a third dose of SARS-CoV-2 vaccine in SOTRs and after two doses of an mRNA-based vaccine in HCs using a research assay (MSD) with FDA-verified seropositivity cutoffs. No participants included in this study had a positive anti-N response before or after a third dose of vaccine (Figure S1). Prior to a third dose of vaccine, 17 (36%) and 11 (23%) SOTRs were seropositive for anti-RBD and anti-S, respectively (Figure S1). After the third dose, these numbers increased to 36 (77%) and 34 (72%), respectively, and there was a significant increase in the median total anti-S (1.6 fold-change) and anti-RBD (1.5 fold-change) IgG levels compared to matched pre-third dose samples (Figure S1). Despite these increases, the median anti-RBD and anti-S IgG values of SOTRs receiving a third dose remained significantly lower than the median responses in fully vaccinated HCs after the

TABLE 1 Clinical and demographic characteristics of SOTRs and healthy controls

	Overall, $n = 62$	SOTR, $n = 47$	Healthy controls, $n = 15$
Age, years			
20–39	10 (16)	3 (6)	7 (47)
40–59	26 (42)	18 (38)	8 (53)
60–79	26 (42)	26 (55)	0 (0)
Sex			
Female	31 (50)	26 (55)	5 (33)
Male	31 (50)	21 (45)	10 (67)
Race			
White	57 (92)	46 (98)	11 (73)
Asian	4 (6)	1 (2)	3 (20)
African American	1 (2)	0 (0)	1 (7)
Graft transplanted			
Kidney[a]	-	30 (64)	-
Liver	-	10 (21)	-
Heart	-	4 (9)	-
Lung	-	2 (4)	-
Pancreas	-	1 (2)	-
Anti-rejection medication[b]			
Prednisone	-	22 (47)	-
Calcineurin Inhibitors	-	36 (77)	-
mTOR inhibitors	-	7 (15)	-
anti-metabolites	-	30 (64)	-
Type of the third dose vaccine			
mRNA	-	33 (70)[c]	-
Ad26.COV2.S	-	14 (30)	-
Days between second dose and third dose vaccine	-	102 (70–124)	-
Days between transplant and third dose vaccine	-	1778 (930–4419)	-
Days post second dose vaccine	-	-	8 (7–10)

Note: All study participants received mRNA vaccine for the first two doses. Categorical variables were presented in *n* (%), and continuous variables were presented in median (interquartile range).

[a]1 person had both kidney and pancreas transplanted and has been grouped into kidney category.

[b]Anti-rejection medication use was not mutually exclusive.

[c]10 (30%) of the 33 participants received a third mRNA vaccine that differed from their initial two-dose series.

two-dose series (Figure 1A). In comparison to all other transplant recipients, KTRs had significantly lower anti-S IgG (Figure S2). In exploratory analysis, median IgG levels did not differ by other key clinical or demographic parameters such as age, sex, or type of third dose received, though subgroup sizes were small (Figure S2). Notably, seven female KTRs had the lowest post third dose IgG levels of all SOTRs in the study. All were taking anti-metabolite maintenance immunosuppression, but they did not otherwise share clinical or demographic factors.

Next, we investigated the neutralizing potential of SOTR plasma versus SARS-CoV-2 VOCs after three vaccine doses using pseudoneutralization (ACE2 inhibition) with comparison to that of healthy individuals after two vaccine doses. There was a significant increase in the median pseudoneutralization of all variants after a third vaccine dose among SOTRs: fold-changes were 2.5, 2.2, 1.6, 1.5, and 2.5 for vaccine, Alpha, Beta, Gamma, Delta variants, respectively (Figure 1B). However, pseudoneutralization of all variants was significantly lower than that of HCs after two doses of an mRNA-based vaccine (Figure 1C). For example, only three (6%) SOTRs had pseudoneutralization values for the Delta variant above the first quartile of the HC pseudoneutralization values; the majority were below 20% inhibition for all variants. When stratified by type of organ received, KTRs had significantly lower ACE2 inhibition versus the vaccine strain and Alpha variant compared to all other organs (Figure S3). There were no significant differences in pseudoneutralization when stratifying the cohort by age, sex, or vaccine platform (Figure S3).

We also examined the correlation between anti-S IgG and pseudoneutralization for all the variants. There was a strong correlation between anti-S IgG and pseudoneutralization, but the relationship only became linear around 4 $\log_{10}$(arbitrary unit, AU) IgG (Figure 1D).

Finally, live-virus neutralization (nAb) was assessed using 50% neutralization titer (NT50) and area under the curve (AUC) against the vaccine strain and the Delta variant before and after a third vaccine dose in SOTRs and in two-dose vaccinated HCs. For SOTRs, median (IQR) NT50s were 40 (10–120) versus vaccine strain and 20 (10–40) versus Delta (Figure 2A), with median (IQR) AUC of 50 (2–145) and 9 (3–50), respectively (Figure 2B) after a third vaccine dose. This corresponded to a fold-change in NT50 of 1.6 and 1.3, and a fold-change in AUC of 50.2 and 8.4, versus the vaccine strain and Delta variant, respectively. Compared to HCs, NT50s and AUC versus vaccine and Delta variant strains were significantly lower among SOTRs (Figure 2C,D). 32% of SOTRs had nAb NT50s at or below the limit of detection versus the Delta variant after a third vaccine dose (as compared to 0% of HCs). There were, however, two female liver transplant recipients with very high nAbs, even beyond those of the HCs.

Interassay correlation was assessed for both the vaccine strain (Figure 3A) and Delta variants (Figure 3B) among clinical (EUROIMMUN) and research (MSD) anti-spike IgG assays, as well as pseudoneutralization and nAb AUC for SOTRs and HCs. For the vaccine strain, EUROIMMUN and MSD IgG showed excellent positive correlation, particularly above the clinical manufacturer threshold for seropositivity (1.1 AU). Correlation of pseudoneutralization with both IgG assays was strong above a threshold of 20% ACE2 blocking. Below this, there was marked variation in corresponding IgG levels among SOTRs, particularly

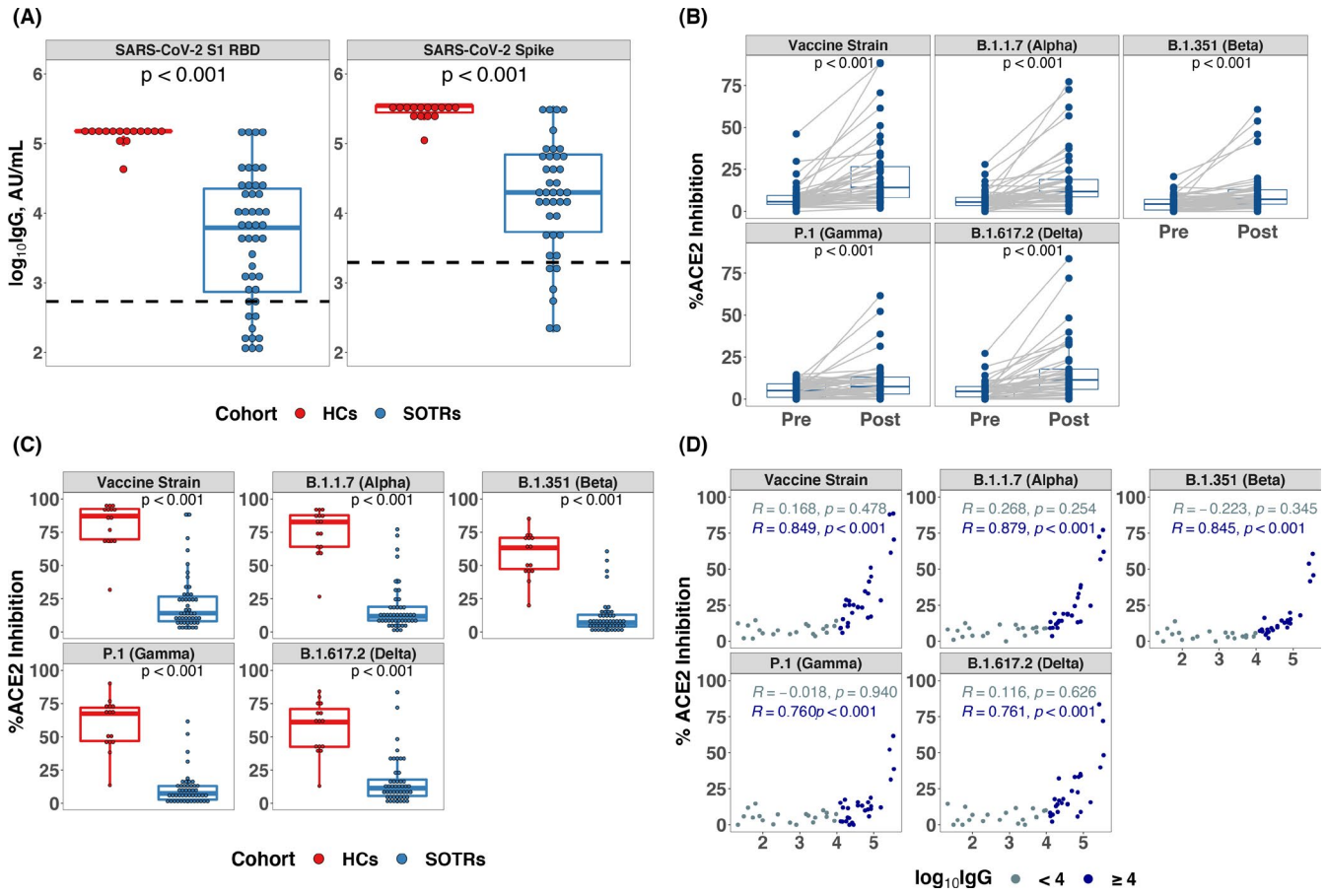

FIGURE 1 Changes in SARS-CoV-2 specific IgG and pseudoneutralization after a third dose of SARS-CoV-2 vaccine. (A) Total SARS-CoV-2 S1 RBD- (left) and Spike- (right) specific IgG in fully mRNA vaccinated healthy controls (HCs, red) ($n = 15$) and SOTRs (blue) after a third dose of vaccine ($n = 47$). (B) Pseudoneutralization of full-length SARS-CoV-2 Spike variants (indicated in top header of each panel) before and after a third dose of vaccine among SOTRs. (C) Pseudoneutralization of full-length SARS-CoV-2 Spike variants (indicated in top header of each panel) in SOTRs ($n = 47$) after a third dose of vaccine compared to fully vaccinated healthy controls ($n = 15$). (D) Correlation between total SARS-CoV-2 Spike IgG and pseudoneutralization of full-length SARS-CoV-2 Spike variants among SOTRs receiving a third dose of vaccine. In panels A–C, the boxplots represent the IQR. The median is represented by a horizontal line in the box. The lower and upper whiskers represent 1.5x the IQR beyond the quartiles. Each dot represents an individual sample. Statistical differences between groups were determined by Wilcoxon signed rank test for panel B, and Wilcoxon rank sum test for panels A and C. Pearson correlation coefficient were generated for panel D. Values of $p < .05$ were considered significant [Color figure can be viewed at wileyonlinelibrary.com]

versus Delta (e.g., EUROIMMUN IgG ranged 0–7.5 AU) (Figure S4). Correlation of both IgG assays and nAb AUC was moderate, though markedly improved when restricting to higher IgG cutoffs (4 AU on the EUROIMMUN assay and 4 $\log_{10}$(AU) on the MSD assay). Overall correlation of pseudoneutralization and nAb was stronger, particularly when restricting to a single patient group (SOTR or HC). These cross-correlation patterns were similar when considering the Delta variant, although pseudoneutralization and nAb AUC correlation was stronger as compared to the vaccine strain, reflecting reduction in ACE2 blocking for HCs.

All participants responded to at least one follow-up survey regarding COVID-19 diagnoses, including the 87% of respondents who returned surveys in November 2021. None reported a breakthrough infection by a median (IQR) of 130 (140–158) days after the third vaccine dose.

4 | DISCUSSION

Here, we provide evidence that a third dose of SARS-CoV-2 vaccine increases plasma neutralization against VOCs for some SOTRs, including versus the Delta variant. This was robustly characterized using a combination of clinical and research IgG assays, pseudoneutralization, and gold-standard live-virus neutralization. Although median plasma neutralizing capacity did increase for SOTRs, levels were generally far below that of HCs after the two-dose mRNA series and 32% showed no nAb against the Delta variant using the live-virus assay.

Other key findings include lower neutralization among KTRs versus other transplant recipients, potentially reflecting heavier maintenance immunosuppression. Other factors previously associated with improved seroresponse such as younger age or third dose vaccine platform (i.e., mRNA vs. adenoviral vector) were not clearly associated with response. Importantly, although there was significant variability

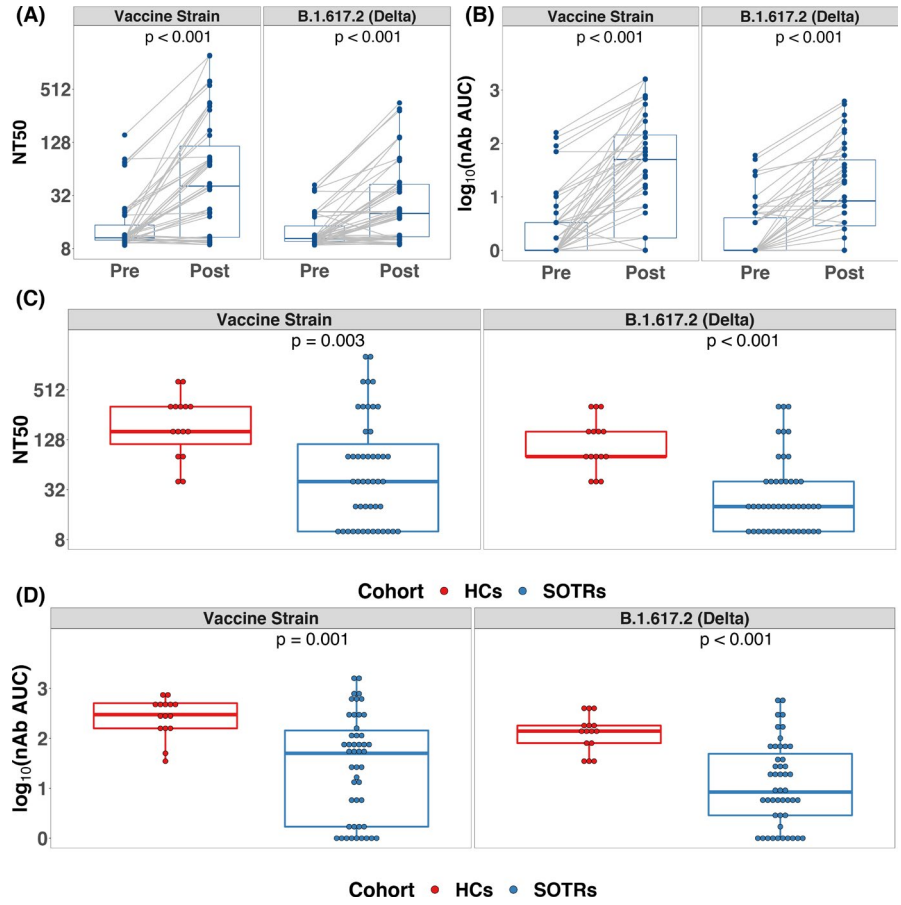

FIGURE 2 Neutralizing antibody (nAb) versus SARS-CoV-2 vaccine strain and Delta variant. (A) nAb NT50 versus SARS-CoV-2 vaccine strain and Delta variant before and after a third dose SARS-CoV-2 vaccine among SOTRs. (B) nAb area under curve (AUC) versus SARS-CoV-2 vaccine strain and Delta variant before and after a third dose SARS-CoV-2 vaccine among SOTRs. (C) Comparison of nAb reciprocal NT50 versus SARS-CoV-2 vaccine strain and Delta variant between SOTRs after a third dose of SARS-CoV-2 vaccine and HCs after two mRNA vaccine doses. (D) Comparison of nAb AUC of SARS-CoV-2 vaccine strain and Delta variant between SOTRs after a third dose of SARS-CoV-2 vaccine and HCs after two mRNA vaccine doses. In panels A–D, the boxplots represent the IQR. The median is represented by a horizontal line in the box. The lower and upper whiskers represent 1.5x the IQR beyond the quartiles. Each dot represents an individual sample. Statistical differences between groups were determined by Wilcoxon signed rank test for panels A and B, and Wilcoxon rank sum test for panels C and D [Color figure can be viewed at wileyonlinelibrary.com]

in IgG responses in SOTRs, we found evidence through Pearson correlation analysis between assays that certain IgG cutoffs were associated with clear increases in ACE2 blocking, as well as in nAb. This is an early step toward establishing thresholds for high-throughput assays that may indicate protection from COVID-19, though this will need to be tested by assessing risk of infection in real-world cohort and clinical trial settings. Notably, not all antigen-specific antibodies provide effective neutralization, which is likely why the correlation between anti-spike IgG and neutralizing activity is less robust at lower values of IgG.[24] The function and importance of non-neutralizing anti-spike antibody in SOTRs is an open area of investigation.

The humoral response to additional vaccine doses in this high-risk group was poor, yet highly variable, with a small minority of SOTRs producing antibody response on par with HCs. Though we identified kidney transplantation as a risk factor for decreased responsiveness, consistent with prior observations,[8] the mechanism underlying this association is unknown. Additional investigations and deeper immunological analyses are warranted to understand in a personalized fashion why some SOTRs respond to additional antigen exposure, while others do not.

It is not yet clear whether these antibody responses will be adequate to protect SOTRs from symptomatic COVID-19. Associations between neutralizing activity and clinical protection were not evaluable in this study due to a lack of reported clinical breakthrough infections after a median of 4.5 months of follow up. Recent work does suggest that peri-infection neutralizing antibody response is important in reducing cases,[25] whether this will have an impact on hospitalization in SOTRs as it does on the general population remains to be seen.[26] Regardless, the overall poor humoral response to a three-dose vaccine regimen indicates that alternative strategies, such as immunosuppressive modulation, use of emerging vaccine platforms, or long-acting anti-spike monoclonal antibodies, may be necessary to induce a protective response versus SARS-CoV-2. This study was limited by its observational nature and small number of participants with demographic and immunosuppressive heterogeneity. Additionally, HC comparators were younger than SOTRs, which may contribute to observed differences in humoral response. Although patient survey and anti-N IgG were used to rule out prior COVID-19, it is possible that

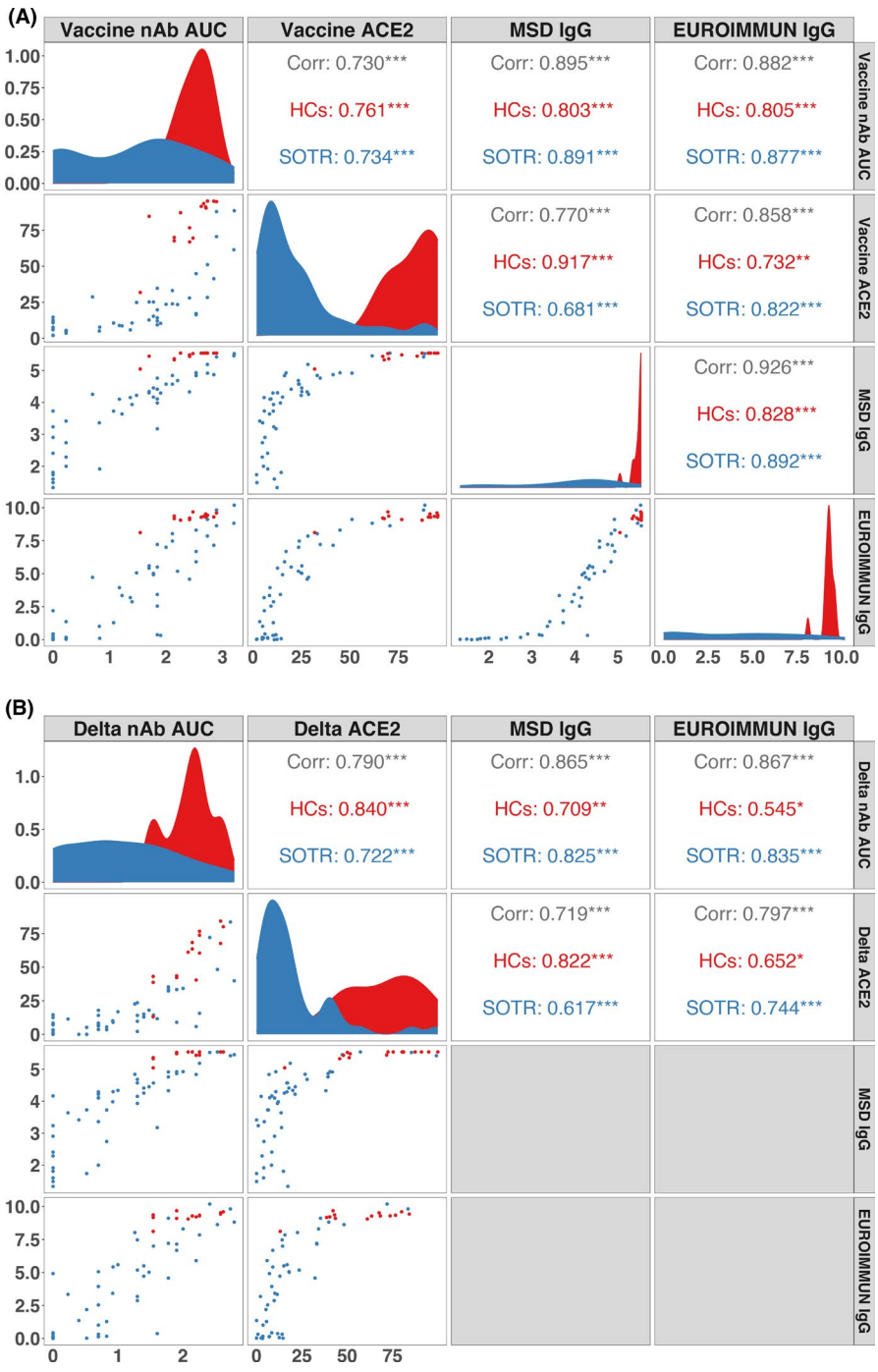

FIGURE 3 Correlations between neutralizing antibody (nAb), percent ACE2 inhibition, MSD anti-spike IgG, and EUROIMMUN anti-spike IgG of SARS-CoV-2 among SOTRs and HCs. (A) Correlations between neutralizing and IgG assays versus the SARS-CoV-2 vaccine strain among SOTRs after a third dose of vaccine and HCs after two doses. (B) Correlations between neutralizing and IgG assays versus the Delta variant among SOTRs after a third dose of vaccine and HCs after two doses. Each point on the scatter plots represents an individual sample. Pearson correlation coefficients between assays are presented in the upper panels. "Corr" represents the correlation across all samples. "HCs" (in red) represents the correlation among only HCs. "SOTR" (in blue) represents the correlation among only SOTRs. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. Density plots of SOTRs and HCs are shown in diagonal panels. Unit of analysis: nAb AUC, $\log_{10}$AUC; ACE2: percent ACE2 inhibition; MSD IgG, $\log_{10}$IgG AU/ml; EUROIMMUN IgG, AU/ml [Color figure can be viewed at wileyonlinelibrary.com]

subclinical infections occurred in some patients before or after vaccination. Furthermore, mucosal and cellular immune responses were not characterized in this study.

In summary, a third dose of a SARS-CoV-2 vaccine increases anti-spike IgG levels and plasma neutralizing capability, including against the Delta variant, in some SOTRs. Yet, a significant portion of SOTRs have limited or no neutralizing activity against the dominant VOC indicating that a third dose of vaccine may not be a fully effective strategy for a large portion of immunocompromised patients. These data also inform how research and clinical anti-spike IgG measurements might be used to estimate neutralizing ability and potential sero-protection thresholds. This is novel information regarding the potential improvement of immune protection against SARS-CoV-2 variants in a highly vulnerable population amidst ongoing community surges.

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DISCLOSURE

The authors of thismanuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. DLS has the following financial disclosures: consulting and speaking honoraria from Sanofi, Novartis, CSL Behring, Jazz Pharmaceuticals, Veloxis, Mallinckrodt, Thermo Fisher Scientific. AHK has received consulting fees from Roche. None of the other authors have any relevant competing interests.

DATA AVAILABILITY STATEMENT

Reasonable requests for deidentified data to the corresponding author will be granted.

ORCID

Andrew H. Karaba https://orcid.org/0000-0003-2785-317X
Aura T. Abedon https://orcid.org/0000-0001-8083-6964
Jennifer L. Alejo https://orcid.org/0000-0003-3137-9271
Brian J. Boyarsky https://orcid.org/0000-0001-6902-9854
Dorry L. Segev https://orcid.org/0000-0002-1924-4801
William A. Werbel https://orcid.org/0000-0003-2943-5895

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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