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Serologic Responses to COVID-19 Vaccination in Pediatric Kidney Transplant Recipients

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Background. There are limited data describing the immune responses to COVID-19 vaccination in pediatric kidney transplant recipients, and expanding upon this information could help inform vaccination strategies in this unique population. **Methods.** We performed a prospective, observational, single-center cohort study using remnant blood samples of pediatric kidney transplant recipients from routine clinic visits to examine longitudinal serological responses after COVID-19 vaccination. We enrolled 61 pediatric kidney transplant recipients who had at least 1 sample available for analysis. Sera or plasma were analyzed for ancestral SARS-CoV-2 and Omicron (B.1.1.529; BA.1) spike IgG and nucleocapsid IgG using a Meso Scale Discovery platform. **Results.** One month after a 3-dose COVID-19 vaccination series, the IgG geometric mean titer to the SARS-CoV-2 ancestral spike was 684 binding antibody units/mL (95% confidence interval, 269-1739), but titers waned by 4–6 mo. A fourth dose of the COVID-19 vaccine boosted IgG geometric mean titer to 1606 binding antibody units/mL (95% confidence interval, 868-2972), and titers persisted through 6 mo. IgG titers against Omicron (B.1.1.529; BA.1) were overall lower than ancestral SARS-CoV-2. They were higher in participants with prior infection and were not significantly impacted by receipt of belatacept. **Conclusions.** Additional doses of the COVID-19 vaccine bolstered durable serologic responses in pediatric kidney transplant recipients, and this study broadens our understanding of immune responses to COVID-19 vaccinations in this population.

(*Transplantation Direct* 2025;11: e1756; doi: 10.1097/TXD.0000000000001756.)

From fall 2020 to spring 2024, approximately 234 000 US children were hospitalized with COVID-19.[1] Although COVID-19 vaccines have been shown to safely and effectively protect against symptomatic SARS-CoV-2 infection, including severe COVID-19 and disease caused by emerging variants of concern,[2-4] the optimal timing of COVID-19 vaccination and immunological responses are not well characterized for children who have received solid organ transplants (SOTs). Data regarding COVID-19 vaccine immunogenicity and its correlation with clinical outcomes for the pediatric

Received 9 October 2024. Revision received.

Accepted 31 October 2024.

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This study was funded by Children's Healthcare of Atlanta and Emory University.

E.J.A. has consulted for Pfizer, Sanofi Pasteur, GSK, Janssen, Moderna, and Medscape, and his institution receives funds to conduct clinical research unrelated to this article from MedImmune, Regeneron, PaxVax, Pfizer, GSK, Merck, Sanofi Pasteur, Janssen, and Micron. He has also served on a safety monitoring board for Kentucky BioProcessing Inc and Sanofi Pasteur. He has served on a data adjudication board for WIRB-Copernicus Group and Applied Clinical Intelligence Clinical. His institution has also received funding from National Institutes of Health to conduct clinical trials of COVID-19 vaccines. He is currently employed by Moderna, Inc. C.A.R.'s institution has received funds to conduct clinical research unrelated to this article from BioFire Inc, GSK, MedImmune, Micron, Janssen, Merck, Moderna, Novavax, PaxVax, Pfizer, Regeneron, and Sanofi Pasteur. Her institution has received funding from National Institutes of Health to conduct clinical trials of Moderna and Janssen COVID-19 vaccines. She is a coinventor of patented respiratory syncytial virus vaccine technology, which has been licensed to Meissa Vaccines Inc with royalties received. The other authors declare no conflicts of interest.

K.P.G., R.L., E.J.A., A.L.S., R.G., R.P.G., and C.A.R. participated in research design. K.P.G., E.S., E.Y., M.K., M.A.P., and H.-M.H. participated in investigation and data curation. M.A.P., H.-M.H., L.S.D., and C.A.R. participated in data analysis. K.P.G., L.S.D., and C.A.R. participated in writing—original draft preparation. All authors participated in writing—review and editing. E.J.A. and C.A.R. participated in supervision and funding acquisition. All authors have read and agreed to the published version of the article.

Data will be made available upon reasonable request to the authors.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantationdirect.com).

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ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001756

SOT population are sparse, and the literature suggests that children may respond differently to COVID-19 infection and immunization than their adult counterparts.[5,6] To address the limited data on pediatric SOT recipients (SOTRs), we enrolled a diverse cohort of pediatric kidney transplant recipients (PKTRs) at our institution in a prospective observational cohort study to assess the magnitude, breadth, and longevity of serological responses to COVID-19 vaccination using remnant blood samples collected at routine transplant clinic visits. Expanding our understanding of their immunologic responses may inform recommendations to help optimize outcomes for PKTRs in the setting of ongoing SARS-CoV-2 circulation and the emergence of novel variants of concern.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board (STUDY00000723) at Emory University, and informed consent and assent were obtained from all participants, as indicated by age. PKTRs up to 21 y of age presenting for routine care to the kidney transplant clinic at Children's Healthcare of Atlanta who had received or were eligible to receive a COVID-19 vaccine based on the standard of care at that time were eligible for the study. Participants were enrolled from October 7, 2021, to July 21, 2022. Demographic data, COVID-19 history, and vaccination history were collected through patient interviews and medical chart abstraction and entered into a REDCap database. Residual serum or plasma samples were collected longitudinally at sequential follow-up visits from October 2021 to September 2022. Samples were stored at –80 °C until analysis.

Samples were analyzed for ancestral SARS-CoV-2 and Omicron (B.1.1.529; BA.1) spike IgG and nucleocapsid IgG using the Meso Scale Discovery (MSD) V-PLEX platform (Meso Scale Diagnostics, LLC, Rockville, MD), which quantitatively measures antibodies using electrochemiluminescence. Antibody concentrations were measured in arbitrary units per milliliter (AU/mL) and were converted to standardized binding antibody units per milliliter (BAU/mL) for ancestral SARS-CoV-2 spike IgG. Because blood samples constituted a convenience sample with a varying number of available samples per time period, only vaccine time periods with >4 samples were included in the analyses (**Table S1, SDC,** http://links.lww.com/TXD/A736). If a participant provided multiple samples on the same date, results were averaged; if a participant provided multiple samples within the same time period on different dates, the sample dated closest to the median of the time period was used. Included time periods were defined as follows: "1 mo" 14–60 d (median, 37 d), "2 mo" 61–120 d (median, 91 d), "4 mo" 121–180 d (median, 151 d), and "6 mo" 181–240 d (median, 211 d). Geometric mean titers (GMTs) were calculated, and log-transformed titers were compared using nonparametric Kruskal-Wallis with Dunn's multiple comparisons test, Wilcoxon with matched-pair signed rank test, or Mann-Whitney test. *P* values of ≤0.05 were considered statistically significant. All statistical analyses were performed using GraphPad Prism version 9.5.1.

To conduct this analysis, we selected a threshold IgG antibody titer to compare participants and provide a frame of reference for possible immune protection. Although correlates of protection have been defined for numerous prior vaccines[7,8] and this has been an area of research for COVID-19 vaccines, a universally agreed-upon correlate of protection has not yet been determined. Several adult studies, however, have proposed such serologic endpoints, which may be useful in understanding immunogenicity data. For example, Goldblatt et al[9] proposed an estimated mean protective antispike IgG protective threshold of 154 BAU/mL for original SARS-CoV-2 and 168 BAU/mL for Alpha variant based on 6 different vaccine regimens. Feng et al[10] reported 80% vaccine efficacy at 264 BAU/mL against the majority Alpha variant with AZD1222 vaccination, whereas Gilbert et al[11] reported 90% vaccine efficacy at 298 BAU/mL for variants circulating during the pivotal phase 3 COVE trial of mRNA-1273. Based on these available data, we used a threshold of 298 BAU/mL as a reference point of an IgG titer at which significant protection against COVID-19 might be expected. For nucleocapsid positivity, a threshold of 1704 AU/mL was used based on published data by Asamoah-Boaheng et al,[12] which specifically evaluated the performance of the MSD V-PLEX nucleocapsid assay and distinguished between unvaccinated and vaccinated adults. A similar threshold of nucleocapsid positivity using this assay has not been established in children, who may have reduced seroresponse compared with adults.[13]

RESULTS

Seventy PKTRs were enrolled, of whom 61 had at least 1 sample available for analysis (Table 1). The median age of participants was 16 y (interquartile range [IQR], 14–18). Thirty-six percent were women. Thirty-two PKTRs (52%) identified as White, 26 (43%) as Black, and 11 (18%) as Hispanic/Latinx. The standard induction regimen at our center includes basiliximab and intravenous methylprednisolone pulse. At the time of this study, most study participants were receiving triple immunosuppression. Tacrolimus + mycophenolate mofetil + low-dose prednisone was the most common regimen, followed by belatacept + mycophenolate mofetil + low-dose prednisone. Fifty-nine participants (97%) were receiving an antimetabolite and 20 (33%) were receiving belatacept as part of their regimen. The median time since transplant was 1483 d (4 y; IQR, 482–3464), and 37 (61%) were ≥3 y from receipt of transplant at the time of their first COVID-19 vaccination. Patients were vaccinated from April 2021 to September 2022. The majority of participants (95%) received all BNT162b2 (Pfizer Inc) vaccinations, but others did receive mRNA-1273 (Moderna Inc) and Ad26.COV2.S (Janssen) vaccines. Twenty-six participants (43%) reported a history of COVID-19 before vaccination or during the study.

We compared ancestral SARS-CoV-2 spike IgG titers before and after the second, third, and fourth COVID-19 vaccine doses. At baseline, we observed a range of titers with 2 of 7 samples (29%) above the putative threshold of protection before vaccination (Figure 1A), and 4 of 7 prevaccination samples (57%) were seropositive for SARS-CoV-2 nucleocapsid IgG, suggesting prior natural infection (data not shown). From our convenience sample, the next time period with sufficient samples available for analysis was 1 mo post-dose 2, at which time antibody titers were increased and 71% of participants had ancestral SARS-CoV-2 spike IgG titers >298 BAU/mL (putative threshold of protection). Titers returned to near prevaccination levels by 4 mo (GMT, 13; 95% CI,

TABLE 1.

Clinical characteristics of study participants

Clinical characteristic	Pediatric kidney transplant recipients (N = 61)
Age, y, median (range)	16 (2–20)
Female sex, n (%)	22 (36)
Race, n(%)	
White	32 (52)
Black	26 (43)
Asian	2 (3)
Native Hawaiian or Pacific Islander	1 (2)
Ethnicity, n (%)	
Hispanic/Latinx	11 (18)
Days from transplant at the time of first COVID-19 vaccine, median (range)	1483 (15–6041)
≥3 y from transplant at time of first COVID-19 vaccine, n (%)	37 (61)
Vaccine type, n (%)	
Pfizer only regimen	58 (95)
mRNA vaccine only regimen (Pfizer or Moderna/Pfizer combo)	60 (98)
Janssen (Ad26.COV2.S vector vaccine) included in regimen	1 (2)
Immunosuppression includes antimetabolite	59 (97)
Immunosuppression includes belatacept	20 (33)
Reported history of COVID-19 at any time before or during vaccination	26 (43)

2-100; $P > 0.9999$). With a third COVID-19 vaccine dose, SARS-CoV-2 ancestral spike IgG titers again increased, and at 1 and 2 mo after the third dose, 83% (GMT, 684 BAU/mL; 95% CI, 269-1739; $P = 0.2832$) and 84% of participants (GMT, 550 BAU/mL; 95% CI, 332-911; $P > 0.9999$) had titers >298 BAU/mL, respectively. GMTs waned over 6 mo, and only 46% of participants maintained protective titers (GMT, 309 BAU/mL; 95% CI, 90-1058; $P > 0.9999$). After a fourth dose, ancestral SARS-CoV-2 spike IgG titers significantly increased (GMT, 1606 BAU/mL; 95% CI, 868-2972 at 1 mo; $P = 0.012$, and GMT 3625 BAU/mL; 95% CI, 2193-5994 at 2 mo; $P < 0.0001$), and nearly all participants (≥89%) had titers >298 BAU/mL. Protective antibody titers were maintained for at least 4 mo after dose 4 with 100% of participants above the threshold (GMT 2453 BAU/mL; 95% CI, 984-6111; $P = 0.0299$).

SARS-CoV-2 IgG Omicron (B.1.1.529; BA.1) variant titers were significantly lower than ancestral titers at all but 2 time points postvaccination (Figure 1B). Nevertheless, the third and fourth doses boosted IgG antibodies to the Omicron variant (**Figure S1, SDC,** http://links.lww.com/TXD/A736). In each time period, participants with presumed hybrid immunity, as evidenced by SARS-CoV-2 nucleocapsid seropositivity, had higher SARS-CoV-2 ancestral spike IgG antibody titers than participants who were negative for SARS-CoV-2 nucleocapsid. This difference was statistically significant in a quarter of the time periods (3/12; Figure 2A), and most participants with presumed hybrid immunity maintained protective levels of antibodies for up to 4–6 mo postvaccination after each dose. When participants were stratified by treatment with or without belatacept, no significant differences were observed (Figure 2B; $P > 0.05$ for all time periods).

DISCUSSION

In this prospective longitudinal analysis, we leveraged convenience samples from a uniquely diverse clinical cohort to better characterize the serological responses to COVID-19 vaccination in PKTRs. We found that SARS-CoV-2 spike IgG titers were boosted by third and fourth vaccine doses (significantly so with a fourth dose) and remained elevated above a putative protective threshold for at least 4 mo after the fourth dose. The third and fourth dose vaccinations also improved the breadth of antibody responses against the Omicron (B.1.1.529; BA.1) variant. These data suggest that vaccination with third and fourth COVID-19 vaccine doses improves the magnitude, breadth, and durability of antibody responses in PKTRs. Participants with evidence of prior infection (hybrid immunity) also had superior antibody responses compared with those without prior infection.

Existing literature in PKTRs demonstrates a suboptimal antibody response after 2 vaccine doses with an improved response after a third dose. Seroconversion rates after the second vaccine dose range from about 50%–75%, with variation likely related to small sample sizes and methodology (including population, detection methods, exclusion of prior COVID-19 infection, etc).[14-19] Small studies in adolescent and young adult SOTRs support a third SARS-CoV-2 mRNA vaccine dose. Qin et al[20] found that, for SOTRs aged 12–18 y, antibody positivity increased from 74.4% to 88.4% with a third dose, and 87% of participants remained positive after 3 mo.[20] In their study of pediatric and adolescent KTRs, Crane et al[16] found an increase in seroconversion from 56% to 85% from the second to third dose, and of the 16 who did not seroconvert after a second dose, 12 (75%) seroconverted after a third dose.[16] Furthermore, McAteer et al[21] demonstrated in their study of adolescent SOTRs that all participants (n = 34) had positive anti-RBD antibody titers 6 mo after a third dose of the BNT162b2 mRNA vaccine. Our study sheds further light on the value of third and fourth vaccine doses, specifically in PKTRs.

Prior studies have also examined how the composition and timing of immunosuppressive regimens may affect immune response in SOTRs. Having received a transplant within the past 3 y, multiple immunosuppressive agents and antimetabolite drugs have been associated with poor antibody responses in pediatric SOTRs.[17,22] Our institution uses belatacept, a specific inhibitor of T-cell activation, which has

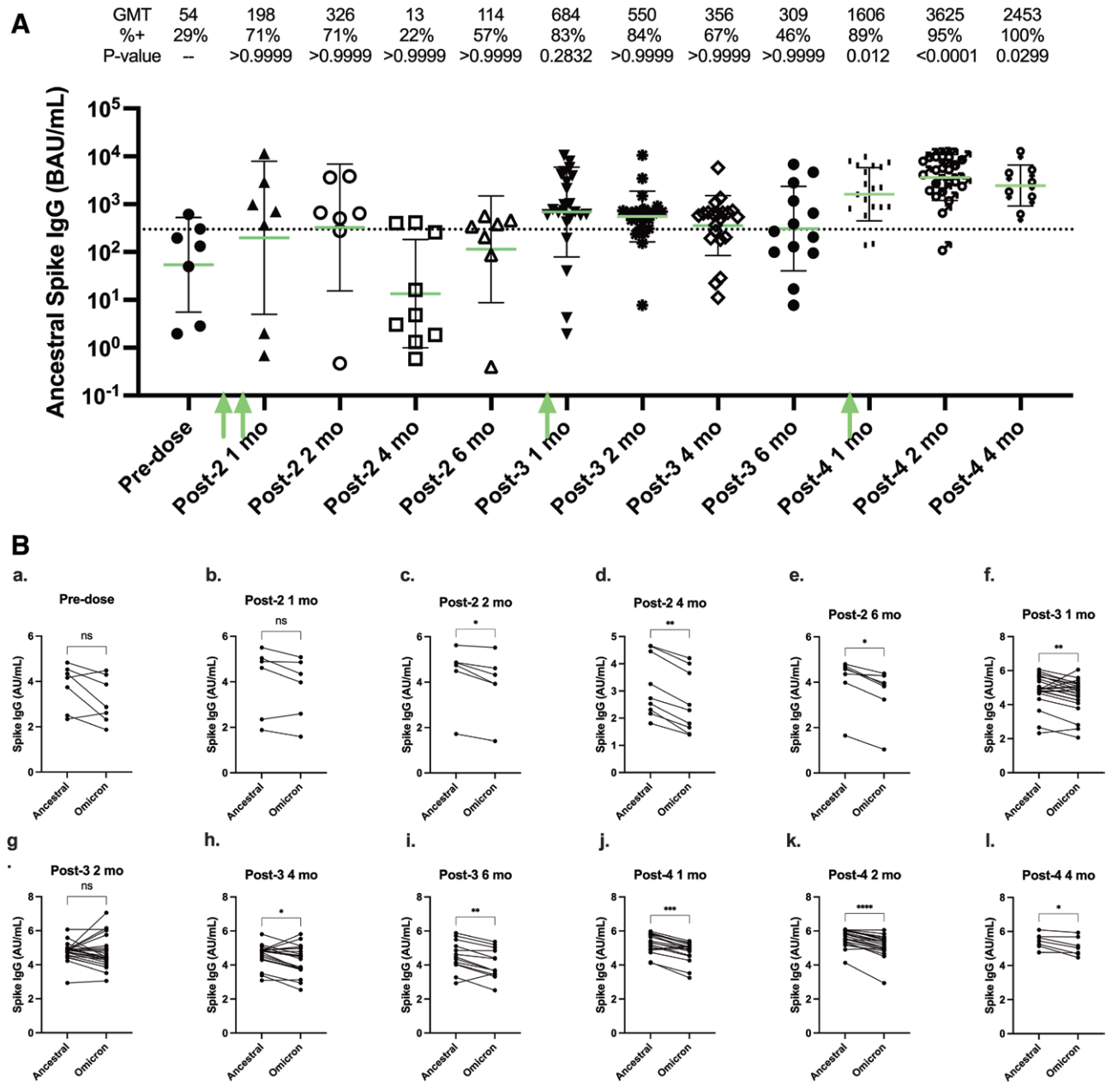

FIGURE 1. Spike IgG titers pre- and post-COVID-19 vaccination in pediatric kidney transplant recipients (A) against ancestral SARS-CoV-2 over time and (B) against Omicron (B.1.1.529; BA.1) at selected time periods pre-dose (a), 1 mo post-dose 2 (b), 2 mo post-dose 2 (c), 4 mo post-dose 2 (d), 6 mo post-dose 2 (e), 1 mo post-dose 3 (f), 2 mo post-dose 3 (g), 4 mo post-dose 3 (h), 6 mo post-dose 3 (i), 1 mo post-dose 4 (j), 2 mo post-dose 4 (k), and 4 mo post-dose 4 (l). A, Dashed line represents 298 BAU/mL (threshold reported to correlate with protection). Green lines represent GMTs with geometric SDs. Green arrows represent vaccinations. Statistical comparisons of log-transformed titers were made with prevaccine titers using the nonparametric Kruskal-Wallis test with Dunn's multiple comparisons test. *P* values of ≤0.05 were considered statistically significant. B, The respective time period is entitled above each graph. Statistical comparisons of log-transformed titers were made using the Wilcoxon test. *P* values of ≤0.05 were considered statistically significant. **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001, and *****P* ≤ 0.0001. %+, percent of participants whose titer was >298 BAU/mL; AU/mL, arbitrary units per milliliter; BAU/mL, binding antibody units per milliliter; GMT, geometric mean titer.

been associated with poor humoral and cellular responses in adult SOT and KTRs after a 3-dose primary COVID-19 vaccine series when compared with those who had not received belatacept.[23,24] However, in our study, we found that participants who received belatacept as part of their immunosuppressive regimen had similar serological responses to participants who did not receive belatacept. Although these findings may suggest that belatacept does not hamper the immune response to COVID-19 vaccination in PKTRs, cellular immune responses were not measured. The sample size may have limited statistical power to ascertain small differences, and time elapsed since belatacept receipt may have diminished the observed immunosuppressive effects.

Since the initiation and completion of this study, the predominant circulating variants have changed from initially Delta, and then Omicron to the currently circulating strains of the Omicron JN.1 lineage, including KP.2 and KP.3. Updated antigen-specific COVID-19 vaccines have also become

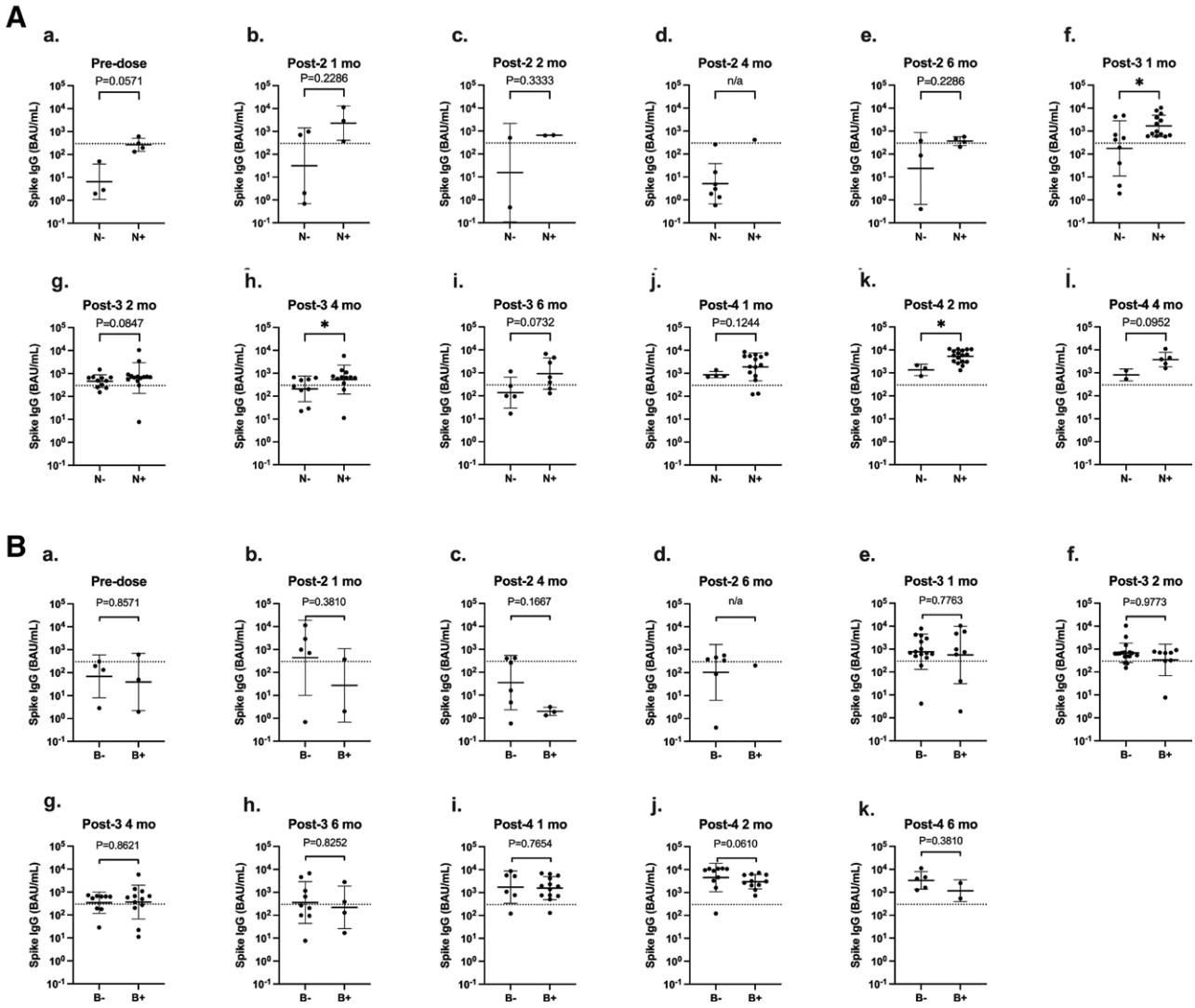

FIGURE 2. SARS-CoV-2 ancestral spike IgG titers pre- and post-COVID-19 vaccination in pediatric kidney transplant recipients (A) comparing those with (N+) and without (N–) nucleocapsid seropositivity, as evidence of prior infection pre-dose (a), 1 mo post-dose 2 (b), 2 mo post-dose 2 (c), 4 mo post-dose 2 (d), 6 mo post-dose 2 (e), 1 mo post-dose 3 (f), 2 mo post-dose 3 (g), 4 mo pos-dose 3 (h), 6 mo post-dose 3 (i), 1 mo post-dose 4 (j), 2 mo post-dose 4 (k), 4 mo post-dose 4 (l); and (B) comparing those who received (B+) or did not receive (B–) belatacept pre-dose (a), 1 mo post-dose 2 (b), 4 mo post-dose 2 (c), 6 mo post-dose 2 (d), 1 mo post-dose 3 (e), 2 mo pos-dose 3 (f), 4 mo post-dose 3 (g), 6 momo post-dose 3 (h), 1 mo post-dose 4 (i), 2 mo post-dose 4 (j), and 4 mo post-dose 4 (k). The respective time period is entitled above each graph. The solid black horizontal lines represent the geometric mean, and the error bars demonstrate the geometric SD. The dashed black line represents 298 BAU/mL (threshold reported to correlate with protection). Statistical comparisons of log-transformed titers were made using the Mann-Whitney test. *P* values of ≤0.05 were considered statistically significant. **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001, and *****P* ≤ 0.0001. If only 1 sample was available for comparison, statistical analysis was not completed and listed as "n/a." BAU/mL, binding antibody units per milliliter.

available, which now include monovalent vaccines targeting the Omicron JN.1 lineage.[25] Although this study only evaluated the prototype versions of COVID-19 vaccines, the importance of vaccine boosters to expand the magnitude and breadth of immunity for PKTRs remains relevant for currently circulating and newly emerging strains. It will be valuable to perform similar analyses in this population in the context of newly circulating variants and recommended vaccines.

Overall, the findings of this study may be limited by a small sample size and single-center analysis that relied upon remnant specimens collected at convenient time points during a limited time period in the COVID-19 pandemic, which may have introduced enrollment bias. As a result, longitudinal samples were not available for many individuals, and limited samples were available at certain time points postvaccination. We also present our data in comparison with a putative threshold of protection for ancestral spike IgG and an adult seropositivity threshold for nucleocapsid IgG based on prior literature because there are no definitive thresholds for these variables. This may over- or underestimate the significance of the antibody response and may underestimate prior natural infection in our pediatric population, as children have been shown to produce a lesser nucleocapsid IgG response than adults.[13] Nearly all participants in this study received Pfizer BNT162b2 mRNA vaccines, and immune responses may vary between the 2 mRNA vaccines and non-mRNA vaccines.[26,27] Functional antibody responses, such as neutralization, and cellular responses were not measured. The sample size and immunosuppressive regimens did not allow for subanalysis of antimetabolite treatment effects on immune responses, as

95% of participants were receiving an antimetabolite. Clinical outcomes in relation to disease severity, immunogenicity data, and vaccine safety and reactogenicity data were not available. These remain important areas for future research.

In conclusion, we found that most PKTR were able to achieve protective antibody titers to ancestral SARS-CoV-2 after a 3-dose primary series. Although antibody titers to the Omicron variant were lower than ancestral titers at each time period assessed postvaccination, a fourth dose improved the magnitude, seroresponse rate, breadth, and durability of SARS-CoV-2 antibodies in PKTRs. As SARS-CoV-2 variants continue to emerge and cause clinical disease, these data may help inform COVID-19 vaccination recommendations to protect this immunocompromised population.

ACKNOWLEDGMENTS

The authors thank patients and their families for generously participating in this study and donating blood to further our understanding of immune responses to COVID-19 vaccination in PKTRs.

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