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CLINICAL RESEARCH

Humoral and Cellular Immunogenicity of 3 Doses of BNT162b2 in Children With Kidney Diseases

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Introduction: Patients with severe kidney diseases are at risk of complications from COVID-19; however, little is known about the effectiveness of COVID-19 vaccines in children and adolescents with kidney diseases.

Methods: We investigated the immunogenicity and safety of an accelerated 3-dose primary series of COVID-19 vaccination among 59 pediatric patients with chronic kidney disease (CKD) (mean age 12.9 years; 30 male) with or without immunosuppression, dialysis, or kidney transplant. Dosage was 0.1 ml BNT162b2 to those aged 5 to 11 years, and 0.3 ml BNT162b2 to those aged 11 to 18 years.

Results: Three doses of either vaccine type elicited significant antibody responses that included spike receptor-binding domain (S-RBD) IgG (90.5%–93.8% seropositive) and surrogate virus neutralization (geometric mean sVNT% level, 78.6%–79.3%). There were notable T cell responses. Weaker neutralization responses were observed among those on immunosuppression, especially those receiving higher number of immunosuppressants or on mycophenolate mofetil. Neutralization was reduced against Omicron BA.1 compared to wild type (WT, i.e., ancestral) (post-dose 3 sVNT% level; 82.7% vs. 27.4%; $P < 0.0001$). However, the T cell response against Omicron BA.1 was preserved, which likely confers protection against severe COVID-19. Infected patients exhibited hybrid immunity after vaccination, as evidenced by the higher Omicron BA.1 neutralization response among these infected patients who received 2 doses compared with those who were uninfected. Generally mild or moderate adverse reactions following vaccines were reported.

Conclusion: An accelerated 3-dose primary series with BNT162b2 is immunogenic and safe in young children and adolescents with kidney diseases.

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Adult patients with CKD and kidney failure are at risk of severe disease and mortality in the COVID-19 pandemic period.[1–4] Generally, children with CKDs and COVID-19 infection seem to experience a milder clinical course[5–7]; however, some children with moderate or severe infection are at risk of developing severe complications, including acute kidney injury and death.[8] Timely vaccination is likely to offer protection to these vulnerable children. BNT162b2 is an mRNA vaccine that has demonstrated 90% to 100% efficacy against symptomatic COVID-19 caused by the

pre-Omicron variants in healthy children and adolescents aged 5 to 15 years. A 3-dose mRNA vaccine primary series has been recommended for immunocompromised children and adolescents due to their vulnerability and suboptimal immunogenicity in many places that include our locality, Hong Kong.[9]

Our previous publication demonstrated that antibody responses which were elicited by 2 doses of BNT162b2 were diminished in adolescents with kidney diseases.[10] Otherwise, research on the immunogenicity and safety of COVID-19 vaccines among the pediatric population remains extremely scarce.[11,12] Importantly, data concerning the humoral and cellular immunogenicity to the 3-dose primary series of COVID-19 vaccines among young children with kidney diseases, as well as immunogenicity against the Omicron variant, are lacking.

We initiated a 3-year nonrandomized study to investigate the use of COVID-19 vaccines in children and adolescents in Hong Kong. In the present interim analysis, we recruited younger children and adolescents aged 5 to18 years with advanced CKD (stage 3 or above) and those on immunosuppressive therapy, chronic dialysis, and post-kidney transplant, because these are clinically vulnerable patients. We evaluated the immunogenicity and safety in participants who had initiated or completed a 3-dose primary series of either 0.1 ml doses of BNT162b2 for patients of age 5 to 11 years, and 0.3 ml doses for patients of age 11 to 18 years.

METHODS

Study Design

COVID-19 Vaccination in Adolescents and Children (COVAC; registered as NCT04800133 at clinicaltrials.gov on March 16, 2021) is a nonrandomized study investigating the safety and immunogenicity of BNT162b2 in healthy children and adolescents or those with pediatric illnesses as previously described.[10,13,14] The study was approved by the University of Hong Kong/Hong Kong West Cluster Hospital Authority Institutional Review Board (UW21-157) and adheres to the Declaration of Helsinki.

Participants

The current analysis included children and adolescents, aged 5 to18 years, with advanced CKD (stage 3 or above) and those on immunosuppressive therapy, on chronic dialysis or post-kidney transplant. Participants with no known history of kidney diseases were excluded. All patients received at least 2 doses of COVID-19 vaccine at the time of analysis.

Procedures

Patients were recruited by physicians from the Pediatric Nephrology Centre, Hong Kong Children's Hospital and the Department of Pediatrics and Adolescent Medicine, Queen Mary Hospital in Hong Kong. The Pediatric Nephrology Centre in Hong Kong Children's Hospital is the territory-wide, designated pediatric referral center for complicated kidney diseases and chronic kidney replacement therapy, including dialysis and transplant. Participants aged 18 years provided informed consent. For those aged <18 years, informed assent was obtained from the participants and consent from their respective parents or legally acceptable representatives. Participants were either unvaccinated or partially vaccinated at the time of enrollment. Demographic information was reported by participants and the clinical details were extracted from their electronic health records. In this study, 0.1 ml BNT162b2 was offered for those aged 5 to 11 years because the original pediatric formulation was not available in Hong Kong, and 0.3 ml BNT162b2 for those aged 11 to 18 years. Participants who received an alternative COVID-19 vaccine as the first or second dose prior to joining the study would complete the remaining doses with the vaccine brand indicated for their age for this study protocol. Additional consent and assent were obtained for dose escalation to 0.3 ml for participants who became 12 years old after initiating the primary series. BNT162b2 was administered via the intramuscular route to the deltoid or thigh, or for some, 0.3 ml BNT162b2 by the intradermal inoculator (MicronJet600, NanoPass Technologies, Nes Ziona, Israel) to the deltoid. Doses 2 and 3 were given at least 14 and 28 days after the preceding dose. Vaccination was deferred in patients with SARS-CoV-2 infection and was resumed or initiated 28 days later. All participants were observed by a nurse and physician for at least 15 minutes after each dose. Blood sampling and safety data collection were performed pre-dose 1, pre-dose 2, post-dose 2 (14–42 days after dose 2), pre-dose 3 (blood was obtained for participants who received dose 3 more than 56 days after their post-dose 2 visit only), and post-dose 3 (14–42 days after dose 3).

Immunogenicity

Humoral

Primary outcomes on humoral immunogenicity included WT S-RBD IgG enzyme-linked immunosorbent assay and WT sVNT. Whereas the S-RBD IgG is a binding antibody assay, the sVNT is a functional antibody assay that reflects blocking of S-RBD and human ACE2 receptor by vaccinee sera and correlates with the gold-standard plaque reduction neutralization test.[15] The secondary outcome was Omicron BA.1

sVNT. In-house WT S-RBD IgG enzyme-linked immunosorbent assay was carried out as previously published.[13,16] sVNT was performed according to the manufacturer's instructions (GenScript Inc, Piscataway, NJ).[16]

Cellular

Primary outcomes on cellular immunogenicity included antiviral cytokine-expressing (IFN-γ^+ or IL-2^+) helper ($CD4^+$) or cytotoxic ($CD8^+$) T cell responses against SARS-CoV-2 S (and N and M) proteins. These were assessed by intracellular cytokine staining on flow cytometry after stimulation with SARS-CoV-2 15-mer peptide pool(s) (Miltenyi Biotec, Bergisch Gladbach, Germany) as described previously.[13,14,17] The SARS-CoV-2 S peptide pool was used for BNT162b2. Secondary outcomes on cellular immunogenicity included T cell responses to stimulation by BA.1 S (and N and M) mutation pools, which consisted of 15-mer peptides spanning the regions with BA.1-associated mutations only. These were compared with WT reference pools made up of WT sequences in the same regions (Miltenyi Biotec, Bergisch Gladbach, Germany for S mutation pools; and ChinaPeptides, Shanghai, China for N and M mutation pools).[14,17]

Analysis

Outcome data on immunogenicity were transformed by applying natural logarithm of the values and compared longitudinally using paired *t* test. Negative values, that is, those below the limit of detection, limit of quantification or cut-off, were imputed as half the limit or cut-off and included in the analyses. Certain WT T cell and Omicron-specific antibody and T cell responses could only be performed in participants who could provide sufficient blood sample volume. Samples obtained after an infection were excluded from analysis unless otherwise stated. Additional details are available in Supplementary Methods. Relationships between immunogenicity and clinical variables or use of immunosuppressives were explored by multiple or simple linear regression. *P*-values are adjusted with Bonferroni correction.

Safety and Reactogenicity

Participants reported prespecified adverse reactions in an online or paper-based diary for 7 days after vaccination. Unsolicited adverse events (AEs) were captured for up to 28 days after each dose. Severe AEs, including life-threatening complications, unanticipated or prolonged hospitalizations, disabilities, deaths and birth defects of their offspring, or breakthrough COVID-19, would be monitored for 3 years after vaccination. We also monitored graft rejection among kidney transplant recipients and disease flare among those with glomerular disease, if any. AEs reported were reviewed by investigators, who determined the possibility of causal relationship with the study vaccine.

RESULTS

Participant Composition

A total of 59 children and adolescents with kidney diseases (mean age 12.9 years, interquartile range 10.4–16.4 years, and 30 males) received at least 1 dose of COVID-19 vaccine, including 25 aged 5 to 11 years for 0.1 ml BNT162b2, and 34 aged 11 to 18 years for 0.3 ml BNT162b2 (Table 1). Twelve patients were on dialysis, 15 patients underwent kidney transplant, and 29 patients were on immunosuppressants alone at the time of dose 1. All participants were Asian. The details of these patients, grouped according to their treatment, are summarized in Supplementary Table S1. Four patients aged 14 to 18 years received dose 3 BNT162b2 intradermally. Trial completion is depicted in Supplementary Figure S1.

Primary Immunogenicity Analysis of Longitudinal Antibody Responses by Vaccine Type

We studied antibody responses because they have been shown to correlate with vaccine efficacy against symptomatic COVID-19.[18] We tracked antibody responses in uninfected patients against WT SARS-CoV-2, including S-RBD IgG for binding antibody and sVNT for neutralization, longitudinally from the pre-vaccine baseline to post-dose 3 by vaccine type as shown in Figure 1a and b, respectively. S-RBD IgG and sVNT responses rose significantly for all 2 vaccine types/age groups after 3 doses when compared to pre-dose 1 on paired t test after natural logarithmic transformation. For patients who received the 3-dose primary series of BNT162b2, 70.6% and 93.8% of the participants aged 5 to 11 years (who received 0.1 ml BNT162b2) were seropositive (above limit of detection of 0.5, considered 'seropositive') after doses 2 and 3, with geometric mean sVNT% levels of 48.6% and 78.6%, respectively. These results were similar to the 11 to 18 years age group (who received 0.3 ml BNT162b2), in which 85.7% and 90.5% of the participants were seropositive, with geometric mean sVNT% levels of 63.6% and 79.3% after doses 2 and 3, respectively. Compared with 2 doses only, an additional third dose of 0.1 ml and 0.3 ml BNT162b2 both elicited significant increases in S-RBD IgG and sVNT. Three patients who received 2 intramuscular and 1 intradermal dose of 0.3 ml BNT162b2 had a high geometric mean sVNT% level of 95.6% post-dose 3. S-RBD IgG and sVNT responses were also presented by treatment (Table 2). sVNT responses were further

Table 1. Participant profile by vaccine type and age eGFR (estimated glomerular filtration rate) is estimated by modified Schwartz equation for participants below age 18 years and CKD-EPI formula for those above

Clinical variables	All patients	BNT162b2, 5–11 yrs, 0.1 ml	BNT162b2, 11–18 yrs, 0.3 ml
Number of participants, *N*	59	25	34
Age mean (interquartile range)	12.9 (10.4–16.4)	9.1 (8.6–12.1)	15.7 (10.5–16.4)
Sex male n: female *n*	30:29	12:13	18:16
Number of participants completing 2 doses *n* (%)	59 (100%)	25 (100%)	34 (100%)
Number of participants completing 3 doses *n* (%)	46 (78%)	17 (68%)	29 (85%)
Primary kidney diagnosis *n* (%)			
CAKUT	9 (15%)	4 (16%)	5 (15%)
Glomerular diseases	33 (56%)	15 (60%)	18 (53%)
Hereditary	10 (17%)	4 (16%)	6 (18%)
Miscellaneous	7 (12%)	2 (8%)	5 (15%)
Treatment modality *n* (%)			
No KRT	32 (54%)	16 (64%)	16 (47%)
On immunosuppression	29 (49%)	14 (56%)	15 (44%)
KRT			
Kidney transplant	15 (25%)	7 (28%)	8 (24%)
Dialysis	12 (20%)	2 (8%)	10 (29%)
Vintage of KRT mean (range)	5.8 (1–18)	6.2 (2–10)	5.6 (1–18)
Concurrent immunosuppression within 3 months n (%)			
Mycophenolate mofetil	31 (53%)	13 (52%)	18 (53%)
Prednisolone	37 (63%)	15 (60%)	22 (65%)
Azathioprine	3 (5%)	0 (0%)	3 (9%)
Cyclosporine A	2 (3%)	1 (4%)	1 (3%)
Tacrolimus	28 (47%)	13 (52%)	15 (44%)
Everolimus	4 (7%)	3 (12%)	1 (3%)
Hydroxychloroquine	6 (10%)	1 (4%)	5 (15%)
Rituximab	2 (3%)	0 (0%)	2 (6%)
0 immunosuppressants	11 (19%)	3 (12%)	8 (24%)
1 immunosuppressants	11 (19%)	7 (28%)	4 (12%)
2 immunosuppressants	11 (19%)	7 (28%)	4 (12%)
3+ immunosuppressants	26 (44%)	8 (32%)	18 (53%)
Immunosuppression within 1 yr n (%)			
Rituximab	8 (14%)	2 (8%)	6 (18%)
Kidney function and proteinuria mean (range)			
eGFR (ml/min per 1.73 m^2)	87.5 (20–184)	91.2 (20–184)	84.0 (24–145)
Urine protein/creatinine ratio (mg/mmol)	34.8 (1.6–140)	29.8 (1.6–140)	40.2 (6–108)
Blood count and comorbidities			
Hypertension *n* (%)	34 (58%)	12 (48%)	22 (65%)
Absolute lymphocyte count ($\times 10^6$/ml) mean (range)	2.4 (0.48–5.59)	2.9 (1–5.59)	2.0 (0.48–5.18)

CAKUT, congenital anomalies of kidney and urinary tract; eGFR, estimated glomerular filtration rate; KRT, kidney replacement therapy.

broken down by both vaccine type and treatment, showing longitudinal increases across all groups (Supplementary Figure S2A and B).

Primary Immunogenicity Analysis of Longitudinal T Cell Responses by Vaccine Type

We also studied T cell responses because they protect against progression to severe COVID-19.[19] After 3 doses of BNT162b2, we detected significant increases in all S-specific IFN-γ^+ and IL-2^+ $CD4^+$ and $CD8^+$ T cell responses (Figure 2) except for S-specific IL-2^+ $CD8^+$ T cell response in those aged 5 to 11 years who received 0.1 ml BNT162b2 (Figure S3). After 3 doses, moderate proportions of participants mounted a positive S-specific IFN-γ^+ $CD4^+$ (68.8% for 0.1 ml BNT162b2 and 65.0% for 0.3 ml BNT162b2) and $CD8^+$ T cell response (43.8% for 0.1 ml BNT162b2 and 40.0% for 0.3 ml BNT162b2) (Figure 2). Patients who received an intradermal dose 3 of 0.3 ml BNT162b2 had a similar level of T cell responses as those who received intramuscular dose 3. Longitudinal trends of S-specific IFN-γ^+ $CD4^+$ and $CD8^+$ T cell responses were broken down by both vaccine type and treatment (Supplementary Figure S4A–C, Supplementary Figure S5A–C).

Antibody and T cell Responses Against Omicron BA.1

As a secondary outcome, Omicron BA.1 neutralization was evaluated by sVNT. When compared with paired WT sVNT, we found a significantly lower sVNT%

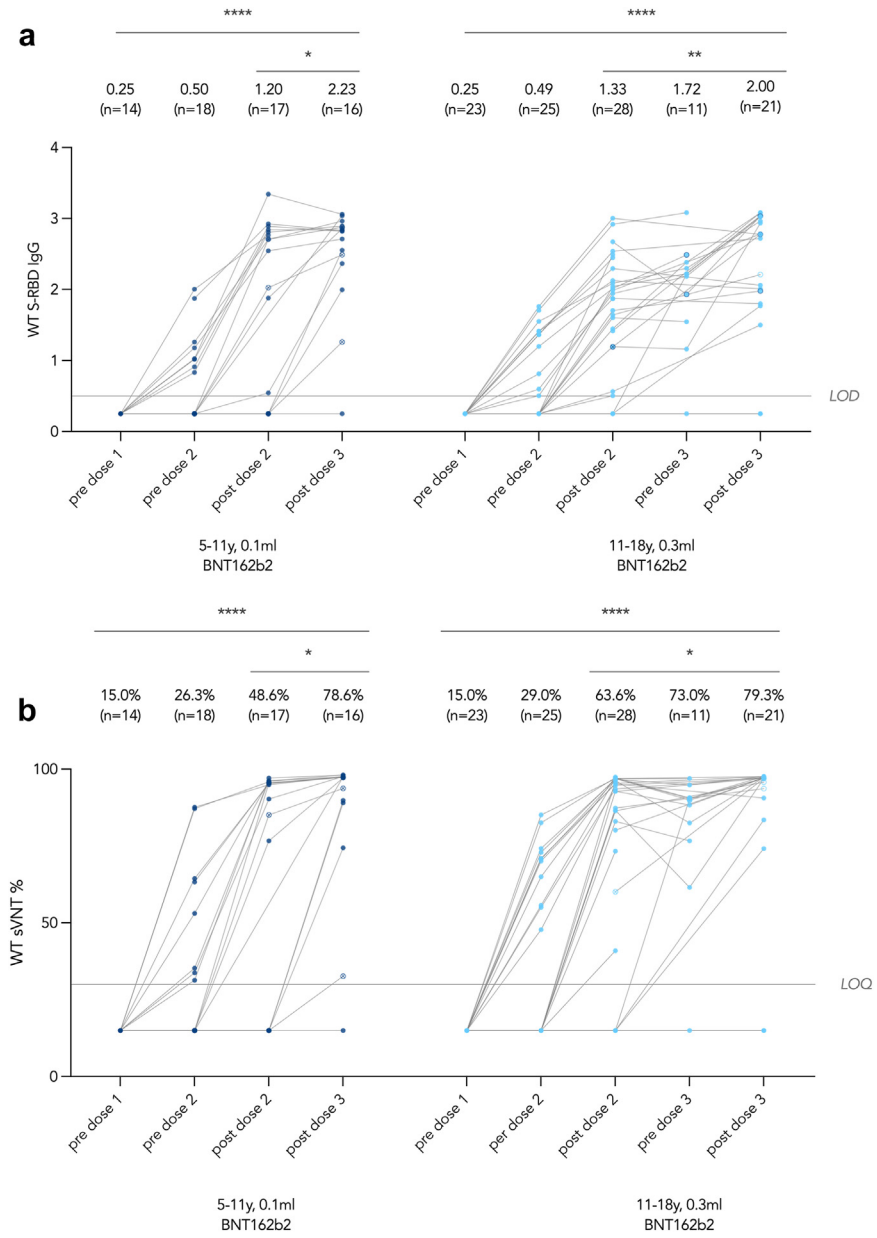

Figure 1. Antibody responses against wild-type SARS-CoV-2 including (a) S-RBD IgG for binding and (b) surrogate virus neutralization test (sVNT) for neutralization. Matched pre-dose 1 or post-dose 2 tests were compared with post-dose 3 by paired t test after natural logarithmic transformation, and *P* values are denoted by asterisks (*, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$; ns, not significant). Geometric means (GM) are shown with center lines and stated above each column. Limits of detection and quantification were drawn as grey lines. Data points of patients who received intradermal 0.3 ml BNT162b2 as dose 3 were hollow.

level against Omicron BA.1 following dose 2 (68.8% vs. 15.7%, $P < 0.0001$) and dose 3 (82.7% vs. 27.4%, $P < 0.0001$) (Supplementary Figure S3A), indicating BA.1 markedly evades neutralization compared to WT in kidney patients. However, we observed a significant increase in geometric mean BA.1 sVNT% level with dose 3 (post-dose 2 vs. post-dose 3, 15.7% vs. 27.4%, $P < 0.0001$).

We then assayed IFN-γ^+ $CD4^+$ and $CD8^+$ T cells responses against Omicron BA.1 mutations in S, N, and M after 2 doses of vaccine. Notably, T cell responses against Omicron BA.1 and WT S protein were comparable after 2 doses of any vaccine (Figure 3b), suggesting T cell responses were not diminished by mutations in Omicron BA.1.

Correlation of Immunogenicity With Clinical Variables

We sought to investigate whether clinical variables associated with immune responses after vaccination (Table 3) as a secondary analysis. We focused on sVNT and S-specific IFN-γ^+ $CD4^+$ and $CD8^+$T cells after 2 doses. Using multiple linear regression, we found that both age and disease category did not affect immune

Table 2. Antibody responses including S-RBD IgG and surrogate virus neutralization test (sVNT) against wild-type SARS-CoV-2 by treatment status

	No KRT			On KRT	
Timepoint	**No IS**	**Any IS**	**Rituximab**	**Dialysis**	**Transplant**
S-RBD IgG seropositivity (%)					
Pre-dose 2	2/2 (100%)	10/21 (48%)	0/4 (0%)	7/11 (64%)	0/9 (0%)
Post-dose 2	2/2 (100%)	18/22 (82%)	2/4 (50%)	9/9 (100%)	7/12 (58%)
Post-dose 3	2/2 (100%)	17/18 (94%)	2/3 (67%)	6/6 (100%)	9/11 (82%)
Geometric mean sVNT% level (95% CI)					
Pre-dose 2	62.2 (8.3–467.9)	28.2 (20.3–39.3)	15.0 (15.0–15.0)	38.9 (23.1–65.5)	15.0 (15.0–15.0)
Post-dose 2	96.6 (91.5–101.8)	63.0 (45.8–86.7)	30.0 (7.7–116.1)	92.8 (86.6–99.4)	31.1 (17.5–55.1)
Post-dose 3	97.8 (94.8–100.9)	86.0 (69.1–107.1)	52.1 (3.6–757.3)	96.2 (93.3–99.2)	59.4 (35.9–98.2)

CI, confidence interval; IS, immunosuppressants; KRT, kidney replacement therapy; S-RBD, spike receptor-binding domain.
Number of seropositive patients by S-RBD IgG were given by *n*/*N* (%) and geometric mean sVNT with 95% confidence intervals were shown.

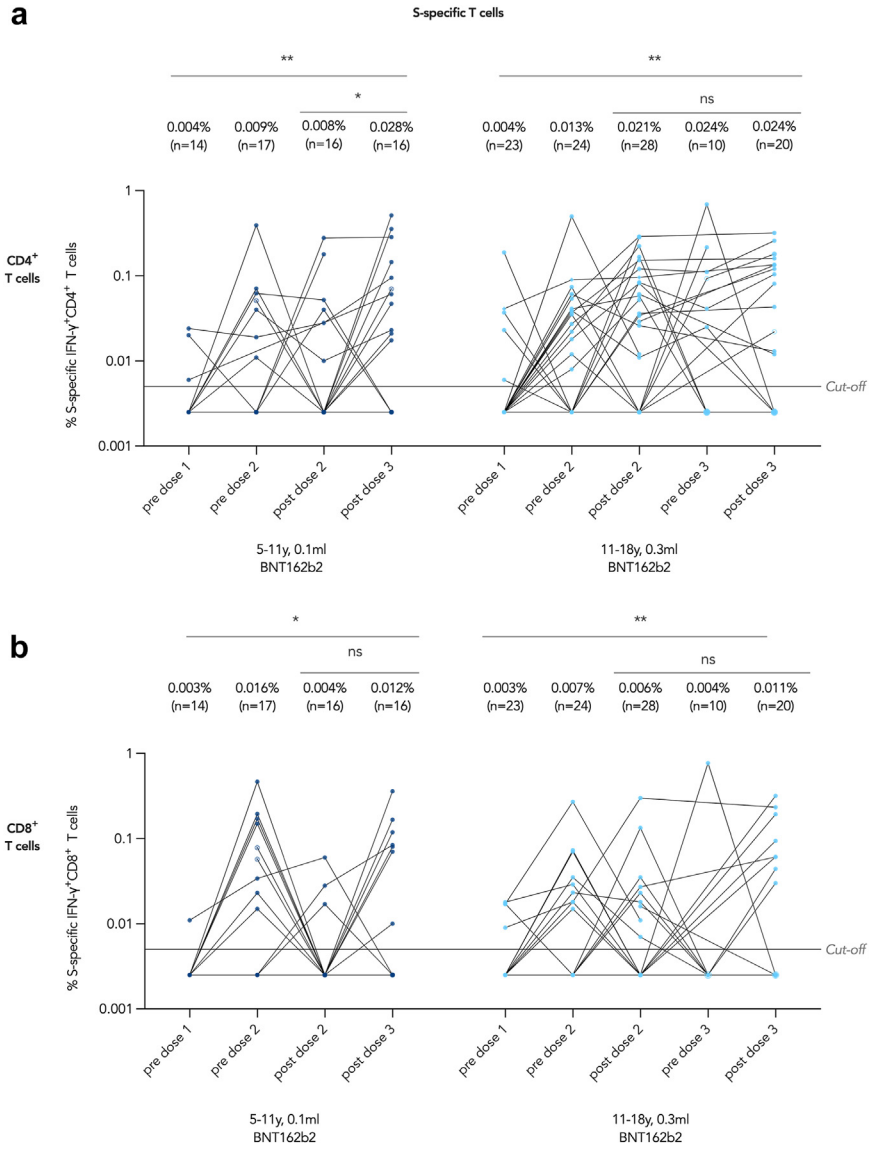

Figure 2. IFN- γ^+ T cell responses against wild-type SARS-CoV-2 proteins. T cell responses against S protein were tested. Matched pre-dose 1 and post-dose 2 tests were compared to post-dose 3 by paired t test after natural logarithmic transformation, and *P*-values are denoted by asterisks (*, $P < 0.05$; **, $P < 0.01$; ns, not significant). Geometric means (GM) are shown with center lines and stated above each column. Cut-offs were drawn as grey lines. Data points of patients who received intradermal 0.3 ml BNT162b2 as dose 3 were hollow.

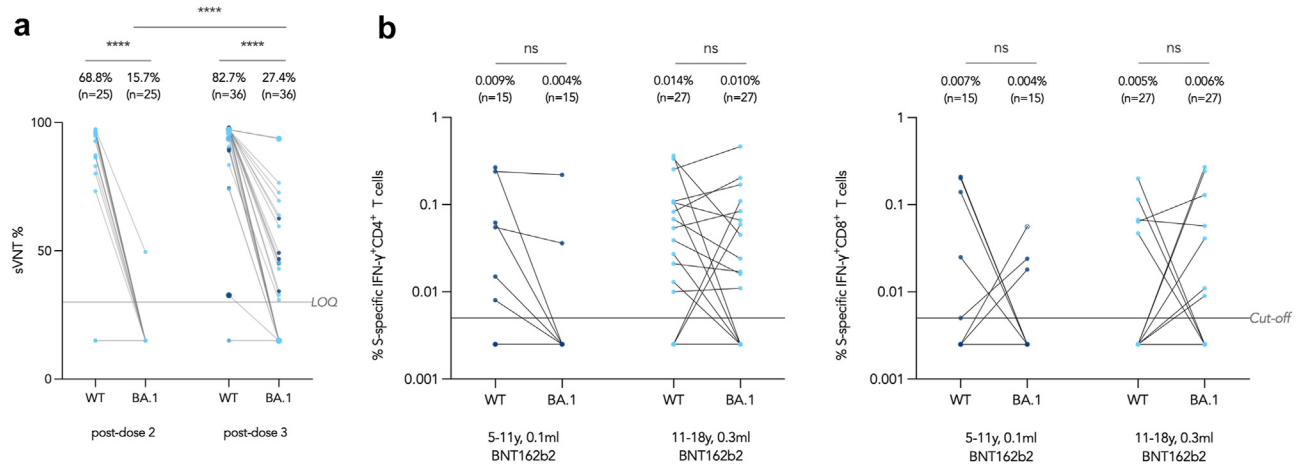

Figure 3. Antibody and T cell response against Omicron BA.1. Matched WT and BA.1 tests were compared by paired t test after natural logarithmic transformation, and the *P*-values are denoted by asterisks (****, $P < 0.0001$; ns, not significant). Geometric means (GM) are shown with center lines and stated above each column. Limit of quantification and cut-offs were drawn as grey lines. Data points were colored navy blue and cyan according to vaccine type and age, referring to 5–11 years 0.1 ml BNT162b2 and 11–18 years 0.3 ml BNT162b2, respectively. Data points of patients who received intradermal 0.3 ml BNT162b2 as dose 3 were hollow. Dots representing identical values, including a substantial proportion of undetectable responses, overlapped in the figure.

responses, whereas patients who were not on kidney replacement therapy but received any immunosuppressive therapy had a weaker sVNT response ($P = 0.0045$). We then further analyzed whether specific immunosuppressants would lead to poorer responses (Supplementary Table S2), and we found that mycophenolate mofetil use was linked to lower sVNT ($P = 0.001$). Using simple linear regression, we screened for associations with additional clinical variables and found that the number of immunosuppressants was associated with poorer sVNT responses ($P = 0.0044$) (Supplementary Table S3).

Breakthrough COVID-19 Cases and Hybrid Immunity

Since January 2022, Hong Kong has been afflicted with the first major wave of COVID-19 resulting from the Omicron BA.2 variant. Eleven patients (17% of all participants) reported COVID-19 during that time, including 7 (11%) before and 4 (6%) after initiating COVID-19 vaccination. All patients reported mild COVID-19 without the need for hospitalization. No mortality was reported. Two patients had a longer clinical course with rapid antigen test positivity for 14 and 17 days. They were both unvaccinated and treated with immunosuppressive therapy for underlying glomerular disease. Two patients had breakthrough COVID-19 after completing 3 doses, and both had a high WT sVNT% level above 90% (93.7% and 90.6%) tested shortly before breakthrough infection. Both patients were managed conservatively and had no complications.

We also investigated the effect of hybrid immunity, which refers to the synergized immune response from both vaccination and infection in patients who were infected before vaccination (Supplementary Figure S6A–D). Before vaccination, only 2 of 7 patients seroconverted to infection alone, and only 1 of them had a positive but weak neutralizing response (Supplementary Figure S6A). After receiving 2 doses of vaccine, infected patients showed significantly boosted S-RBD IgG and WT sVNT responses (Supplementary Figure S6A). When compared to uninfected patients who received 2 doses of vaccine, infected and vaccinated patients also had higher Omicron BA.1 sVNT (Supplementary Figure S6B). Among infected patients, S-specific IFN-γ^+ $CD4^+$ T cell responses were significantly increased after 2 doses compared to pre-dose 1; and S-specific IFN-γ^+ $CD8^+$ T cells showed an upward trend as well (Supplementary Figure S6C). Omicron BA.1 S-specific IFN-γ^+ T cell responses appeared comparable between infected and uninfected participants (Supplementary Figure S6D).

Safety and Reactogenicity

We tracked adverse reactions for 7 days and AEs for 28 days after each dose. COVID-19 vaccination appeared tolerable for these patients. Patients receiving either vaccine type mainly reported mild and moderate adverse reactions (Figure 4). Two and 5 AEs were reported within 28 days after 0.1 ml and 0.3 ml BNT162b2, respectively, including 2 reports after dose 1 and 5 reports after dose 2. These AEs included chest discomfort ($n = 1$), palpitations ($n = 2$), tachycardia ($n = 1$), epistaxis ($n = 1$), insomnia ($n = 1$), and tinnitus ($n = 1$). All AEs were graded as mild except for tinnitus, which was moderate. No kidney transplant recipient developed graft rejection during the follow-

Table 3. Correlation of immunogenicity with clinical variables

Clinical variables	Estimate	95% CIs	*P*-value
sVNT			
Age group			
5–11 yrs	−0.3849	−0.8213 to 0.05158	0.6568
Disease category			
Hereditary	−0.4511	−1.245 to 0.3431	1.0000
CAKUT	−0.8266	−1.762 to 0.1086	0.6512
Miscellaneous	−0.5723	−1.426 to 0.2814	1.0000
Treatment			
Dialysis	0.4288	−0.3781 to 1.236	1.0000
Transplant	−0.3934	−1.176 to 0.3894	1.0000
No KRT any IS	−1.124	−1.878 to −0.3713	0.036[a]
No KRT no IS	0.9398	−0.3103 to 2.190	1.0000
S-specific IFN- γ^+ CD4$^+$ T cells			
Age group			
5-11 years	−0.2777	−1.500 to 0.9443	1.0000
Disease category			
Hereditary	0.002199	−2.158 to 2.162	1.0000
CAKUT	1.898	−0.8035 to 4.600	1.0000
Miscellaneous	−0.2511	−2.574 to 2.072	1.0000
Treatment			
Dialysis	1.457	−0.7400 to 3.655	1.0000
Transplant	0.4193	−1.710 to 2.549	1.0000
No KRT any IS	1.819	−0.2358 to 3.874	0.6472
No KRT no IS	−0.4854	−3.919 to 2.948	1.0000
S-specific IFN-γ^+ CD8$^+$ T cells			
Age group			
5–11 yrs	−0.02061	−0.8521 to 0.8109	1.0000
Disease category			
Hereditary	0.03374	−1.436 to 1.503	1.0000
CAKUT	1.766	−0.07267 to 3.604	0.4736
Miscellaneous	1.100	−0.4801 to 2.681	1.0000
Treatment			
Dialysis	−0.2493	−1.744 to 1.246	1.0000
Transplant	−1.009	−2.458 to 0.4400	1.0000
No KRT any IS	1.449	0.05151 to 2.847	0.34
No KRT no IS	−2.057	−4.393 to 0.2789	0.66

CAKUT, congenital anomalies of the kidney and urinary tract; Cis, confidence intervals; IS, immunosuppressants; KRT, kidney replacement therapy.
[a]*P*-value is adjusted with Bonferroni correction, with asterisk denoting significance ($P < 0.05$).
Associations of age group, disease category, and treatment modality with sVNT and S-specific IFN-γ^+ CD4$^+$ and CD8$^+$T cells were analyzed with multiple linear regression. *P*-value is adjusted with Bonferroni correction, with asterisk denoting significance ($P < 0.05$).

up period. Of the 31 nondialysis patients with glomerular disease, 2 (6.4%) developed disease relapse within 28 days after vaccination and responded to medical management. Two other patients experienced relapse more than 28 days after vaccination, which could be attributable to acute COVID-19 and B cell reconstitution 12 months after rituximab, respectively. Four patients reported a nonfatal, resolved severe AE after study vaccine administration, including 1 for nephrotic syndrome relapse, 1 with suspected myopericarditis, 1 with prolonged postvaccine fever and 1 with catheter-associated infection. All patients with a serious AEs recovered without complications. More details regarding AEs are available in the Supplementary Information.

DISCUSSION

In this study, we demonstrate that an accelerated 3-dose COVID-19 vaccine schedule with BNT162b2 at age-appropriate dosing (5–18 years) elicits favorable antibody and T cell responses. However, there are weaker antibody responses for patients who are on immunosuppressive therapies, especially those receiving a higher number of immunosuppressants or on mycophenolate mofetil. Although Omicron BA.1 mutations lead to partial neutralization escape, dose 3 significantly enhances neutralization capacity. Importantly, T cell responses against Omicron BA.1 are comparable to that with WT, suggesting vaccines could effectively protect patients from severe COVID-19 caused by Omicron.

We showed a significant induction of S-RBD IgG and WT sVNT antibody responses regardless of vaccine type and age. Antibody response is the first line of defense against infection and correlates with efficacy against COVID-19.[18] Therefore, our results suggest that 3 doses of COVID-19 vaccines could offer protection against COVID-19 in children and adolescents with kidney diseases. In contrast to healthy adolescents who have 100% seropositivity after a single dose of BNT162b2,[13] 4 of 47 (9%) patients failed to develop antibody responses after 3 doses of vaccine. Similarly, studies on adult patients who are on dialysis or post-kidney transplant also demonstrated weaker antibody responses, which was related to their suppressed immunity due to kidney failure and immunosuppressive therapy.[20,21] In our study, patients on immunosuppressive therapy, especially those receiving a higher number of immunosuppressants or on mycophenolate mofetil, were identified to have lower antibody responses. Our finding that mycophenolate mofetil use is associated with weaker antibody response is in line with previous reports.[22–24] Conversely, our data did not show that the use of rituximab was associated with worse immunogenicity, compared to other immunosuppressants. This may be partially explained by our strict adherence to published recommendations that vaccination should be provided at least 4 weeks before initiation or resumption of rituximab therapy to ensure maximal immunogenicity.[25] However, given that this reassuring result may have been confounded by other concurrent immunosuppressants or due to a small number of patients, we would recommend patients receiving rituximab have booster vaccination to ensure adequate protection against COVID-19. Therefore, the timing of vaccination and rituximab should be individualized, balancing the risk of infection and disease relapse. Fortunately, our findings did not support an increased

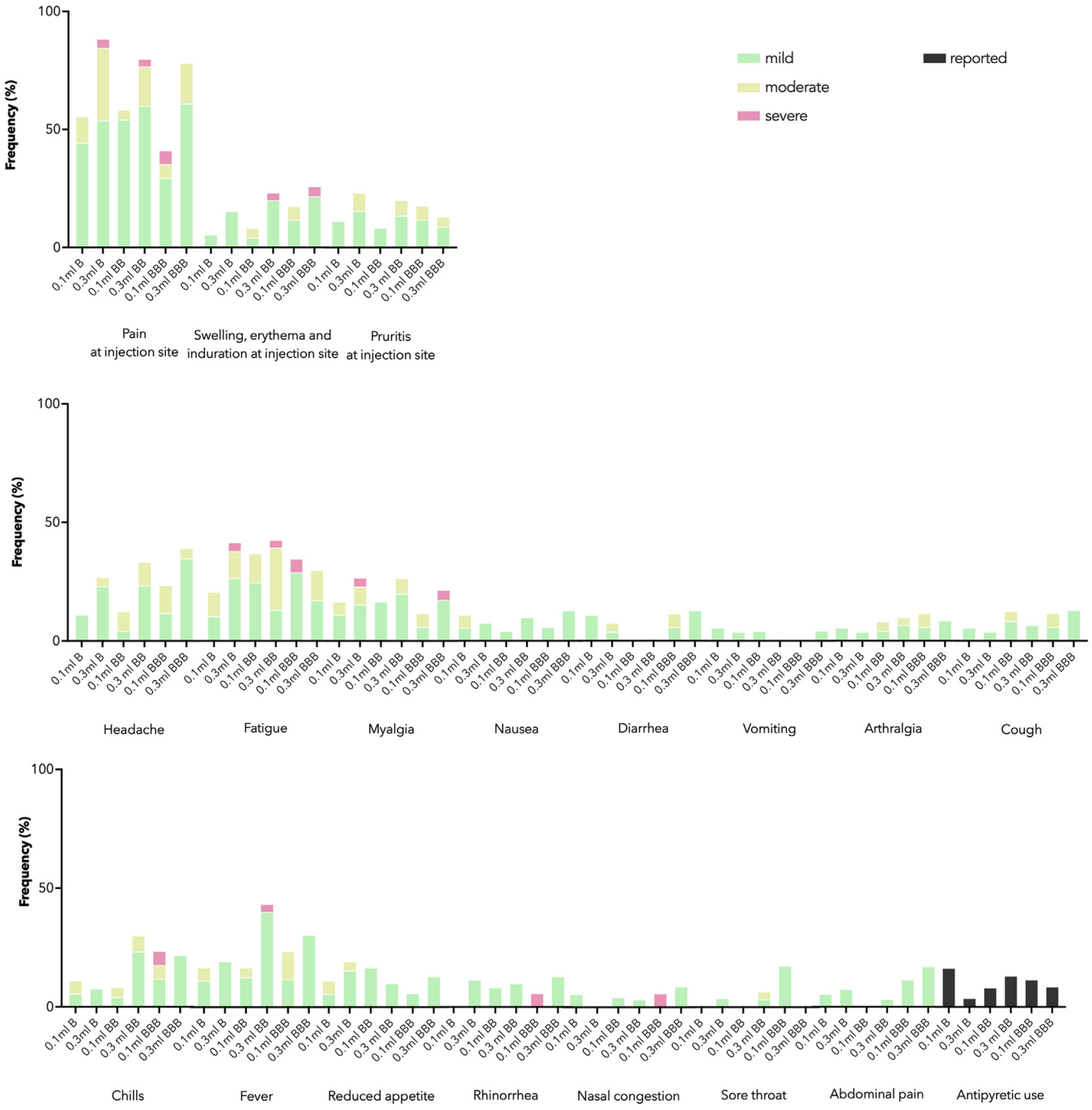

Figure 4. Adverse reactions and antipyretic use reported 7 days after each dose by vaccine type. Stacked bar chart shows adverse reactions by maximal severity in different colors. 0.1 ml or 0.3 ml B–1 dose BNT162b2, BB–2 doses and BBB–3 doses.

risk of graft rejection and disease relapse following vaccination, indicating the safety of COVID-19 vaccination in children and adolescents with kidney diseases.

The SARS-CoV-2 Omicron variant has caused repeated surges in COVID-19 cases and hospitalizations across the globe, with more than 30 mutations in the S protein that are associated with extensive neutralization escape and reduction in the effectiveness of COVID-19 vaccines.[26–28] According to our data, when compared with WT sVNT, we found a significantly lower sVNT% level against Omicron BA.1 following doses 2 and 3, signifying partial neutralization escape. However, neutralization capacity was significantly enhanced by dose 3 from 18.5% to 27.2%. We postulate that further booster doses may further optimize the immunogenicity and protection in children and adolescents with kidney diseases, because a fourth dose can lead to seroconversion in adult kidney transplant recipients who are seronegative after 3 doses.[29] Interestingly, our data suggest that T cell responses remain robust against Omicron even with a

WT vaccine; and this is in line with observations in healthy adolescents.[14,17] T cell responses against SARS-CoV-2, including the non-S proteins,[30] may protect against progression to severe COVID-19 after breakthrough infection, even against variants of concerns that are capable of major neutralization escape.[19]

We only observed mild COVID-19 after vaccination in our cohort. It has been known that either a post-vaccine breakthrough infection or vaccination following infection would magnify the immune response, leading to hybrid immunity that can be longer-lasting with cross-reactivity toward novel variants.[31] Our data showed only 28% of patients seroconverted to infection alone. However, Omicron BA.1 sVNT after 2 doses among infected patients was significantly higher than the uninfected but vaccinated patients. This further supports the importance of vaccination in pediatric patients with kidney diseases even after SARS-CoV-2 infection, which potentiates hybrid immunity against future variants. Taken together, we recommend all patients to complete 3 doses of vaccination regardless of their history of COVID-19 to ensure maximal protection. In addition, we advocate for the continuation of personal hygienic practices in these vulnerable children and adolescents with kidney diseases to minimize the chance of severe breakthrough COVID-19.

Furthermore, due to the Omicron BA.2 wave during the study period, we administered the vaccines using an accelerated regimen, with a 14-day interval between doses 1 and 2, and a 28-day interval between doses 2 and 3. Although longer prime-boost intervals are associated with better immunogenicity in general,[32] seronegativity after just 1 or 2 doses was common in our study. We aimed for our patients to attain a higher level of protection as soon as possible before they would be exposed and suffer from severe COVID-19 during the Omicron BA.2 wave. We demonstrated that the accelerated regimen was safe and effective in eliciting antibody and T cell responses for children and adolescents with kidney diseases. It had been speculated but not yet demonstrated until this study that immune responses in these patients would be further enhanced by breakthrough infections and subsequent booster vaccinations. An important caveat is that this study assessed antibody and T cell responses for experimental research purposes, and at this time we do not recommend routine SARS-CoV-2 antibody testing and decision-making based on these results by clinicians in their usual daily practice. In our cohort, 2 patients who were vaccinated with 3 doses and had a high WT sVNT antibody response (>90%) still became infected with SARS-CoV-2 afterward although their symptoms were mild. Therefore, the antibody level in an individual patient cannot be used to predict susceptibility to infection nor serve as a reliable guidance in deciding on the need for another booster dose.

Our study has strengths and limitations. This study provided data on both antibody and T cell responses longitudinally in a relatively large cohort of vaccinated children and adolescents with different kidney diseases and treatments aged 5 to 18 years. We provided the first Omicron-specific neutralization and T cell responses in this patient population. On the other hand, we could not assess the clinical effectiveness, or compare immunogenicity of vaccine type in the same age groups, because pediatric kidney patients are rare. Regarding the accelerated vaccine regimen, there was no control arm consisting of the usual intervals of vaccination to allow head-to-head comparison. The sample numbers available for a particular test and timepoint was limited by the blood volume collected and completion of study visit by the participant. Considering that we used 0.1 ml dose of the adult formulation for BNT162b2, reactogenicity and immunogenicity could potentially differ from that of the actual pediatric formulation. We used cryopreserved peripheral blood mononuclear cells for T cell testing to reduce variations between runs; however, this might affect assay sensitivity. Lastly, we did not utilize serology to exclude undiagnosed infection. However, all school children in Hong Kong were mandated to undergo daily rapid antigen testing, and negative polymerase chain reaction testing was required to enter the 2 participating hospitals for clinic follow-up or dialysis. Asymptomatic infection would likely be detected by these measures.

In conclusion, this study found an accelerated 3-dose primary series of mRNA COVID-19 vaccines to be safe and immunogenic for children and adolescents with kidney diseases. Although the neutralization response against Omicron was reduced, T cell responses were preserved and may offer protection to children and adolescents with kidney diseases from severe COVID-19. Further studies in the future are needed to shed light on the immunogenicity of a fourth dose and hybrid immunity in immunocompromised children.

DISCLOSURE

All the authors declared no competing interests.

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Data Availability Statement

All requests to share the pseudonymized data underlying the conclusions of this paper from researchers will be facilitated by the authors, subject to ethics approval. Enquiries should be addressed to lauylung@hku.hk.

AUTHOR CONTRIBUTIONS

YLL conceptualized the study. YLL, MP, WT, DL, JSRD, and XM designed the study. YLL led the acquisition of funding. YLL, WT, and MP supervised the project. SMC, DL, XM, SMSC, IYST, and JHYL led the study's administrative procedures. AL-TM, YLL, JSRD, EY-HC, SC, FT-WH, P-CT, W-ML, and MHLL provided study-related clinical assessments and follow-up. DL, SMC, STKS, JHYL, JSRD, and YLL collected clinical safety data. SMSC, LCHT, KKHK, and MP developed and performed S-RBD IgG and sVNT. XM, YC, HHWW, AMTL, WYL, and WT developed and performed the T cell assays. DL, DHLL, and JHYL curated, analyzed, and visualized the data. DL, XM, SMSC, JSRD, DHLL, JHYL, and SMC validated the data. DL and EY-HC drafted the manuscript and were supervised by AL-TM, JSRD, and YLL, with input from XM, and SMSC. All authors reviewed and approved the final manuscript.

SUPPLEMENTARY MATERIALS

Supplementary File (PDF)

Supplementary Methods.

Figure S1. Trial completion diagram.

Figure S2. Surrogate virus neutralization test (sVNT) antibody responses against wild-type SARS-CoV-2 by vaccine type and treatment.

Figure S3. IL-2$^+$ T cell responses against wild-type SARS-CoV-2 proteins.

Figure S4. IFN-γ^+ CD4$^+$ T cell responses against wild-type SARS-CoV-2 proteins against wild-type SARS-CoV-2 by vaccine type and treatment.

Figure S5. IFN-γ^+ CD8$^+$ T cell responses against wild-type SARS-CoV-2 proteins against wild-type SARS-CoV-2 by vaccine type and treatment.

Figure S6. Hybrid sVNT and IFN-γ^+ T cell responses in patients infected prior to vaccination.

Table S1. Participant profile by treatment.

Table S2. Correlation of immunogenicity with immunosuppressives.

Table S3. Correlation of immunogenicity with additional clinical variables.

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