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ORIGINAL ARTICLE

SARS-CoV-2-specific humoral and cell-mediated immune responses after immunization with inactivated COVID-19 vaccine in kidney transplant recipients (CVIM 1 study)

Jackrapong Bruminhent[1,2] | Chavachol Setthaudom[3] | Pongsathon Chaumdee[4] | Sarinya Boongird[2,5] | Sasisopin Kiertiburanakul[1] | Kumthorn Malathum[1] | Arkom Nongnuch[2,5] | Angsana Phuphuakrat[1] | Sopon Jirasiritham[2,6] | Chitimaporn Janphram[7] | Sansanee Thotsiri[7] | Supparat Upama[8] | Montira Assanatham[2,5] | The Ramathibodi Transplant Infectious Diseases (RTID) Study Group

[1]Division of Infectious Diseases, Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[2]Excellence Center for Organ Transplantation, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[3]Immunology Laboratory, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[4]Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[5]Division of Nephrology, Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[6]Division of Vascular and Transplant Surgery, Department of Surgery, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[7]Somdech Phra Debaratana Medical Center, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[8]Samitivej Sukhumvit Hospital, Bangkok, Thailand

Correspondence
Montira Assanatham, Division of Nephrology and the Excellence Center for Organ Transplantation, Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, 270 Rama VI Road, Ratchathewi, Bangkok, 10400, Thailand.
Email: montira.asa@mahidol.ac.th

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Immunogenicity following inactivated SARS-CoV-2 vaccination among solid organ transplant recipients has not been assessed. Seventy-five patients (37 kidney transplant [KT] recipients and 38 healthy controls) received two doses, at 4-week intervals, of an inactivated whole-virus SARS-CoV-2 vaccine. SARS-CoV-2-specific humoral (HMI) and cell-mediated immunity (CMI) were measured before, 4 weeks post-first dose, and 2 weeks post-second dose. The median (IQR) age of KT recipients was 50 (42–54) years and 89% were receiving calcineurin inhibitors/mycophenolate/corticosteroid regimens. The median (IQR) time since transplant was 4.5 (2–9.5) years. Among 35 KT patients, the median (IQR) of anti-RBD IgG level measured by CLIA after vaccination was not different from baseline, but was significantly lower than in controls

Abbreviations: ACE2, angiotensin-converting enzyme 2; AE, adverse events; CI, confidence interval; CMI, SARS-CoV-2-specific cell-mediated immunity; CNI, calcineurin inhibitors; COVID-19, coronavirus disease 2019; ELISpot, enzyme-linked immunospot assay; HMI, SARS-CoV-2-specific humoral immunity; IFN-γ, interferon-γ; IgG, immunoglobulin G; IQR, interquartile range; KT, kidney transplant; M, SARS-CoV-2 membrane protein; MMF, mycophenolate mofetil; MPS, mycophenolate sodium; mRNA, messenger ribonucleic acid; N, SARS-CoV-2 nucleoprotein; ORF, open reading frame; PBMC, peripheral blood mononuclear cell; RBD, receptor-binding domain; RT-PCR, reverse-transcription polymerase chain reaction; S, SARS-CoV-2 spike glycoprotein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFU, spot forming units; SNMO, SARS-CoV-2 spike protein, nucleoprotein, membrane protein, and ORF-3a and ORF-7a proteins; SOT, solid organ transplantation; sVNT, SARS-CoV-2 surrogate virus neutralization test.

Jackrapong Bruminhent and Chavachol Setthaudom contributed equally to this work.

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(2.4 [1.1–3.7] vs. 1742.0 [747.7–3783.0] AU/ml, $p < .01$) as well as percentages of neutralizing antibody inhibition measured by surrogate viral neutralization test (0 [0–0] vs. 71.2 [56.8–92.2]%, $p < .01$). However, the median (IQR) of SARS-CoV-2 mixed peptides-specific T cell responses measured by ELISpot was significantly increased compared with baseline (30 [4–120] vs. 12 [0–56] T cells/10^6 PBMCs, $p = .02$) and not different from the controls. Our findings revealed weak HMI but comparable CMI responses in fully vaccinated KT recipients receiving inactivated SARS-CoV-2 vaccination compared to immunocompetent individuals (Thai Clinical Trials Registry, TCTR20210226002).

KEYWORDS
coronavirus, immunocompromised, neutralizing antibody, receptor binding domain, renal transplant, spike protein, vaccine

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a recently emerged pathogen causing coronavirus disease 2019 (COVID-19), which spread worldwide. The clinical manifestations vary from asymptomatic to mild upper or severe lower respiratory tract disease.[1] Solid organ transplant (SOT) recipients are among those who are potentially compromised for this particular infection, resulting in significant morbidity and substantial mortality in this demographic.[2–5] Vaccination against SARS-CoV-2 is recommended to ameliorate this potentially serious infection and its unfavorable consequences. Various COVID-19 vaccines have been developed across a range of platforms and have been deployed among immunocompetent individuals. However, immunogenicity and safety data following COVID-19 vaccination among SOT recipients receiving immunosuppressants remain limited.

A messenger RNA (mRNA)-based COVID-19 vaccine has been shown to produce immune responses and adequate efficacy to prevent natural infection in immunocompetent recipients.[6,7] However, recent studies focusing on immunogenicity following a two-dose, 4-week interval mRNA-based COVID-19 vaccination strategy revealed suboptimal immune responses among immunocompromised patients. Only 17% and 54% of participants generated robust immune responses after single and double doses, respectively, of the mRNA-based COVID-19 vaccine.[8–10] An inactivated SARS-CoV-2 vaccine has been shown to be primarily adequate to prevent death (86% efficacy), with reportedly lower effect against clinical infection (65.9%).[11] However, a study focusing on immunogenicity and safety following vaccination with an inactivated whole-virus SARS-CoV-2 vaccine among SOT recipients has not been assessed. Furthermore, safety concerns for these immunocompromised patients have not been investigated.

Herein, we decided to conduct an immunogenicity study among kidney transplant (KT) recipients following a full course of inactivated SARS-CoV-2 vaccine. Both SARS-CoV-2-specific humoral (HMI) and cell-mediated immune (CMI) responses were investigated along with the safety profile.

2 | MATERIALS AND METHODS

2.1 | Study design

Between April 2021 and July 2021, we performed a prospective cohort study of adult KT recipients who received a two-dose, 4-week interval vaccination with an inactivated whole-virus SARS-CoV-2 vaccine, CoronaVac® (Sinovac Biotech Ltd.), which contains 3 μg of inactivated whole-virus SARS-CoV-2 in 0.5 ml, given intramuscularly into the deltoid muscle.

HMI and CMI were measured before, 4 weeks after the first dose, and 2 weeks after the second dose, using a SARS-CoV-2 immunoglobulin G (IgG) assay that tests for antibodies against the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein, SARS-CoV-2 surrogate virus neutralization test (sVNT), and an enzyme-linked immunospot (ELISpot) assay for interferon-γ (IFN-γ), respectively (Figure S1).

Participants were eligible if they were KT recipients aged 18–59 years old, at least 1 month post-transplant, and stable in their allograft function and immunosuppressive regimen. KT recipients who attended the outpatient care during the study period were evaluated by their transplant nephrologist and recruited for vaccination. In addition, patients with suspected respiratory tract infection in the preceding 3 days, concurrent active infection, recent diagnosis of allograft rejection requiring intense immunosuppressants (methylprednisolone pulse therapy with 500 mg IV daily for 3 days, antithymocyte globulin therapy within 3 months, rituximab therapy within 6 months, or prednisolone more than 15 mg/day), receiving other vaccination within 4 weeks, previous history of COVID-19, or prior administration of COVID-19 vaccine were excluded. All included patients were screened for active respiratory tract infection, recent COVID-19 exposure, and comorbidities by history. Nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR) were not performed before vaccination. There were no dose adjustment or withheld of immunosuppressants in all participants. Demographic and transplant

characteristics were extracted, including age, sex, number of transplants, allograft type, onset from transplant, and dosing regimen of immunosuppressants. Low C_0 level of calcineurin inhibitors (CNI) was defined as tacrolimus ≤5 ng/ml or cyclosporine ≤150 ng/ml.[12] A low therapeutic dose of mycophenolic acid was defined as mycophenolate mofetil (MMF) ≤1 g/day or mycophenolate sodium (MPS) ≤720 mg/day.[13]

Healthy controls aged 18–59 years old who did not receive immunosuppressants were voluntarily recruited and referenced as a control. They also received the same type and interval of COVID-19 vaccination and were assessed for immunity as described above.

2.2 | SARS-CoV-2 humoral immune responses

SARS-CoV-2 anti-RBD IgG antibodies were measured using the Abbott SARS-CoV-2 IgG II Quantification assay (Abbott SARS-CoV-2 IgG II Quant assay; Abbott). Plasma samples were run on the Abbott Alinity instrument following the manufacturer's instructions. The assay is a chemiluminescent microparticle immunoassay for the quantitative detection of IgG in human serum against the RBD of the SARS-CoV-2 spike protein. The quantitative results of anti-RBD IgG were reported in arbitrary units (AU)/mL. Those with anti-RBD antibody levels of ≥50 AU/ml were characterized as having a seroconversion.[14]

The function of the anti-SARS-CoV-2 spike protein S1 RBD antibody was determined by using a SARS-CoV-2 NeutraLISA surrogate neutralization test assay (Euroimmun). The neutralizing antibodies in plasma compete for binding with the biotinylated angiotensin-converting enzyme 2 (ACE2) receptor for the S1/RBD domain of the SARS-CoV-2 spike protein. Later, further incubation with peroxidase-labeled streptavidin catalyzed a color reaction of the bound ACE2, which catalyzes a color reaction. The intensity of the formed color is inversely proportional to the concentration of neutralizing antibodies in the sample. The neutralizing antibody inhibition was reported in percent, and those with ≥35% were considered as having a positive result.[15]

2.3 | SARS-CoV-2-specific cell-mediated immune responses

According to the manufacturer's protocol, heparinized whole blood samples from participants were collected, and peripheral blood mononuclear cells (PBMCs) were isolated using the EasySep™ Direct Human PBMC Isolation Kit (Stemcell Technologies). Isolated cells were counted, and the cell suspension was normalized at a final concentration of 2.5×10^6 cells/ml in AIM V media (Gibco), followed by manual plating of cells into strip plates (2.5×10^6 PBMCs/well) for stimulation with peptide pool or cell stimulation cocktail.

ELISpot assays assessed IFN-γ production by activated PBMCs using a human IFN-γ ELISpot plus ALP kit (Mabtech, Stockholm, Sweden). ELISpot plates were washed four times with 200 μl/well Dulbecco's PBS (Gibco) and were blocked with AIM V media for at least 30 min. Two hundred and fifty thousand PBMCs in 100 μl AIM V were stimulated under five conditions including AIM V negative control, SARS-CoV-2 S1 domain (S1) of the spike protein scanning peptide pool (Mabtech), SARS-CoV-2 S2 domain of the spike protein, and the nucleoprotein (S2N) peptide pool (Mabtech), SARS-CoV-2 spike protein, nucleoprotein, membrane protein, open reading frame (ORF)-3a and ORF-7a proteins (SNMO) peptide pool (Mabtech), and anti-CD3 antibodies as a positive control. The final concentration was 2 μg/ml of each peptide. After incubation for 40 h at 37°C and 5% CO_2, cells were removed, and IFN-γ production was determined using biotinylated anti-human IFN-γ mAb 7-B6-1 (1 μg/ml in AIMV; Mabtech) for 2 h at room temperature, followed by incubation with streptavidin alkaline phosphatase (1:1000 in AIM V), and finally treatment with 100 μL ready-to-use BCIP®/NBTLiquid substrate (Gibco). After each step, plates were washed five times with distilled water. Emerged spots were counted using an ImmunoSpot analyzer (Cellular Technology Limited), and spot quality was checked using ImmunoSpot Software v5.0.9.15. Results were reported as median and interquartile range (IQR) of IFN-γ-producing spot forming units (SFUs) per 10^6 PBMCs for each peptide pool.[16]

2.4 | Safety

All patients underwent vital signs measurement and physical examination before vaccination and were then monitored for immediate adverse events (AEs) up to 30 minutes after each vaccination, including local and systemic adverse reactions (Figure S2). In addition, solicited AEs were monitored by a phone call on days 3 and 7 after each vaccination (Figure S3), and the patients were encouraged to report unsolicited AEs recorded in their diary (Figure S4). We then determined the causal association between vaccination and AEs. Participants were also encouraged to contact us to report any possible infections, especially those who developed respiratory symptoms. Those in need of medical attention were asked to visit our facility for further evaluation of adverse reactions or investigation of COVID-19 diagnosis. Nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 RT-PCR were performed if needed to confirm the diagnosis, and treatment was provided according to a standard of care.

2.5 | Statistical analyses

Categorical variables were presented as absolute, and frequencies and continuous variables were expressed as median with interquartile range (IQR). Accordingly, the chi-square test, Fisher exact test, and Mann-Whitney U test were used to assess differences between categorical and continuous variables as appropriate. In addition, the distribution of anti-RBD IgG level, the percentage of neutralization inhibition, and SARS-CoV-2-specific IFN-γ-producing SFUs/10^6 PBMCs were presented as a dot plot with a bar representing median with IQR. These were generated by GraphPad

Prism 6.0 (GraphPad Software, Inc.). The Mann-Whitney U test was performed to compare immunogenicity between KT recipients and controls, and the Wilcoxon signed-rank test was performed to compare median ranks between time points within each KT and control group. p values <.05 were considered significant. Statistical analyses were performed with Stata statistical software, version 15 (StataCorp, LLC).

2.6 | Ethics approval

All patients provided written consent. The Institutional Review Board of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, reviewed and approved the study protocol (approval number: MURA2021/242). The study was registered with the Thai Clinical Trials Registry, TCTR20210226002.

3 | RESULTS

3.1 | Clinical characteristics of kidney transplant recipients and controls

A prospective study was conducted between April and July 2021. A total of 75 adult patients were vaccinated, including 37 KT recipients and 38 healthy controls. Among the former, two were excluded owing to denial participation and prior COVID-19 diagnosis (Figure S1). Clinical characteristics of KT recipients are shown in Table 1. Among 35 eligible participants, the median (IQR) age was 50 years (42–54), and 60% were male. All (100%) had received a deceased allograft and the majority (97%) had undergone first KT. The median (IQR) time since transplant was 4.5 (2–9.5) years. The maintenance immunosuppression regimen included tacrolimus (68%), cyclosporine (29%), corticosteroids (97%), mycophenolic acid (97%), sirolimus (3%), and everolimus (3%).

The controls' median (IQR) age was 39 (34–42) years, which was significantly younger compared to the KT group ($p < .01$). Of those, 82% were female and 47% were health care workers.

TABLE 1 Clinical characteristics of kidney transplant recipients

Clinical characteristics, *n* (%)	*N* = 35
Age, years, median (IQR)	50 (42–54)
Male	21 (60)
Asian	35 (100)
Onset from transplant	
Within 6 months	1 (3)
7–12 months	1 (3)
After 1 year	33 (94)
Deceased allograft	35 (100)
First transplant	34 (97)
Immunosuppressants	
Tacrolimus	22 (63)
C_0 level, ng/ml, median (IQR)	6 (5–7.3)
Cyclosporine	11 (31)
C_0 level, ng/ml, median (IQR)	86 (66–106)
Low C_0 level of calcineurin inhibitors (tacrolimus ≤5 ng/ml or cyclosporine <150 ng/ml)[a]	20 (60)
Mycophenolate mofetil	21 (60)
Dose, mg/day, median (IQR)	1500 (625–2000)
Mycophenolate sodium	13 (37)
Dose, mg/day, median (IQR)	1080 (720–1080)
Low therapeutic dose of mycophenolic acid (mycophenolate mofetil ≤1 g /day or mycophenolate sodium ≤720 mg/day)[b]	14 (41)
Sirolimus	1 (3)
Everolimus	1 (3)
Prednisolone	34 (97)
Dose, mg/day, median (IQR)	5 (5–5)

Abbreviations:: IQR, interquartile range; KT, kidney transplant.
[a]Evaluated in 33 patients.
[b]Evaluated in 34 patients.

3.2 | SARS-CoV-2-specific HMI responses

An anti-RBD antibody level in KT recipients from before to after the first and second doses in KT recipients compared to the controls is present in Figure 1 and Table 2. At 4 weeks after a single dose of the vaccine, the anti-RBD IgG level was not significantly different compared with before vaccination in KT group. Additionally, at 2 weeks post-second dose of the vaccine, anti-RBD IgG antibody was not significantly increased from the baseline (2.4 [1.1–3.7] vs. 1.6 [0.8–2.7] AU/ml, $p = .07$) among KT recipients.

In comparison with healthy controls, the median (IQR) anti-RBD IgG level was significantly lower in the KT group at 2 weeks post-second dose (1742.0 [747.7–3783.0] vs. 2.4 [1.1–3.7] AU/ml, $p < .01$; Table 2). Similarly, seroconversion significantly occurred less in KT recipients compared to healthy controls (9% vs. 100%, $p < .01$). Seroconverted KT recipients were more likely to receive low therapeutic doses of mycophenolic acid (100% vs. 33%, $p < .01$) and cyclosporine-based regimen (75% vs. 26%, $p = .08$) as a maintenance immunosuppression compared to non-seroconverted individuals. There was no significant difference in the proportion of participants with and without seroconversion in terms of onset after transplant and C_0 level of CNI (Table S1).

The median (IQR) percentages of neutralizing antibody inhibition measured by sVNT were also significantly lower in the KT group at 2 weeks post-second dose compared to 26 evaluable healthy controls (0 [0–0] vs. 71.2 [56.8–92.2]%, $p < .01$; Figure 2). The neutralizing antibody inhibition of ≥35% was present in 1 of 35 KT recipients and in 23 of 26 healthy controls (3% vs. 88%, $p < .01$).

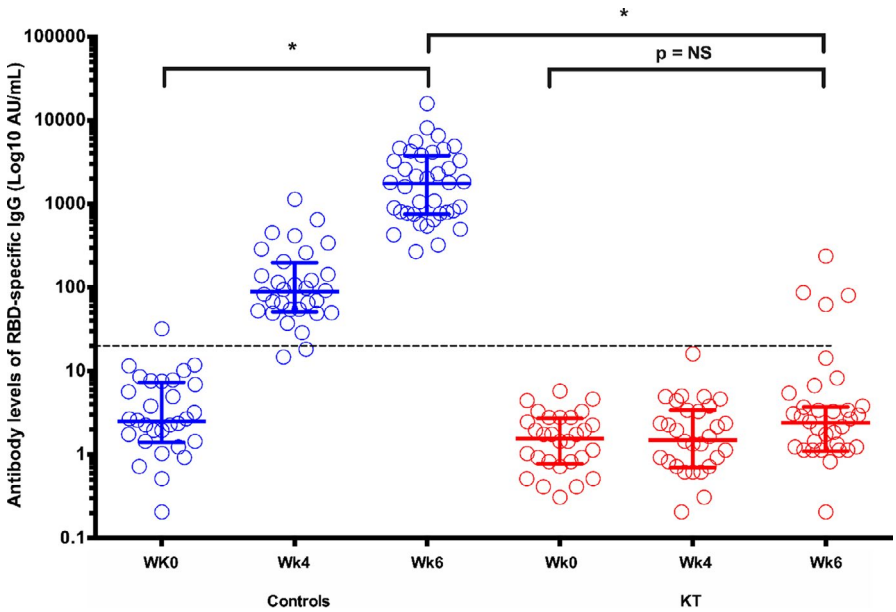

FIGURE 1 The prevalence of SARS-CoV-2 RBD-specific IgG antibody level before, 4 weeks post-first dose, and 2 weeks post-second dose in healthy controls and KT recipients. Bar represents median with IQR. Dash horizontal line indicated SARS-CoV-2 RBD-specific IgG antibody level of 50 AU/ml (seroconversion). *p value < .05 [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 SARS-CoV-2-specific HMI responses represented by anti-RBD IgG in KT recipients and healthy controls vaccinated with inactivated SARS-CoV-2 vaccine

Anti-RBD IgG (AU/ml), median (IQR)	KT recipients (n = 35)	Healthy controls (n = 38)	p value
Before vaccination	1.6 (0.8–2.7)	2.5 (1.4–7.3)	.01
Four weeks post-first dose	1.5 (0.7–3.4)	89.2 (51.2–198.5)	<.01
Two weeks post-second dose	2.4 (1.1–3.7)	1742.0 (747.7–3783.0)	<.01
Anti-RBD IgG >50 AU/ml, n (%)	4 (9)	38 (100)	<.01

Abbreviations: AU, arbitrary unit; CI, confidence interval; IgG, immunoglobulin G; IQR, interquartile range; KT, kidney transplant; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

3.3 | SARS-CoV-2-specific CMI response

A change in SARS-CoV-2-specific CMI in KT recipients compared to the controls is described in Figure 3 and Table 3. Thirty-one KT recipients were evaluated for SARS-CoV-2-specific CMI at 4 weeks after a single dose of vaccine. A median (IQR) of S1 and SNMO-specific T cell responses were not significantly different compared with before vaccination. However, S2N-specific T cell responses were significantly decreased compared with the baseline (13 [5–21] vs. 32 [17–48] specific T cells/10^6 PBMCs, p = .03).

At 2 weeks post-second dose of the vaccine, median (IQR) SNMO-specific T cell responses were significantly increased compared with before vaccination (30 [4–120] vs. 12 [0–56] specific T cells/10^6 PBMCs, p = .02). However, there was no significant increase in S1- or S2N-specific T cell responses at 2 weeks post-second dose of vaccine observed compared to baseline levels or at 4 weeks after the first dose of vaccine.

Median (IQR) of S1, S2N, and SNMO-specific T cell responses after the second dose of vaccines was similar to those achieved by healthy controls (p = .36, .09, .97), respectively.

3.4 | Safety

No severe local or systemic AEs were observed immediately within 30 min after each dose of the vaccine. Solicited AEs are presented in Table S2. On day 3 after the first dose, 16 (46%) of participants reported no AEs, and the remainder reported AEs including fever (17%), pain at the injection site (14%), sleepiness (9%), muscle aches (6%), increased appetite (3%), and others (6%). Two (6%) unsolicited AEs were reported, including asthmatic attack (n = 1) and subconjunctival hemorrhage (n = 1). Both were evaluated and deemed not related to the vaccine. On day 7 after the first dose, the majority of participants (94%) reported no AEs. On day 3 after the second dose, 18 (51%) reported no AEs. However, the remainder reported the following: fever (11%), pain at the injection site (9%), muscle aches (9%), sleepiness (14%), and others (6%). On day 7 after the second dose, 34 (97%) reported no AEs. No acute rejection episodes occurred in those who were fully vaccinated. All observed AEs were mild (grade 1) in severity and recovery occurred within 48–72 h, except for one patient with asthma who required an outpatient visit and was prescribed a bronchodilator inhaler (grade 3).

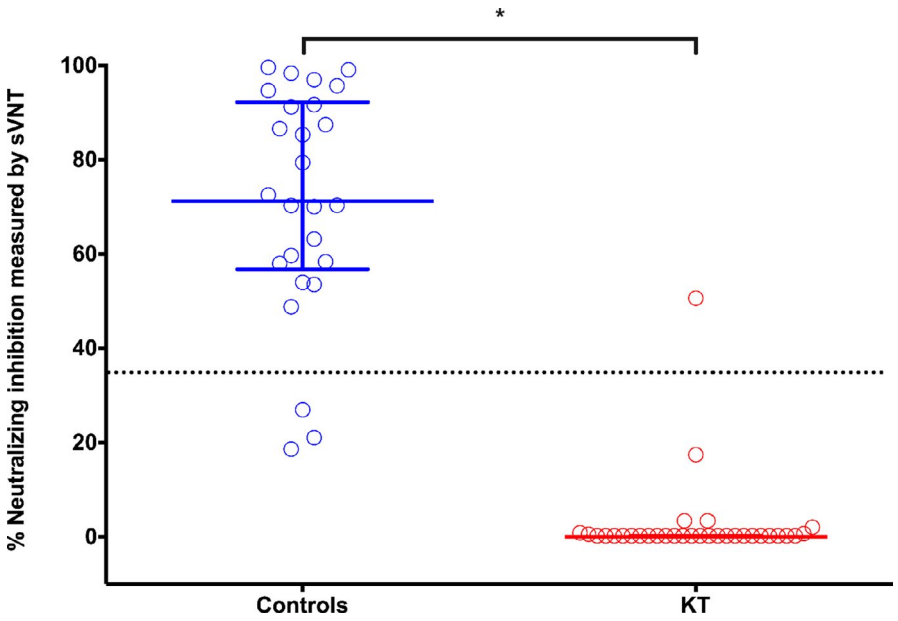

FIGURE 2 The prevalence of neutralizing antibody inhibition measured by surrogate virus neutralization test (sVNT) at 2 weeks post-second dose in healthy controls and kidney transplant recipients. Bar represents median with IQR. Dash horizontal line indicated the percentage of neutralizing antibody inhibition of 35% (positive test). *p value < .05 [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

We herein present a pilot study investigating immunogenicity, focusing on immune responses specific to SARS-CoV-2 after vaccination with an inactivated whole SARS-CoV-2 vaccine administered at an interval of 28 days in patients who previously underwent KT and were receiving immunosuppressive agents. The development of HMI, indicated by anti-RBD IgG levels and the percentage of neutralizing antibodies inhibition, was not adequately achieved after two doses of vaccine and was poor compared with healthy individuals. Conversely, a significant increasing trend was observed for CMI, quantified by SARS-CoV-2-specific IFN-γ-producing T cells after stimulation with SARS-CoV-2 mixed peptides. In addition, the short-term safety and clinical profile was acceptable but warrant further study.

SOT recipients are considered to have comorbidities and are at greater risk of severe respiratory tract disease.[17] Among several COVID-19 vaccines available, the total anti-SARS-Cov-2 antibodies seroconversion rate after a two-dose regimen of SARS-CoV-2 mRNA vaccine in SOT recipients was 40%, and a third dose was required to boost a more significant response to 68%.[18] KT recipients receiving adenovirus-vectored vaccine could still be vulnerable to infection, reflecting a possible inadequate immune response in a small recent study.[19] Our study also confirmed a weak HMI response, although no threshold has been established for protective immunity. In our cohort, 9% of KT recipients seroconverted while 100% of healthy controls seroconverted. Anti-RBD antibody levels were well below those observed in immunocompetent patients vaccinated with CoronaVac® in phase 1 and 2 studies.[20] However, a direct comparison may not be possible due to the lack of standardization among assays and patients' demographics variations.

We observed increasing SARS-CoV-2-reactive T cell responses to an isolated S1 domain of the spike protein in KT recipients parallel and comparable with healthy controls. Surprisingly, SARS-CoV-2-reactive T cell responses to spike protein combined with nucleocapsid protein significantly decreased 1 month after the first dose and later increased 2 weeks after complete vaccination. Furthermore, we also observed significantly increased responses to mixed peptides (SNMO) after the second dose in KT recipients and the controls which could be due to a natural characteristic of the whole virus we selected. Although S1-specific T cell responses would expect to be more significant among those receiving mRNA-based or viral vector vaccines, this postulate may need further investigation since SARS-CoV-2-specific CMI responses did not show a significant difference between immunocompetent vaccinee who received inactivated vaccine and mRNA-based vaccine contrast to more excellent humoral immune responses elicited from the latter.[21]

Moderate generation of IFN-γ-producing T cell responses among those receiving 3–6 μg inactivated virus-containing vaccines was 3.4 and 1.2 SFU/10^6 PBMCs, respectively (the former produced more), in a relatively new cohort, which was lower compared with our results even in those with intact immunity.[20] Our study also revealed a relatively comparable CMI after immunization to the control group and supported that 3 μg inactivated virus-containing vaccine to robust CMI should be adequate.

Although immunosuppressive agents could blunt our patients' immunity, we observed more HMI effects than CMI. The responses of CMI in KT recipients were not statistically significant compared to the controls could be explained by a wash step of ELISpot assay, which attempts to decrease the effect of T cell immunosuppressants on their responses. This assumption was probably supported by comparable numbers of IFN-γ-producing T cells after stimulation with anti-CD3 antibodies (p = NS) in both groups. CMI response was also detectable after mRNA-based vaccination in SOT recipients in a recent study.[22] We believe an intact CMI induced by memory T cells is essential and could be activated during natural infection, thus decreasing the severity of the disease.

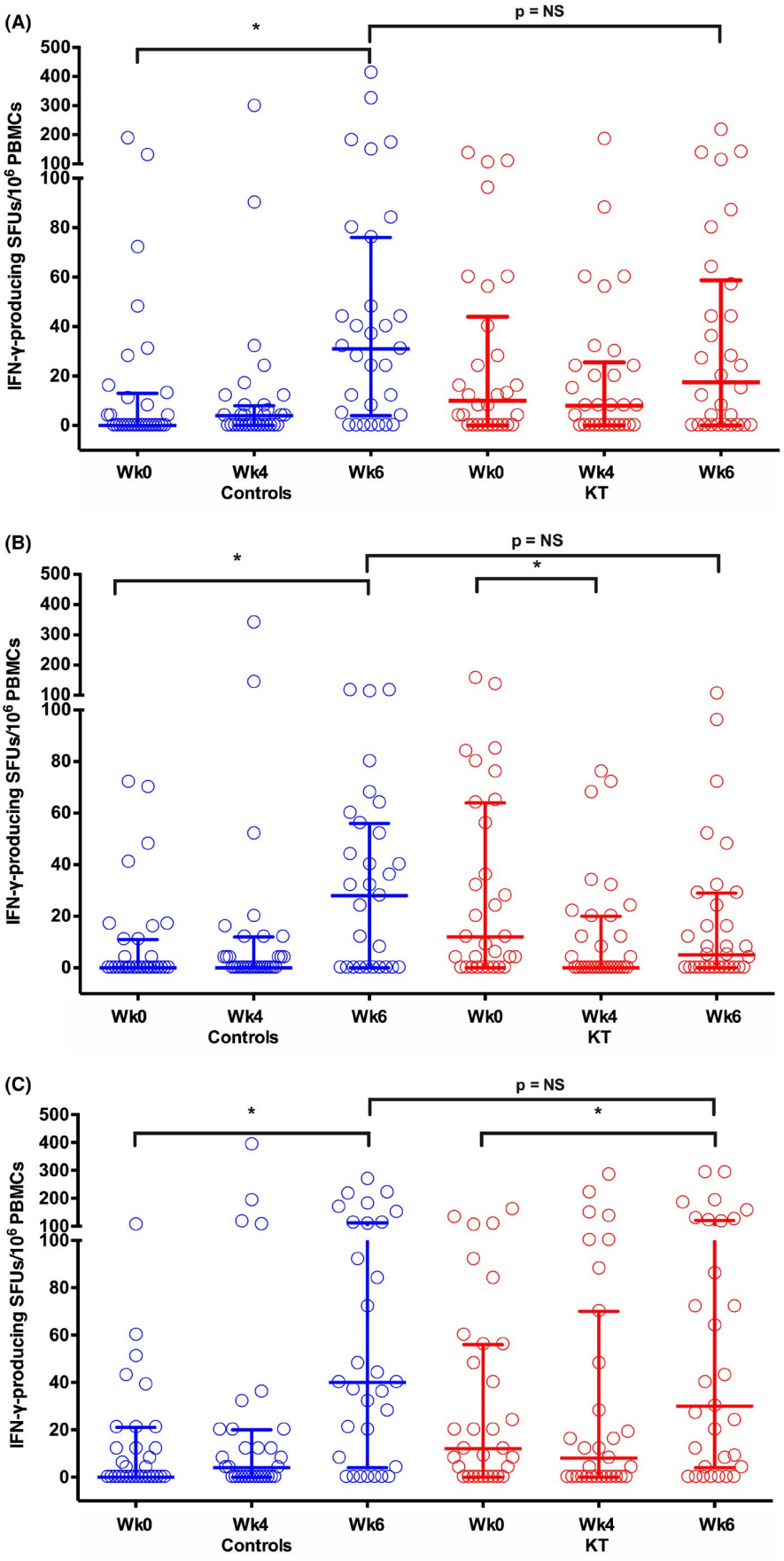

FIGURE 3 SARS-CoV-2-specific IFN-γ-producing T cell responses reactive to the S1 protein (A), S2N protein (B), and the SMNO protein (C) detected by IFN-γ ELISpot assay before vaccination, 4 weeks post-first dose, and 2 weeks post-second dose in KT recipients. Bar represents median with IQR. *p value < .05. IFN-γ, interferon-γ; PBMC, peripheral blood mononuclear cell; S, spike glycoprotein; S1, S1 domain of spike protein; S2N, spike and nucleoproteins; SFU, spot forming unit; SNMO, peptide pool of spike protein, nucleoprotein, membrane protein, and open reading frame proteins [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 SARS-CoV-2-specific T cell responses assessed by the IFN-γ ELISpot assay in KT recipients and healthy controls vaccinated with inactivated SARS-CoV-2 vaccine

SARS-CoV-2-reactive T cells SFUs/10^6 PBMCs, median (IQR)	KT recipients ($n = 31$)				Healthy controls ($n = 31$)			
	Before vaccination	Four weeks post-first dose	Two weeks post-second dose		Before vaccination	Four weeks post-first dose	Two weeks post-second dose	
				Positive control				Positive control
S1 protein	12 (0–40)	8 (0–30)	20 (0–64)	5340 (4472–6268)	0 (0–13)	4 (0–8)	31 (4–76)	4524 (3668–5476)
	Ref.	0.87	0.17		Ref.	0.95	0.02	
			Ref.				0.36	
S2N protein	12 (0–64)	0 (0–20)	5 (0–29)		0 (0–11)	0 (0–12)	28 (0–56)	
	Ref.	0.03	0.13		Ref.	0.41	<0.01	
			Ref.				0.09	
SNMO protein	12 (0–56)	8 (0–70)	30 (4–120)		0 (0–21)	4 (0–20)	40 (4–112)	
	Ref.	0.59	0.02		Ref.	0.20	<0.01	
			Ref.				0.97	

Abbreviations: CI, confidence interval; ELISpot, enzyme-linked immunospot assay; IFN-γ, interferon-γ; IQR, interquartile range; KT, kidney transplant; PBMC, peripheral blood mononuclear cell; Ref., reference; S1, S1 domain of spike protein; S2N, spike and nucleoproteins; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFU, spot forming unit; SNMO, peptide pool of spike protein, nucleoprotein, membrane protein, and open reading frame proteins.

Several factors could diminish immune responses after vaccination in SOT recipients; not least, immunosuppressive agents must maintain renal allografts, especially antimetabolites.[9] More specifically, mycophenolate mofetil treatment greater than 1 g per day and mycophenolate sodium greater than 720 mg per day have been reported in the literature as a critical factor to blunt an immune response along with our result.[13] Furthermore, we observed a slightly better trend of immune responses in those receiving a cyclosporine-based immunosuppressive regimen. However, a low plasma C_0 concentration of CNIs was not correlated.

The virus contained in the CoronaVac® vaccine should be more than 3 μg to produce adequate immunogenicity in patients receiving immunosuppressants. These data are compatible with immunogenicity generated following a standard dose of inactivated influenza vaccine in KT recipients, which revealed lower antibody levels than non-transplant immunocompromised populations such as patients living with HIV or end-stage renal disease.[23] Therefore, influenza vaccine formulations with a higher dose of hemagglutinin are encouraged for those in need, such as elderly individuals or SOT recipients, to generate a stronger immune response compared with the standard dose.[24–26] A recent study evaluating immunogenicity after triple doses of an mRNA COVID-19 vaccine in SOT recipients indicated that this approach could achieve an optimal response and be promising.[18] There is the possibility that additional vaccine doses would be needed, or switching to another vaccine platform could be intriguing. Heterologous vaccine studies have been more focused on investigation of the immunocompetent population while our specific posttransplant population is often excluded from the study.

Safety is another issue of concern among SOT recipients. Adverse reactions during the early period were reported to be mild, confirmed by a large cohort prospective study of mRNA vaccine provided to SOT recipients. The most common AE reported in a phase 1/2 study of the inactivated whole virus vaccine was injection site pain, reported by approximately one in five participants; this was higher than the rate reported in our study of 14%.[20] Our study confirmed that only minimal and mild adverse reactions were observed following vaccination in these unexplored populations. However, immediate and short-term AEs are tolerable. Long-term adverse events and allograft profiles such as allograft rejection require further follow-up.

Limitations of this study include the small sample size, and the controls were not age matched with KT recipients, although they were all adults within the same age group (<60 years old). A previous study revealed that age older than 80 could impact the ability to neutralize the virus.[27] Future large-scale, with age- and sex-matched control, studies are needed to confirm our findings and further explore independent predictors of inadequate immune responses in this specific population. In addition, neutralizing antibody in our study is measured by a sVNT and rather be described as an ACE2 receptor competing for antibody test. Therefore, neutralizing antibody measured by plaque reduction test is believed to be a valid test to assess protective immunity, although an appropriate cut-off value

to determine those with sufficient neutralizing titer has not yet been established and requires a postmarketing study to prove its effectiveness.[28] The strength of this study is it represents one of the first studies to investigate immunogenicity and safety in SOT recipients vaccinated with an inactivated SARS-CoV-2 vaccine. Although poor anti-RBD antibody and surrogate neutralization antibody responses were observed in the KT recipients compared with immunocompetent individuals, the assumption of inadequate humoral responses cannot yet be completely elucidated, as further studies using standardized plaque reduction neutralization tests are necessary to define a better cut-off antibody level that correlates well with neutralization. However, we instead attempted to assess CMI, which is believed to boost a prolonged protective memory response in our susceptible patients. However, the most important thing is adherence to strict basic infection prevention measures, which remains crucial after immunization.

So far, research focused on the effectiveness of COVID-19 vaccines in SOT recipients has not been fully explored. Our study could not report the effectiveness of this vaccine in preventing natural infection because of the short follow-up period after vaccination. Furthermore, vaccine effectiveness varies depending on the study population, the dynamics of local virus transmission, the dominance of variants of concern, and health care resources. Thus, postmarketing investigations will be required to determine the efficacy of vaccination in SOT recipients. Allograft safety profiles and long-term data on safety also need to be followed up. Nevertheless, we believe our findings could provide preliminary data on SARS-CoV-2 immune responses following whole virus SARS-CoV-2 vaccination and be beneficial in designing an appropriate strategy for vaccination in SOT recipients.

Our study revealed that KT recipients develop weak antibody responses and their neutralizing effect to the spike protein, but with potentially optimal SARS-CoV-2-specific T cell responses after completing a two-dose course of inactivated SARS-CoV-2 vaccine with acceptable adverse reactions and favorable short-term outcomes. Therefore, future directives are encouraged to study the role of the third dose with the same platform or another heterologous vaccine in these vulnerable populations to prevent this potentially devastating infection.

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

DATA AVAILABILITY STATEMENT

Data available on request from authors.

ORCID

Jackrapong Bruminhent https://orcid.org/0000-0003-0930-8936
Arkom Nongnuch https://orcid.org/0000-0003-3876-9432
Montira Assanatham https://orcid.org/0000-0002-3835-175X

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SUPPORTING INFORMATION

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