Determinants of Immune Response to Anti–SARS-CoV-2 mRNA Vaccines in Kidney Transplant Recipients: A Prospective Cohort Study

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Background. Immune response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination has been recently shown to be impaired in kidney transplant recipients (KTRs), but the underlying factors affecting vaccine effectiveness need to be further elucidated. **Methods.** In this prospective cohort study, antibodies against S1 and S2 subunits of SARS-CoV-2 were evaluated using an immunochemiluminescent assay (cutoff 9.5 AU/mL, sensitivity 91.2%, and specificity 90.2%) in 736 KTRs, who were previously either naive or infected with SARS-CoV-2 and vaccinated before or after transplantation. Cellular response was analyzed in a subset of patients using an interferon gamma release assay (cutoff 0.15 IU/mL, sensitivity 92%, and specificity 100%). **Results.** Seroconversion was significantly more impaired in SARS-CoV-2–naive KTRs than in those previously infected (40.1% versus 97.1%; $P<0.001$). Mycophenolate use (odds ratio, 0.15; 95% confidence interval, 0.09-0.24; $P<0.001$) and depleting therapy in the past year (odds ratio, 0.19; 95% confidence interval, 0.05-0.8; $P=0.023$) were found to be among independent factors associated with impaired humoral response. Similarly, the interferon gamma release assay tested in 50 KTRs (cutoff 0.15 IU/mL, sensitivity 92%, specificity 100%) showed that specific T-cell responses against spike protein epitopes are impaired in SARS-CoV-2–naive KTRs, as compared to previously infected KTRs (9.4% versus 90%, $P<0.001$). All 35 KTRs vaccinated on the waiting list before transplantation exhibited sustained seroconversion persisting after transplantation. **Conclusions.** Survivors of coronavirus disease 2019 and those vaccinated while on the waiting list exhibited a marked immune response to mRNA vaccines, contrary to poor response in naive KTRs vaccinated after transplantation (NCT04832841).

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INTRODUCTION

Kidney transplant recipients (KTRs) represent one of the most vulnerable populations to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, as coronavirus disease 2019 (COVID-19) is associated with a several-fold higher case fatality rate compared with the general population.[1] Therefore, an effective vaccination strategy similar to those already implemented in the general

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All authors were responsible for the conceptualization of the study, participated in the acquisition of resources, and contributed to the review and editing of the final article. M.M., I.Z., T.N., J.G., I.M., and P.R. took part in data curation. M.M., I.Z., and I.M. were responsible for formal analysis and validated the results. O.V., M.H., P.S., and I.S. were responsible for funding acquisition. M.M., I.Z., and M.F. conducted the investigation. M.M., I.Z., M.F., I.M., I.S., and O.V. were responsible for the methodology. O.V. and I.S. were responsible for project administration and supervision. T.N., J.G., I.M., and P.R. were responsible for the software support. I.Z. and T.N. had taken part in the visualization. M.M., I.Z., and O.V. wrote the original draft. M.M. and I.Z. contributed equally to this work.

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population[2,3] would seem to be a critical tool necessary for the improvement of COVID-19 outcomes in KTRs.

However, despite the high clinical efficacy of mRNA vaccines[4,5] and sufficient humoral response in the general population,[6] impaired vaccine response has been reported in immunocompromised individuals, such as KTRs.[7,8] Recent study of 308 KTRs found only a 36.4% seroconversion rate after administration of 2 doses of BNT162b2 mRNA vaccine,[9] whereas another indicated anti–SARS-CoV-2 immunoglobulin G (IgG) reactivity in just 8.33% KTRs, and none of the subjects developed neutralizing antibodies.[7] Even more interestingly, humoral response in KTRs seems to be more impaired after vaccination than after a natural infection, as the majority of KTRs develop significant levels of antispike antibodies after a natural infection,[10] as we have previously reported. Therefore, a significant proportion of KTRs still possibly remain at an increased risk of contracting SARS-CoV-2 despite being vaccinated, which resembles previous experiences with other vaccinations in the transplant population.

It is therefore important to better understand the causes of poor immune response to mRNA vaccines in KTRs and, vice versa, to define patient cohorts in which vaccination is more effective. Patients on dialysis developed significantly higher anti–SARS-CoV-2 antibody levels than KTRs,[11] which indicates that immunosuppression is likely a critical factor limiting mRNA vaccine efficacy. Higher calcineurin inhibitor levels, higher doses of mycophenolate,[9] and antithymocyte globulin administered during the last year before vaccination[12] have recently been proposed as possible causes of poor immune response. Additionally, whether previous SARS-CoV-2 infection enhances antibody production after vaccination in KTRs is not known because individuals previously infected with the virus were generally not included in the studies.[7,13] However, enhanced humoral response after SARS-CoV-2 infection was observed in fully vaccinated general population[14] and lung transplant recipients.[15]

To better define the variables affecting the immune response to mRNA vaccines in KTRs, we conducted a large single-center prospective cohort study of 736 KTRs, who had been fully vaccinated with either BNT162b2 or mRNA-1273 vaccines.

MATERIALS AND METHODS

Study Design

A total of 753 KTRs were enrolled in this prospective single-center observational cohort study registered as NCT04832841 between March 18, 2021, and June 3, 2021. All KTRs followed at our center, and all patients on the waiting list for a kidney transplant registered at our center who signed up for vaccination with mRNA vaccines (Pfizer/BioNTech BNT162b2 or Moderna mRNA-1273) were eligible to participate in the study. All patients were adults, and the vaccination was performed either while the patients were on the waiting list or after kidney transplantation. Serum samples were collected at least 14 d after the administration of the second vaccine dose. In KTRs vaccinated while on the waiting list, serum samples were collected at least 14 d after the transplant. The study cohort consisted of KTRs previously infected with SARS-CoV-2 and virus-naive subjects. Previous SARS-CoV-2 infection was defined by a positive polymerase chain reaction (PCR) test performed at any time before the first vaccine dose. To eliminate reporting bias, all previous SARS-CoV-2 infection records were verified in the official government central registry (https://www.uzis.cz/index-en.php), where all positive test results are mandatorily reported from laboratories throughout the country. The exclusion criteria were (1) positive PCR test for SARS-CoV-2 after the first vaccine dose, (2) active SARS-CoV-2 infection at the time of enrollment, and (3) previous treatment with anti–SARS-CoV-2 monoclonal antibodies.

Study Population

Of the 753 KTRs, 17 were excluded because of a positive PCR test for SARS-CoV-2 after the first vaccine dose (Figure 1). Therefore, 736 KTRs were eligible to participate in the study, and they were further divided into predefined subgroups according to vaccination timing (before or after transplant) and previous SARS-CoV-2 infection.

A total of 41 KTRs were vaccinated while being waitlisted for kidney transplantation. Of these patients, 35 (85.4%) had no history of SARS-CoV-2 infection, and 6 KTRs (14.6%) had tested positive for SARS-CoV-2 in the past and were therefore not further analyzed. Out of 35 KTRs vaccinated while on the waiting list, 22 were hemodialysis patients with a median dialysis time of 21 mo (interquartile range [IQR], 11.8–41.7), 7 were peritoneal dialysis patients with a median dialysis time of 20.4 mo (IQR, 4.8–21.6), and 6 patients underwent preemptive transplantation. None of the KTRs vaccinated while on the waiting list were on maintenance immunosuppression at the time of vaccination.

Overall, 695 KTRs were vaccinated after transplantation. This cohort comprised previously infected KTRs (n = 69; 9.9%) and virus-naive KTRs (n = 626; 90.1%). The median time between confirmed SARS-CoV-2 infection and first vaccine dose was 94 d (IQR, 68–125 d).

Written informed consent and consent to personal data processing were obtained from all participants before their enrollment. The ethical board approved this study under No. G-21-07.

Outcome Measures

The primary objective of the study was to assess the humoral response to mRNA vaccines in predefined groups of KTRs. The secondary objectives were to (1) define factors influencing the development of anti–SARS-CoV-2 IgG, (2) evaluate kidney graft function following vaccination, and (3) analyze cellular response to mRNA vaccines in a subset of KTRs.

Anti–SARS-CoV-2 Antibody Detection

All participants were tested for anti–SARS-CoV-2 IgG after the second vaccine dose. Anti–SARS-CoV-2-IgG against the spike protein were analyzed using the LIAISON SARS-CoV-2 S1/S2 IgG chemiluminescence immunoassay (DiaSorin S.p.A., Italy). According to previously published methodological procedures concerning the measurement methods,[16,17] the method was validated using stored frozen samples obtained from subjects before the SARS-CoV-2 pandemic (n = 41) and from patients with

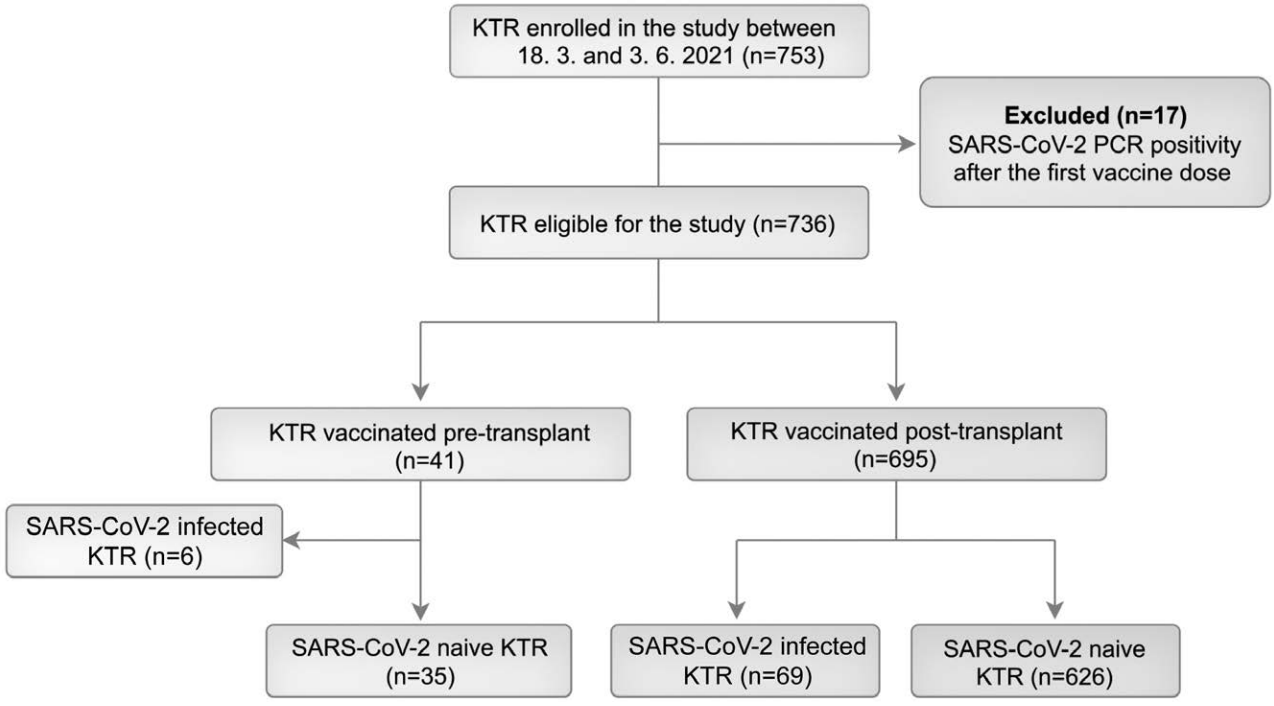

FIGURE 1. Study flowchart. A total of 753 KTRs were enrolled in the study between March 18, and June 3, 2021. Seventeen KTRs with SARS-CoV-2 infection verified by PCR test between the first vaccine dose and antibody testing were excluded from the study. Overall, 736 KTRs were eligible for this study. Participants were further divided according to predefined categories. Of the 41 KTRs vaccinated while on the waiting list, only 6 KTRs had a history of SARS-CoV-2 infection and were not used in the analysis. The other 35 virus-naive KTRs were analyzed for humoral immunity, with 8 of them also tested for cellular immunity. Of the 695 KTRs vaccinated posttransplant, 69 KTRs were previously infected with SARS-CoV-2, whereas 626 had no history of infection. KTR, kidney transplant recipient; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

SARS-CoV-2 infection verified by real-time PCR (n = 34). The cutoff 9.5 arbitrary units (AU/mL) was considered as a positive result with a 91.2% sensitivity (95% confidence interval [CI], 76.3-98.1.) and 90.2% specificity (95% CI, 76.9-97.3) determined using MedCalc Statistical Software version 19.1.

Detection of a Specific Cellular Immune Response to SARS-CoV-2

A total of 50 KTRs were analyzed for cellular immune response; 8 received vaccines while on the waiting list, 32 vaccinated posttransplant were SARS-CoV-2 naive, and 10 were previously SARS-CoV-2 infected (Figure 1). Study subjects were tested for the specific cellular immune response to SARS-CoV-2 using an interferon gamma (IFN-γ) release assay. The principle of this assay is based on the measurement of IFN-γ released by antigen-specific T cells after overnight stimulation with specific SARS-CoV-2 peptides. The IFN-γ release assay test was performed in 2 steps. First, 500 µL of whole blood (Lithium Heparin collection tube, Vacuette, Greiner Bio-One GmbH, Austria) was pipetted into tree tubes containing Roswell Park Memorial Institute-1640 medium (Sigma-Aldrich), supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, 1% penicillin, and 1% streptomycin (Sigma-Aldrich). We used 1 tube containing phytohemagglutinin (Sigma-Aldrich) at a concentration of 25 ng/mL as a positive control and 1 negative control tube without any stimulants. A pool of peptides containing spike protein epitopes, nucleoprotein, membrane protein open reading frame 3a, and open reading frame 7a (0.8 µg/mL) was used for the specific stimulation (Mabtech AB, Nacka Strand, Sweden). The samples were then incubated at 37 °C for 24 h. In the second step, the plasma levels of IFN-γ (IU/mL) were measured using LIAISON QuantiFERON-TB Gold Plus (DiaSorin S.p.A., Saluggia, Italy).

The method was validated using 69 whole blood samples obtained from healthy volunteers. Fifty samples were collected from subjects with a previous SARS-CoV-2 infection or who had been vaccinated with 2 doses of BNT162b2 (Pfizer-BioNTech), and 19 samples were collected from subjects without a history of SARS-CoV-2 infection. The optimized cutoff (0.15 IU/mL) with 92% sensitivity (95% CI, 80.8-97.8) and 100% specificity (95% CI, 82.4-100) was determined using MedCalc Statistical Software version 19.1.

Statistical Analysis

Statistical analysis was performed using SPSS version 22.0 (IBM Corp, Armonk, NY) and R-studio version 1.2.5019 (Development for R. RStudio, Inc., Boston, MA). Normality of data distribution was tested using the Kolmogorov-Smirnov test. Because the majority of the data did not fit the normal distribution, only nonparametric statistical methods were used. Mann-Whitney U or Kruskal-Wallis tests were used to compare continuous variables. Pearson chi-square test or Fischer exact test was used to compare categorical variables. Descriptive statistics are reported as medians and IQRs for continuous variables and numbers and percentages for categorical variables. Logistic regression was used to identify the predictors of antibody detection and cellular immunity. Univariable regression was calculated, and variables with

$P < 0.25$ from univariable analysis or with clear biological importance were entered into the multivariable regression model. Because tacrolimus-based immunosuppression is the standard of care, a categorical variable incorporating other regimens was constructed and tacrolimus-based immunosuppression was used as an indicator/reference variable in the regression analysis. Results are reported as odds ratios (ORs) with 95% CIs. All tests were performed at the 5% level of significance.

RESULTS

Humoral Response in KTRs Following SARS-CoV2 mRNA Vaccination

The primary objective of this study was to assess the humoral immune response to SARS-CoV-2 mRNA vaccines in previously prespecified groups: (1) SARS-CoV-2–naive KTRs vaccinated after transplantation, (2) SARS-CoV-2 previously infected KTRs vaccinated after transplantation, and (3) SARS-CoV-2–naive KTRs vaccinated on the waiting list. The basic characteristics of the study groups are presented in Tables 1 and 2. Prime-boost vaccination was performed with a median 21-d interval between the 2 doses of BNT162b2 vaccines, and with a median of 28 d in case of mRNA-1273 vaccines. The median time from the second dose to antibody testing was 48 d, (IQR, 30–69).

Seroconversion (defined as a detectable level of anti–SARS-CoV-2 IgG antibodies of at least 9.5 AU/mL) was observed in 318 (45.8%) KTRs vaccinated with 2 doses of SARS-CoV-2 mRNA vaccine after transplantation, as opposed to a 100% seroconversion rate observed among KTRs vaccinated while on the waiting list ahead of transplantation.

When the predefined groups of SARS-CoV-2–naive and previously infected KTRs vaccinated after transplantation were added together, the anti–SARS-CoV-2 IgG levels were significantly lower in those KTRs vaccinated after transplantation, as opposed to KTRs vaccinated while on the waiting list (median, 6 AU/mL; IQR, 0–86.7 versus median, 87.4 AU/mL; IQR, 43.4–135 AU/mL; $P < 0.001$). When analyzing only KTRs vaccinated after transplantation (n = 318), in whom seroconversion was observed, the anti–SARS-CoV-2 IgG levels were similar to KTRs vaccinated while on the waiting list (median, 98.8 AU/mL; IQR, 31.6–400 versus median 87.4 AU/mL; IQR, 43.4–135; $P = 0.551$).

Sixty-seven out of 69 (97.1%) KTRs vaccinated after transplantation who were previously infected with SARS-CoV-2 reached seroconversion. This is a significantly higher seroconversion rate than in virus-naive KTRs, where seroconversion was observed in 251 out of 626 KTRs (40.1%; $P < 0.001$). Furthermore, anti–SARS-CoV-2 IgG levels were significantly higher in previously SARS-CoV-2–infected KTRs vaccinated after transplantation than in those who were naive (median 1810 AU/mL; IQR, 261–3070 versus median 4.6 AU/mL; IQR, 0–37.6; $P < 0.001$).

SARS-CoV-2–naive KTRs vaccinated after transplantation had lower anti–SARS-CoV-2 antibody levels than SARS-CoV-2–naive patients vaccinated while on the waiting list. KTRs who were infected with SARS-CoV-2 before vaccination had higher levels of antibodies than those who were SARS-CoV-2 naive, regardless of whether they were vaccinated before or after transplantation. Statistically significant differences were observed among the 3 groups (Figure 2).

TABLE 1.

Characteristics of SARS-CoV-2–naive KTRs vaccinated pretransplant and KTRs vaccinated posttransplant (both SARS-CoV-2–naive and previously SARS-CoV-2–infected)

	Pretransplant vaccinated KTR (SARS-CoV-2 naive) (n = 35)	Posttransplant vaccinated KTR (n = 695)	P[a]
Age (y), median (IQR)	55.8 (47.5–66.5)	64.4 (55–70.7)	<0.001
Sex (male), no. (%)	30 (85.7%)	443 (63.7%)	0.008
Previous infection with SARS-CoV-2, no. (%)	0 (0%)	69 (9.9%)	0.295
BMI, median (IQR)	26.4 (23.1–28.2)	28 (24.9–31.3)	0.003
Diabetes, no. (%)	7 (20%)	203 (29.2%)	0.24
mRNA vaccine type (BNT162b2), no. (%)[b]	35 (100%)	663 (95.4%)	0.394
Tacrolimus-based IS, no. (%)	33 (94.3%)	559 (80.4%)	0.041
Cyclosporine-based IS, no. (%)	0 (0%)	72 (10.4%)	0.04
CNI-free IS, no. (%)	2 (5.7%)	64 (9.2%)	0.486
MMF/MPA, no. (%)	33 (94.3%)	537 (77.3%)	0.018
mTORi, no. (%)	2 (5.7%)	49 (7.1%)	1
Costimulatory blocker,[c] no. (%)	1 (2.9%)	10 (1.4%)	0.42
Depleting induction,[d] no. (%)	5 (14.3%)	296 (42.6%)	0.001
Antirejection therapy, no. (%)	4 (11.4%)	223 (32.1%)	0.01
Time from transplant (mo), median (IQR)	0.63 (0.53–0.93)	71.1 (24.1–142.5)	<0.001
Time from second vaccine dose to antibody testing (d), median (IQR)	71 (46–84)	47 (29–67)	<0.001

[a]*P* values for group comparison based on the Mann-Whitney U test for continuous variables and Pearson chi-square test or Fischer exact test for categorical variables; $P < 0.05$ for significance.
[b]The rest of KTRs were vaccinated with mRNA-1273.
[c]Belatacept and iscalimab.
[d]Antithymocyte globulin and rituximab.
BMI, body mass index; CNI, calcineurin inhibitor; IQR, interquartile range; IS, immunosuppression; KTR, kidney transplant recipient; MMF, mycophenolate mofetil; MPA, mycophenolic acid; mTORi, mammalian target of rapamycin inhibitor; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

TABLE 2.

Characteristics of KTRs with and without previous infection with SARS-CoV-2 vaccinated posttransplant

	SARS-CoV-2 infected (n = 69)	SARS-CoV-2 naive (n = 626)	P[a]
Age (y), median (IQR)	57.1 (49–68.7)	65 (56.1–70.8)	<0.001
Sex (male), no. (%)	45 (65.2%)	398 (63.6%)	0.788
eGFR (mL/min/1.73 m^2), median (IQR)	54.6 (38.7–68.4)	47.4 (34.8–61.2)	0.082
BMI, median (IQR)	27.5 (24.2–30.9)	28 (24.9–31.3)	0.445
Diabetes, no. (%)	16 (23.2%)	187 (29.9%)	0.247
mRNA vaccine type (BNT162b2), no. (%)[b]	55 (79.7%)	605 (96.6%)	<0.001
Tacrolimus-based IS, no. (%)	56 (81.2%)	503 (80.4%)	0.872
Cyclosporine-based IS, no. (%)	8 (11.6%)	64 (10.2%)	0.723
CNI-free IS, no. (%)	5 (7.2%)	59 (9.4%)	0.553
MMF/MPA, no. (%)	55 (79.7%)	482 (77%)	0.61
mTORi, no. (%)	3 (4.3%)	46 (7.3%)	0.355
Costimulatory blocker,[c] no. (%)	1 (1.4%)	9 (1.4%)	0.994
Depleting induction,[d] no. (%)	34 (49.3%)	262 (41.9%)	0.237
Antirejection therapy, no. (%)	21 (30.4%)	202 (32.3%)	0.757
Time from transplant (mo), median (IQR)	60.2 (23.9–127.4)	72.8 (25.1–143.2)	0.562
Time from second vaccine dose to antibody testing (d), median (IQR)	39 (21.5–63)	48 (30–69)	0.004

[a]*P* values for group comparison based on the Mann-Whitney U test for continuous variables, and Pearson chi-square test or Fischer exact test for categorical variables, $P < 0.05$ for significance.
[b]The rest of KTRs were vaccinated with mRNA-1273.
[c]Belatacept and iscalimab.
[d]Antithymocyte globulin and rituximab.
BMI, body mass index; CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; IQR, interquartile range; IS, immunosuppression; KTR, kidney transplant recipient; MMF, mycophenolate mofetil; MPA, mycophenolic acid; mTORi, mammalian target of rapamycin inhibitor; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Determinants of Anti–SARS-CoV-2 IgG Production After the Second Vaccine Dose

Next, factors affecting anti–SARS-CoV-2 IgG production were assessed. This analysis was performed in KTRs vaccinated after transplantation. KTRs were divided into 2 groups based on whether seroconversion was reached after the second vaccine dose (responders) or not (nonresponders). The characteristics of the responders and nonresponders are shown in Table 3. In summary, responders were younger, had higher estimated glomerular filtration rate (eGFR), longer time interval between transplantation and vaccination, had a higher proportion of males, a higher proportion of previous SARS-CoV-2 infections, were more likely to receive mRNA-1273 vaccine, had a higher proportion of cyclosporine and mTOR inhibitor-based therapy, lower use of mycophenolate, and received depleting therapy within the past year less often.

Univariable and multivariable logistic regression analyses were further performed, where seroconversion was used as the dependent variable (Table 4; **Table S1, SDC,** http://links.lww.com/TP/C339). Factors independently associated with higher rates of seroconversion in the multivariable model were previous SARS-CoV-2 infection (OR, 89.89; $P < 0.001$), male sex (OR, 1.97; $P < 0.001$), longer time from transplant (OR, 1.01; $P < 0.001$), and better graft function before vaccination (OR, 1.03; $P < 0.001$). Older age (OR, 0.96; $P < 0.001$), mycophenolate mofetil (OR, 0.15; $P < 0.001$), and depletion therapy within the past year (OR, 0.19; $P = 0.023$) were independently associated with lower seroconversion rates after vaccination.

Renal Graft Function After Vaccination

eGFR (Chronic Kidney Disease Epidemiology Collaboration) was evaluated in the KTRs before and after vaccination. This analysis was performed only in the cohort of KTRs vaccinated after transplantation. There was no significant difference in eGFRs between the 2 measurements performed at median of 70 d (IQR, 52–91 d; prevaccination eGFR median 48 mL/min/1.73 m^2; IQR, 34.8–61.8 versus postvaccination eGFR median 48 mL/min/1.73 m^2; IQR, 33.6–61.8; $P = 0.685$).

Cellular Immune Response in KTRs

Cellular response to the mRNA vaccine was evaluated in 50 KTRs. A positive response was identified in 1 out of 8 (12.5%) SARS-CoV-2–naive KTRs vaccinated pretransplant, 3 out of 32 (9.4%) SARS-CoV-2–naive KTRs vaccinated posttransplant, and 9 out of 10 (90%) SARS-CoV-2 previously infected KTRs vaccinated posttransplant (**Figure S1, SDC,** http://links.lww.com/TP/C339).

Interestingly, 76.9% (10 out of 13) of those who developed cellular immunity also developed a positive antibody response, but only 39.3% (10 out of 26) of those who developed humoral response also developed cellular immunity.

Logistic regression was further used to assess the factors determining the cellular response (**Table S2, SDC,** http://links.lww.com/TP/C339). Previous SARS-CoV-2 infection was identified as the only clear significant factor in univariable regression (OR, 87; $P < .001$). Multivariable regression was not performed because of a low number of events.

Durability of Pretransplant Vaccine-induced Antibodies

Thirty-five patients were vaccinated ahead of their kidney transplantation while still being on the waiting list. The baseline levels of vaccine-induced anti–SARS-CoV-2 antibodies (measured at day 0 of transplantation hospitalization immediately before transplantation) were

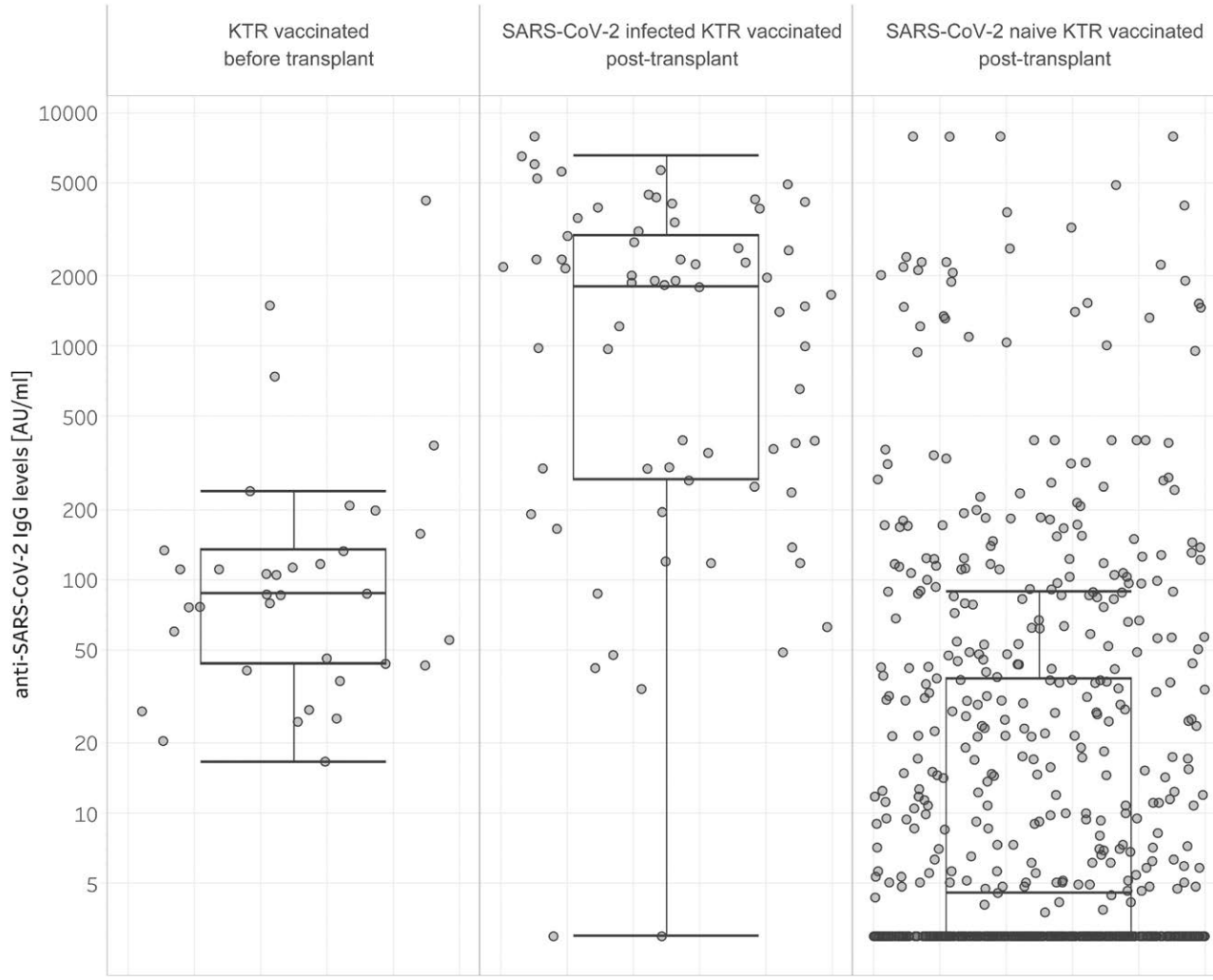

FIGURE 2. Anti–SARS-CoV-2 IgG levels according to previous virus infection and transplantation status. KTRs were divided into predefined groups according to the timing of the vaccination with regards to transplantation and previous SARS-CoV-2 infection to (1) KTRs vaccinated before transplant, all of whom were all SARS-CoV-2 naive; (2) SARS-CoV-2–infected KTRs vaccinated posttransplant; and (3) SARS-CoV-2–naive KTRs vaccinated posttransplant. Statistical differences were assessed using the Kruskal-Wallis test; $P<0.001$ for the overall model. Post hoc test revealed significant differences between the following groups: 1 and 2, $P = 0.019$; 1 and 3, $P<0.001$; and 2 and 3, $P<0.001$. IgG, immunoglobulin G; KTR, kidney transplant recipient; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

measured in 28 of these patients. We analyzed the dynamics of antibody levels between the baseline levels at day 0 and a second later measurement. The median time of the second measurement from the day of transplantation was 20 d (IQR, 17–24 d). There was an apparent decrease in antibody levels between the 2 measurements (median, 136 AU/mL; IQR, 92–177.5 AU/mL at d 0 versus median, 83.8 AU/mL; IQR, 43.6–130 AU/mL; $P = 0.007$). In total, a decrease of >25% from the baseline was observed in 23 (82.1%) KTRs, whereas a decrease of >50% was observed in 7 (25%) KTRs (**Figure S2, SDC,** http://links.lww.com/TP/C339).

Early Durability of Posttransplant Vaccine-induced Antibodies

To estimate the early durability of vaccine-induced anti–SARS-CoV-2 antibodies, we compared the antibody levels in KTRs vaccinated after transplantation, in whom seroconversion after the second dose of mRNA vaccine was achieved. KTRs were divided into 6 groups according to the length of the interval between the second dose of the vaccine and the time of antibody testing (<30 d, 30–60 d, and >60 d), and according to previous SARS-CoV-2 infection status (naive and infected). Lower antibody levels were observed in SARS-CoV-2–naive KTRs tested after >60 d, compared with those tested within 30 d after vaccination ($P = 0.035$). There were no significant differences between previously SARS-CoV-2–infected KTR groups (Figure 3).

Impact of COVID-19 Symptoms on Humoral Response

The severity of clinical symptoms of the 69 KTRs who contracted SARS-CoV-2 before vaccination was scored according to the Clinical Spectrum of SARS-CoV-2 Infection definition.[18] Anti–SARS-CoV-2 IgG levels were significantly higher in patients who experienced moderate to severe symptoms compared with those who had an asymptomatic disease course ($P = 0.014$; Figure 4).

TABLE 3.

Characteristics of KTRs vaccinated posttransplant according to humoral response (n = 695)

	Nonresponders (n = 377)	Responders (n = 318)	P[a]
Age (y), median (IQR)	66.5 (57.3–71.2)	60.8 (52.8–69.4)	<0.001
Sex (male), no. (%)	221 (58.6%)	222 (69.8%)	0.002
Previous infection with SARS-CoV-2, no. (%)	2 (0.5%)	67 (21.1%)	<0.001
BMI, median (IQR)	28.4 (24.9–31.4)	27.5 (24.9–31.1)	0.249
Diabetes, no. (%)	116 (30.8%)	87 (7.6%)	0.325
mRNA vaccine type (BNT162b2), no. (%)[b]	369 (97.9%)	294 (92.5%)	0.01
Tacrolimus-based IS, no. (%)	310 (82.2%)	249 (78.3%)	0.194
Cyclosporine-based IS, no. (%)	31 (8.2%)	41 (12.9%)	0.044
CNI-free IS, no. (%)	36 (9.5%)	28 (8.8%)	0.735
mTORi, no. (%)	18 (4.8%)	31 (9.7%)	0.011
MMF/MPA, no. (%)	326 (86.5%)	211 (66.4%)	<0.001
Costimulatory blocker,[c] no. (%)	10 (2.7%)	0 (0%)	0.002
Depleting induction,[d] no. (%)	170 (45.1%)	126 (39.6%)	0.146
Antirejection therapy, no. (%)	116 (30.8%)	107 (33.6%)	0.418
Depleting therapy in the last y[d]	25 (6.6%)	4 (1.3%)	<0.001
Time from transplant (mo), median (IQR)	54.8 (19–114.5)	91.8 (36.9–168.6)	<0.001
Time from second vaccine dose to antibody testing (d), median (IQR)	43 (29–67.5)	49 (29–67.3)	0.43
eGFR at the time of antibody testing	44.4 (32.7–57.9)	52.2 (40.1–67.8)	<0.001

[a]*P* value for group comparison based on the Mann-Whitney U test for continuous variables and Pearson chi-square test or Fischer exact test for categorical variables; $P < 0.05$ for significance.
[b]The rest of the KTRs were vaccinated with mRNA-1273.
[c]Belatacept and iscalimab.
[d]Antithymocyte globulin and rituximab.
BMI, body mass index; CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; IQR, interquartile range; IS, immunosuppression; KTR, kidney transplant recipient; MMF, mycophenolate mofetil; MPA, mycophenolic acid; mTORi, mammalian target of rapamycin inhibitor; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

TABLE 4.

Determinants of anti–SARS-CoV-2 IgG production after the second vaccine dose in KTRs vaccinated posttransplant

Multivariable model			
Variable	**OR**	**95% CI**	***P***
Previous infection with SARS-CoV-2	89.89	19.76-408.99	**<0.001**
Age (y)	0.96	0.94-0.98	**<0.001**
Sex (male)	1.97	1.33-2.93	**0.001**
Moderna mRNA-1273 vaccine	1.04	0.35-3.08	0.945
Time from transplant (mo)	1.007	1.004-1.01	**<0.001**
eGFR prevaccination (mL/s)	1.034	1.023-1.044	**<0.001**
Pretransplantation diabetes	1.03	0.6-1.77	0.91
MMF/MPA	0.15	0.09-0.24	**<0.001**
Tacrolimus		Ref.	
Cyclosporine A	1.41	0.72-2.75	0.311
CNI-free	0.53	0.26-1.11	0.093
Depleting therapy in the last y[a]	0.19	0.05-0.8	**0.023**

Univariable regression results are to be seen in **Table S1** (**SDC,** http://links.lww.com/TP/C339).
[a]Antithymocyte globulin and rituximab. The *P*-value of variables that reached statistical significance are displayed in bold.
CI, confidence interval; CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; IgG, immunoglobulin G; KTR, kidney transplant recipient; MMF, mycophenolate mofetil; MPA, mycophenolic acid; OR, odds ratio; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

DISCUSSION

The aim of this large prospective cohort study involving 736 subjects was to evaluate the immune response to SARS-CoV-2 vaccination in an immunocompromised population of KTRs. As observed in other recent reports, KTRs exhibit a severely impaired immune response to mRNA vaccines, which impacts both humoral and cellular immunities. We found that patients who received vaccination while on the waiting list for kidney transplantation without immunosuppression and immunosuppressed KTRs who were naturally immunized by SARS-CoV-2 before vaccination mounted higher levels of SARS-CoV-2 antibodies than KTRs who were SARS-CoV-2 naive and vaccinated on immunosuppression. Furthermore, we identified several factors associated with poor antibody response to mRNA vaccination in KTRs, several of which could be potentially targeted to improve humoral response.

Of all KTRs vaccinated after transplantation, seroconversion after the second dose of an mRNA vaccine was reached only in 45.8% KTRs. Furthermore, if previous SARS-CoV-2 infection was considered, the seroconversion rate in the naive population was only 40.1%. This finding contrasts with a 100% seroconversion rate observed among KTRs vaccinated while on the waiting list and is consistent with some previous smaller reports. For example, Bertrand et al[11] demonstrated that in the cohort of 45 KTRs and 10 hemodialysis patients, the mRNA vaccine BNT162b2 induced antispike antibodies in 88.9% hemodialysis patients, compared with just 17.8% rate in KTRs. Although it had been reported that hemodialysis patients display a weaker antibody response to mRNA vaccination compared with healthy controls,[19,20] the evidence from our study shows that these patients respond substantially better than KTRs on immunosuppression.[7,21] However, it remains unclear how long will the waitlisted patients display sufficient immune protection after the start of posttransplant immunosuppression. In our study, most patients who were vaccinated while on the waiting list were repeatedly tested for antibodies following transplantation, and a significant decrease in antibody levels was observed in

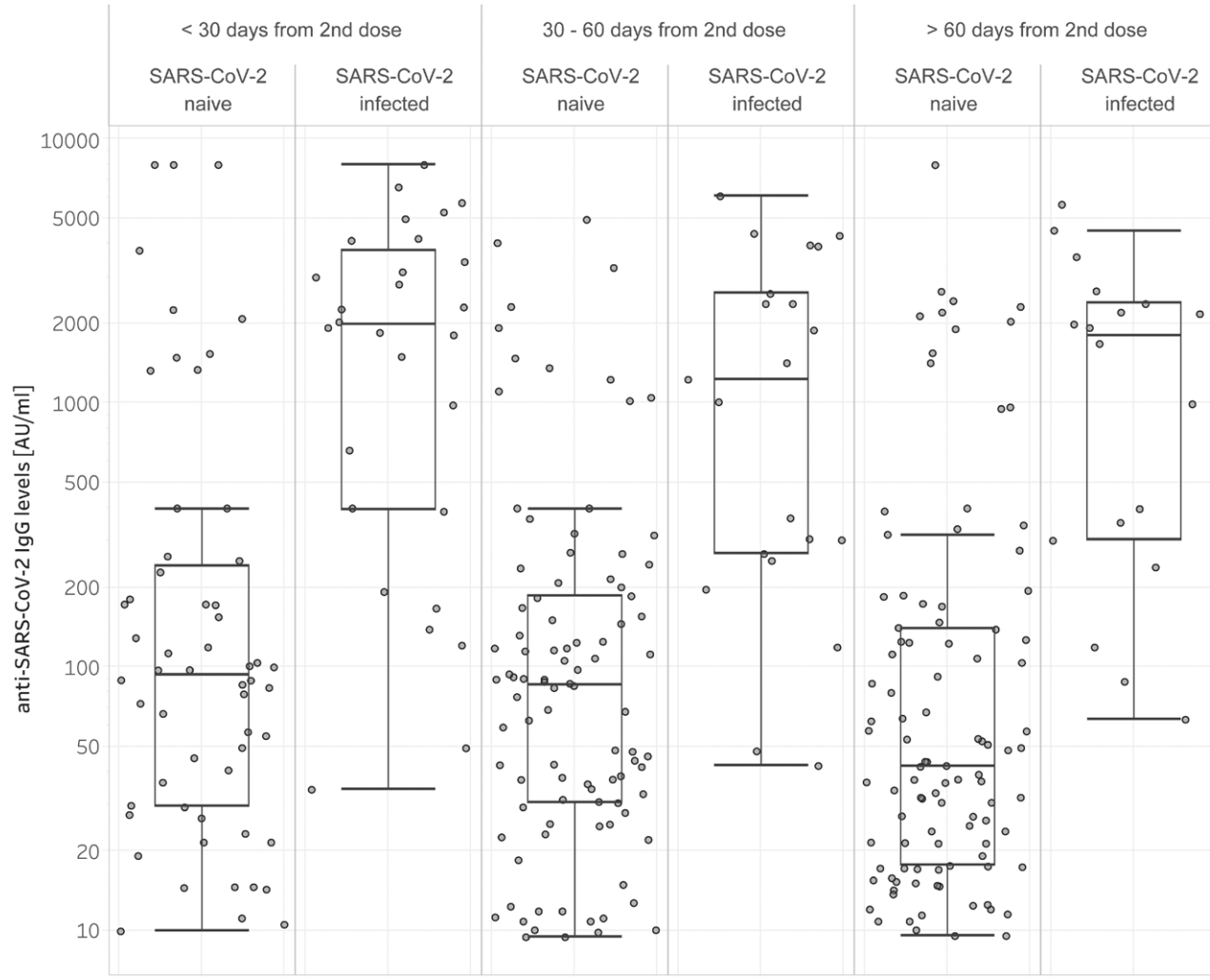

FIGURE 3. Anti–SARS-CoV-2 IgG levels over time. KTRs vaccinated after transplantation in whom seroconversion after the second dose of mRNA vaccine was observed were divided according to previous SARS-CoV-2 infection status and time from the second dose to antibody testing into 3 intervals (<30 d, 30–60 d, and >60 d from the second dose to testing, respectively). Lower antibody levels were observed in SARS-CoV-2–naive KTRs tested >60 d in comparison with <30 d after vaccination, respectively ($P = 0.035$). IgG, immunoglobulin G; KTR, kidney transplant recipient; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

most of these patients when comparing the "day 0" levels and subsequent testing.

It is becoming clear that the immunosuppression burden following transplantation is a major cause of poor vaccine response to SARS-CoV-2 vaccines. Mycophenolate use in particular has been reported as potentially disruptive for vaccine efficacy.[9] This observation is further supported by similar observations in influenza vaccination, where mycophenolate use was associated with reduced humoral immunity in a dose-dependent manner.[22,23] The clear association between mycophenolate use and impaired humoral response poses the question of whether a temporary decrease in dosage or a complete withdrawal of mycophenolate ahead of vaccination would serve as a possible strategy to improve vaccine efficacy in KTRs, at least in patients at low risk of rejection or those at a high risk of severe course of COVID-19. However, such an approach would be problematic as KTRs should not be unnecessarily put at risk of rejection.

Based on the observation of poor humoral response to SARS-CoV-2 mRNA vaccines, it can be assumed that COVID-19 still presents a major risk for vaccinated KTRs. Thus, it is of utmost importance to consider every tool available to protect this vulnerable population, including the continuation of standard regime measures such as social distancing and the use of face masks, vaccination of all waitlisted patients, healthcare providers, and household members, and to consider testing additional booster doses, which could be a promising tool to improve the immunogenicity of vaccines in solid organ recipients.[24-26] Our results are also supportive of recently published expert recommendations.[27]

Natural SARS-CoV-2 infection is described as a strong factor affecting the immune response after mRNA vaccination in the general population.[14] It is well known that previously infected individuals develop higher anti–SARS-CoV-2 neutralizing antibodies and T-cell responses following mRNA vaccination.[14,28-30] In this study, we observed that previous SARS-CoV-2 infection strongly enhances antibody production following vaccination, despite immunosuppression. Vaccination after natural infection has recently been termed as "hybrid immunity to SARS-CoV-2"

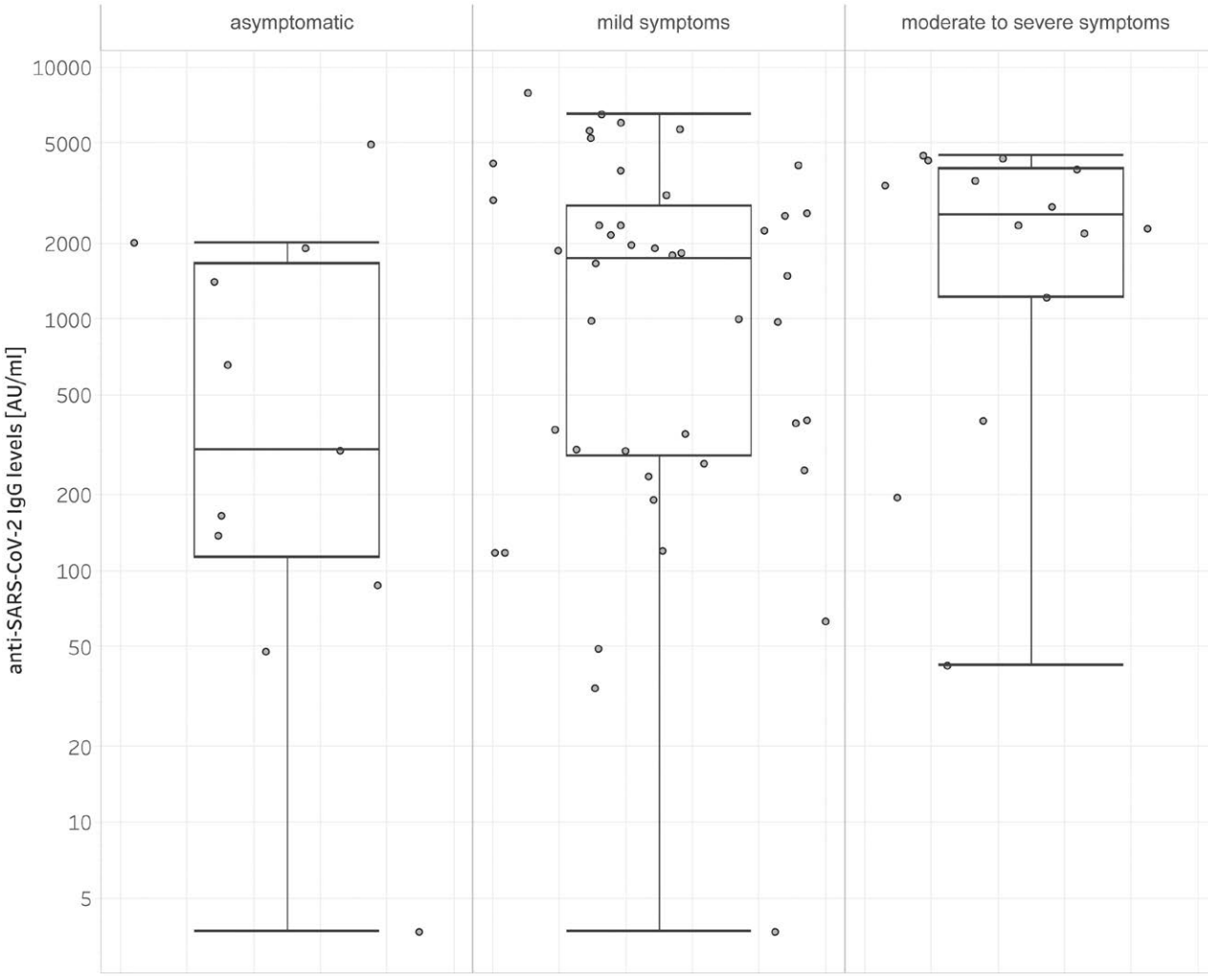

FIGURE 4. Impact of COVID-19 symptoms on anti–SARS-CoV-2 IgG levels after vaccination. KTRs who received vaccination after contracting SARS-CoV-2 were divided on the basis of the severity of clinical symptoms scored according to the Clinical Spectrum of SARS-CoV-2 Infection definition. Statistical differences were assessed using the Kruskal-Wallis test; $P=0.049$ for the overall model. Post hoc test revealed significant differences between asymptomatic KTRs and KTRs with moderate to severe symptoms ($P=0.014$). The differences between the other groups were not significant ($P=0.093$ for asymptomatic and mild symptoms, and $P=0.168$ for mild and moderate to severe symptoms). COVID-19, coronavirus disease 2019; IgG, immunoglobulin G; KTR, kidney transplant recipient; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

and reported to be effective even among solid organ recipients in a previous report.[31] Additionally, it seems that the magnitude of the humoral response is dependent on the severity of clinical symptoms, as KTRs who experienced moderate or severe disease course mounted higher levels of antibodies than asymptomatic individuals.

Furthermore, we recently showed that KTRs mounted a similar level of anti–SARS-CoV-2 antibodies compared with healthcare workers as a result of natural immunization.[10] Using an identical antibody detection method in both studies, the observed SARS-CoV-2 antibody levels after a natural infection[10] were lower than in KTRs who were first infected with SARS-CoV-2 and later received a vaccine. Therefore, mRNA vaccines may serve as a booster of the immune response primed by natural immunization when immunosuppression is absent or reduced. However, mRNA vaccines seem to be far less effective in priming immune responses in immunosuppressed populations. Furthermore, a heterologous approach combining different vaccines might also be a solution to this problem,[32] but so far, this approach has not been tested in a transplantation setting.

Interestingly, no patient on costimulatory inhibitors developed seroconversion after vaccination, including those with previous SARS-CoV-2 infection, who generally developed high antibody levels after vaccination. This observation is consistent with other recent reports.[11]

In this study, we observed that a higher proportion of KTRs vaccinated with mRNA-1273 reached seroconversion, which is a similar finding to that of other reports of higher immunogenicity of mRNA-1273 vaccine in solid organ recipients[33] and the general population.[34] However, we believe that in our cohort, this was because of a higher representation of previously infected SARS-CoV-2 individuals. After adjustment in a multivariable model, there was no difference between these 2 vaccines, and thus we cannot recommend one over the other at this time.

Furthermore, to our knowledge, this is the first study to analyze the immune response to mRNA vaccines in a cohort of KTRs who were vaccinated while on the waiting

list. This was possible because of the high-volume program of our center and the short waiting time for a kidney transplant.[35]

Among the limitations of our study is that, undoubtedly, there were KTRs who were falsely classified as naive to SARS-CoV-2 but were previously infected with the virus. To detect all previous infections, we decided to use the National Registry of Infectious Disease, where the records of every PCR test performed in the country because the first outbreak of COVID-19 are mandatorily reported. In other studies, participants were screened for SARS-CoV-2 antibodies. However, even serological screening has its limitations, as varying proportions of SARS-CoV-2 positive KTRs will not mount antibodies[10,36] or the antibodies might decrease over time. Therefore, even individuals with negative SARS-CoV-2 antibodies might carry an immune memory and bias the measurements. We believe that our approach is a comparable alternative to serological screening in dividing SARS-CoV-2–naive and previously infected individuals.

In conclusion, SARS-CoV-2–naive patients who received both doses of mRNA vaccines while on the waiting list mounted good humoral immune response, which was preserved in the first month following kidney transplantation, contrary to poor response in immunosuppressed KTRs naive to SARS-CoV-2. In contrast, survivors of COVID-19 exhibit abundant humoral and cellular responses to mRNA vaccines despite receiving immunosuppression.

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