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SARS-CoV-2 Spike-specific IFN- γ T-cell Response After COVID-19 Vaccination in Patients With Chronic Kidney Disease, on Dialysis, or Living With a Kidney Transplant

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Background. Studies have shown that coronavirus disease 2019 (COVID-19) vaccination is associated with a lower humoral response in vulnerable kidney patients. Here, we investigated the T-cell response following COVID-19 vaccination in kidney patients compared with controls. **Methods.** Patients with chronic kidney disease (CKD) stage G4/5 [estimated glomerular filtration rate <30 mL/min/1.73 m²], on dialysis, or living with a kidney transplant and controls received 2 doses of the mRNA-1273 COVID-19 vaccine. Peripheral blood mononuclear cells were isolated at baseline and 28 d after the second vaccination. In 398 participants (50% of entire cohort; controls n = 95, CKD G4/5 n = 81, dialysis n = 78, kidney transplant recipients [KTRs] n = 144), SARS-CoV-2-specific T cells were measured using an IFN- γ enzyme-linked immune absorbent spot assay. **Results.** A significantly lower SARS-CoV-2-specific T-cell response was observed after vaccination of patients on dialysis (54.5%) and KTRs (42.6%) in contrast to CKD G4/5 (70%) compared with controls (76%). The use of calcineurin inhibitors was associated with a low T-cell response in KTRs. In a subset of 20 KTRs, we observed waning of the cellular response 6 mo after the second vaccination, which was boosted to some extent after a third vaccination, although T-cell levels remained low. **Conclusion.** Our data suggest that vaccination is less effective in these patient groups, with humoral nonresponders also failing to mount an adequate cellular response, even after the third vaccination. Given the important role of T cells in protection against disease and cross-reactivity to SARS-CoV-2 variants, alternative vaccination strategies are urgently needed in these high-risk patient groups.

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INTRODUCTION

mRNA-based vaccines have been shown to be safe and effective in protecting against coronavirus disease 2019 (COVID-19).^{1,2} However, vaccine efficacy may be lower in specific patient groups, such as patients with severely impaired kidney function and patients on kidney replacement therapy. Recent studies have shown lower percentages of responders to COVID-19 vaccination, based on IgG antibody levels against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) Spike (S)1.³⁻⁶

Neutralizing antibodies are important in protection against infection, whereas other components of the adaptive immune response, specifically T cells, are more important for limiting infection and protection against disease. CD4⁺ T cells contribute to protection by supporting isotype switching of B cells, affinity maturation, and clonal proliferation, whereas CD8⁺ T cells clear virus-infected cells.^{7,8} In patients with mild COVID-19, early induction of interferon (IFN)- γ -secreting SARS-CoV-2-specific T cells was associated with accelerated viral clearance.⁹ Furthermore, the induction of polyfunctional SARS-CoV-2-specific T cells correlated with mild disease.¹⁰ The pivotal phase 1 clinical trial with mRNA-1273 showed induction of CD4⁺ and low CD8⁺ T-cell responses using intracellular cytokine staining after stimulation with Spike antigens.¹¹ Although an impaired cellular response in kidney patients has been observed after vaccination with mRNA vaccines, most studies were small-scale and included only 1 or 2 subgroups of kidney patients without a control group.¹²⁻¹⁴

The aim of this study was to investigate the T-cell response after 2 mRNA-1273 COVID-19 vaccinations in patients with severely impaired kidney function (chronic kidney disease [CKD] G4/5), patients on dialysis, and kidney transplant recipients (KTRs) compared with control subjects without known kidney disease and correlate these results to specific patient characteristics and immunosuppressive agents.

MATERIALS AND METHODS

Our study was designed to investigate the immune response after COVID-19 vaccination in kidney patients (the RECOVAC Immune Response study was published previously).¹⁵ Patients were included and the blood was collected before and 28 d after vaccination between February 1 and May 31, 2021, at the outpatient clinics of 4 university medical centers in The Netherlands (Amsterdam UMC, UMC Groningen, Radboudumc Nijmegen, and Erasmus MC Rotterdam). The primary endpoint was the antibody-based immune response on day 28 after the second vaccination. Results of the primary outcome have been published elsewhere.³ One of the secondary endpoints was the SARS-CoV-2-specific T-cell response. Approval was obtained from the Dutch Central Committee on Research Involving Human Subjects (CCMO, NL76215.042.21), and the local ethics committees of the participating centers. The study is registered at www.ClinicalTrials.gov (NCT04741386).

Study Participants

Four different cohorts were included in the study. Cohort A, the control group, consisted of subjects with normal or mildly disturbed kidney function [estimated glomerular filtration rate (eGFR) >45 mL/min/1.73 m²], cohort B of patients with severely impaired kidney function

(eGFR <30 mL/min/1.73 m² or CKD stages G4/5), cohort C of patients on hemodialysis or peritoneal dialysis, and cohort D of KTRs. The control cohort included partners, siblings or household members of participants in cohorts B, C, and D. The numbers of participants in each cohort were equally divided over the 4 participating centers. All participants received 2 mRNA-1273 COVID-19 vaccinations (Spikevax) with an interval of 28 d according to the manufacturer's instructions. Blood samples were collected at baseline (ie, before first vaccination) and 28 d after the second vaccination.

The T-cell response was measured in a subset of subjects from the RECOVAC Immune Response Study. A flowchart of subject enrollment and selection for measurement of T-cell response is depicted in Figure 1. From the subjects that completed follow-up on day 28 after the second vaccination, we excluded subjects who had previously been infected with SARS-CoV-2, as defined by having baseline SARS-CoV-2 Spike S1-specific IgG serum antibody levels ≥ 10 BAU/mL. Patients who were diagnosed with COVID-19, based on a positive PCR test, before the 28 d post-second dose time point were excluded, as well as patients with missing serology at baseline or low/no peripheral blood mononuclear cell (PBMC) availability. In total, 191 controls, 159 patients with CKD G4/5, 157 dialysis patients, and 288 KTRs were available for a random selection for measurement of T-cell responses. In these subjects, a random sample of 50% was taken using random number generator software stratifying for cohorts and participating centers.

KTRs from this study without a humoral response after 2 vaccinations were asked to participate in a follow-up study (the RECOVAC Repeated Vaccination Study). In this randomized clinical trial, the immunogenicity of alternative vaccination strategies was compared with a standard vaccination. This study is registered at www.ClinicalTrials.gov (NCT05030974). We selected individuals who were included in this follow-up study and received the third vaccination with mRNA-1273 to make comparisons in immunogenicity after 2 and 3 vaccinations.

PBMC Isolation

PBMCs were isolated from 50 mL of venous blood by density gradient centrifugation using Ficoll-paque plus (GE Healthcare) and SepMateTM tubes (STEMCELL) within 24 h of blood collection. PBMCs were washed twice with phosphate buffered saline (PBS) and counted using Türk's solution and checked for viability with Trypan Blue (both Sigma Aldrich). PBMCs were frozen in medium containing RPMI (Gibco), 20% FCS (LPS), and 10% DMSO (Sigma Aldrich) and stored in liquid nitrogen until further use.

IFN- γ ELISpot Assay

SARS-CoV2-specific T cells were measured using an IFN- γ ELISPOT assay. In short, multiScreen HTS IP filter plates (Millipore) activated with 35% ethanol were coated with antihuman IFN- γ antibody (1-D1K, Mabtech; 5 μ g/mL) and incubated overnight at 4 °C. Next, the plates were blocked with X-VIVO (Lonza) medium + 2% Human AB Serum (HS; Sigma) and incubated for 1 h at 37 °C and 5% CO₂. PBMCs were thawed, resuspended in cold IMDM (Gibco) medium + 10% FCS, and centrifuged for 7 min at 375x-g and washed twice. Following aspiration, PBMCs were resuspended in X-VIVO medium +2%HS in a concentration of 4 \times 10⁶ cells/mL and rested for 1 h at 37 °C and 5% CO₂. PBMCs

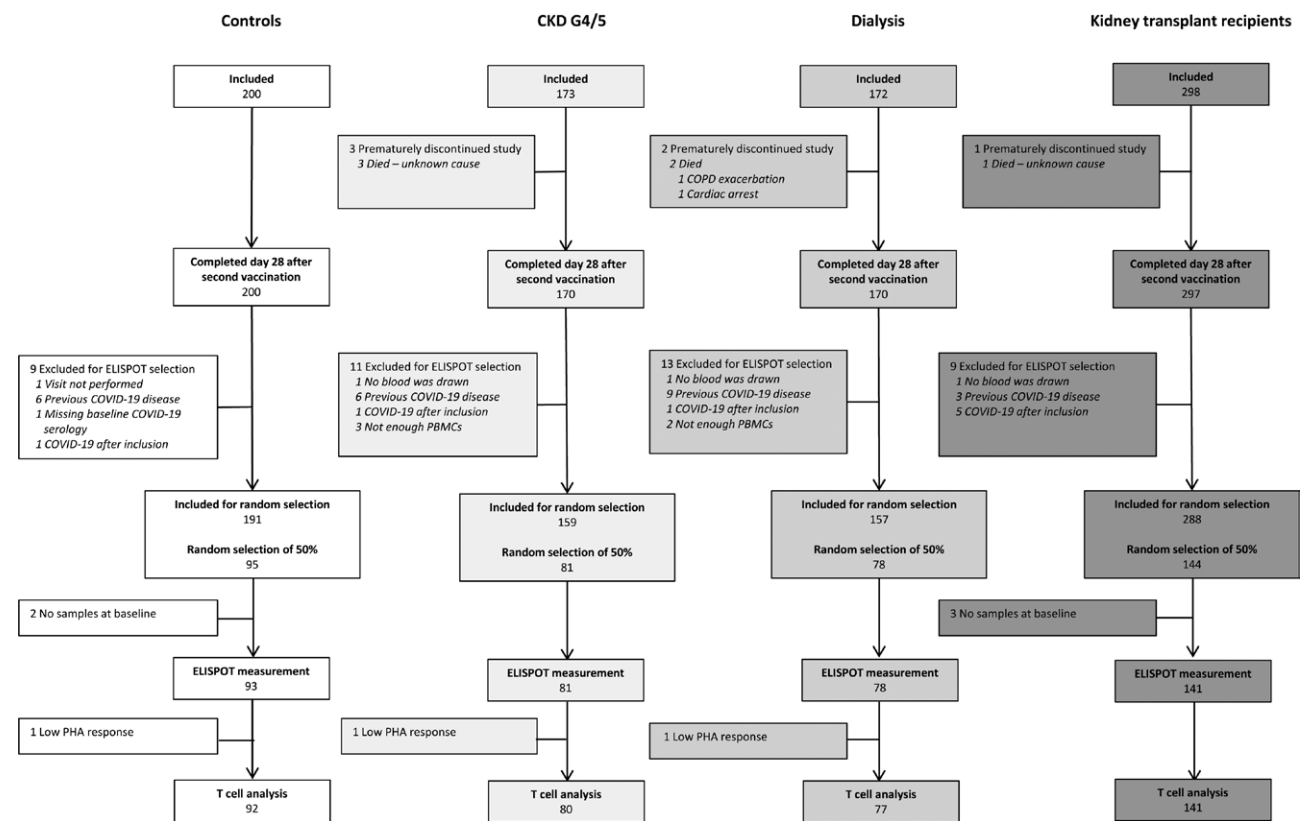


FIGURE 1. Subject enrollment. CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; ELISPOT, enzyme-linked immune absorbent spot; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin.

were counted using Trypan Blue (Sigma) and checked for viability. SARS-CoV-2 S1 and S2 peptide pools (JPT Peptide Technologies) consisting of 15-mer peptides overlapping 11 amino acids that cover the entire S protein were used for stimulation of the PBMCs in a concentration of 0.5 µg/mL. All dilutions were made in in X-VIVO+2%HS and all stimulations were performed in triplicate. A 0.1% DMSO (Sigma) was used as a negative control and PHA (Remel Europe Ltd; 2 µg/mL) as a positive control. PBMCs were seeded at 2×10^5 cells per well and cultured for 20 to 24 h at 37 °C and 5% CO₂. Subsequently, ELISPOT plates were washed with PBS + 0.05% Tween 20. Antihuman biotinylated IFN-γ antibody (7-B6-1, Mabtech; 1:1000) in 0.05% Poly-HRP buffer (ThermoFisher) diluted in PBS was added for 1.5 h at RT, followed by the addition of Streptavidin poly-HRP (Sanquin; 1:6000) in 0.05% Poly-HRP buffer for 1 h at RT (in the dark). Spots were developed using TMB substrate (Mabtech). Spot-forming cells (SFCs) were quantified with the AID ELISPOT/Fluorospot reader and calculated to SFCs/10⁶ PBMCs. The average of the DMSO negative control was subtracted per stimulation. To define the total Spike-specific SFC, the numbers of SFCs of the separate S1 and S2 peptide pools were combined. Individuals with a S-specific response of ≥ 50 SFCs/10⁶ PBMCs after vaccination and a ≥ 2 -fold increase between the 28 d postvaccination and baseline were defined as a responder. This was based on experience in other infectious diseases using values in unvaccinated and uninfected healthy controls.¹⁶ Values were set at 1 SFCs/10⁶ PBMCs in the case of 0 SFCs/10⁶ PBMCs at baseline and/or 28 d post-vaccination. Samples were excluded when the positive control PHA did not reach the expected quality level.

SARS-CoV-2 Spike (S)1-specific IgG antibody response

SARS-CoV-2 Spike (S)1-specific IgG antibodies were measured in serum samples by a validated fluorescent bead-based multiplex-immunoassay with a specificity and sensitivity of 99.7% and 91.6%, respectively, as previously described.^{17,18} Concentrations were interpolated from a reference sample consisting of pooled sera using a 5-parameter logistic fit and NIBSC/WHO COVID-19 reference serum 20 of 136 and expressed as international binding antibody units per mL (BAU/mL). Participants were classified as seropositive based on receiver operator curve analysis and set at S1-specific IgG antibody concentration ≥ 10 BAU/mL.^{18,19}

Statistical Analysis

Continuous data are presented as mean with SD or as median and interquartile interval in the case of nonnormal distribution. Categorical data are presented as percentages. Differences between cohorts or T-cell responders and non-responders were tested, using an independent sample *t* test, a Mann–Whitney U test, or a Pearson chi-square test depending on data distribution, and subsequently corrected for multiple testing. Differences over time within a cohort were tested using a Wilcoxon signed rank test or as stated otherwise. Differences in >2 categories were tested using the 1-way ANOVA, Kruskal–Wallis, or Pearson chi-square test depending on data distribution. To identify independent risk factors for being a T-cell responder, the associations of patient characteristics with T-cell responder status were examined using logistic regression analysis. Odds ratios (ORs), corresponding 95% confidence intervals, and *P* values were reported.

Variables with a P value of <0.1 and with at least 10 cases in univariable analysis were considered as candidate predictors. These candidate predictors were introduced in a multivariable logistic regression model with age and sex as fixed variables. Using a backward elimination procedure, the least significant variables were removed in a stepwise manner until none met the criterion of P value ≥ 0.05 . The association between the T-cell and humoral response after COVID-19 vaccination was tested by performing Spearman correlation between the number of SARS-CoV-2 S-specific SFCs and the level of IgG antibodies. All analyses were performed with the statistical software IBM SPSS statistics, version 23.0 (SPSS Inc, Chicago, IL). Figures were created with GraphPad Prism, version 5.00 (GraphPad Software, San Diego, CA). A 2-sided P value <0.05 was adopted to denote statistical significance and corrected in the case of multiple testing using Bonferroni correction unless stated otherwise.

RESULTS

Baseline Characteristics of the Study Population

A flowchart of the selection of participants included in the RECOVAC Immune response study is depicted in Figure 1. In total, 795 participants were included (controls $n = 200$, CKD G4/5 $n = 173$, dialysis $n = 172$, KTRs $n = 298$), from which we randomly selected 50% per cohort for the IFN- γ ELISPOT measurement, which left 398 participants (controls $n = 95$, CKD G4/5 $n = 81$, dialysis $n = 78$, KTRs $n = 144$). From this random selection, 5 participants were excluded because there were no samples available for measurement. After the measurement, 3 participants were excluded because of low PHA response. This left 92 controls, 80 patients with CKD G4/5, 77 dialysis patients, and 141 KTRs for analysis. Baseline characteristics are shown in Table 1. In CKD G4/5, patients' mean eGFR was 18.2 ± 6.6 mL/min/1.73 m 2 ; in dialysis patients, 64.9% were hemodialysis patients; and in KTRs, the median time after transplantation was 6.5 (2.0–13.0) years. There were some small differences in characteristics of participants included for T-cell analysis compared with participants on which T-cell analysis was not performed: (1) dialysis modality (less hemodialysis in the selected group), (2) azathioprine use (less azathioprine use in the selected group), and (3) S1 IgG antibody level 28 d after vaccination in the controls (lower levels in the selected group) (Table S1, SDC, <http://links.lww.com/TXD/A458>).

SARS-CoV-2-specific IFN- γ T-cell Response

We assessed T-cell responses by stimulating PBMCs from 398 participants with S1 and S2 overlapping peptide pools before and 28 d after second vaccination and measured the number of IFN- γ secreting T cells by ELISPOT. In all groups, the median Spike (S1+S2)-specific SFCs increased 28 d after vaccination ($P < 0.001$ for all groups). Compared with controls, the number of Spike-specific SFCs was similar in CKD G4/5 patients and lower in dialysis patients and KTRs at 28 d after second vaccination (223.3 [77.5–415], 86.7 [34.2–238], 73.3 [16.8–197] versus 200.8 [103.8–421.2] SFCs/10 6 PBMCs; $P = 0.99$, $P = 0.006$, and $P < 0.001$, respectively; Figure 2A). Based on our responder definition, a T-cell response was observed in 70% of CKD G4/5 patients, 54.5% of dialysis patients, and 42.6% of KTRs compared with 76.1% of controls at 28 d after second vaccination ($P = 0.99$, $P = 0.01$, $P < 0.001$, respectively; Figure 2B).

Predictors of T-cell Response in KTRs

Next, we analyzed whether we could identify cohort specific predictors of T-cell response after vaccination. We therefore first assessed differences in characteristics between T-cell responders and nonresponders per cohort (Table 2). There was 1 characteristic significantly different in the control group (diastolic blood pressure) and CKD G4/5 patients (lymphocyte count) and 2 in dialysis patients (diastolic blood pressure and autoimmune disease). In the KTRs, which included the majority of nonresponders, time since transplantation was shorter in nonresponders than in responders (5.0 [1.0–10.0] versus 9.0 [3.0–14.0] y, $P = 0.03$). Additionally, calcineurin inhibitors were used more (90.1% versus 72.9%, $P = 0.008$) and mammalian target of rapamycin inhibitors less often (0% versus 11.9%, $P = 0.001$) in nonresponders. Variables, with at least 10 cases (time since transplantation and calcineurin inhibitors), were subsequently included in a multivariable stepwise backward logistic regression analysis, which showed that the use of calcineurin inhibitors was significantly associated with the risk of being a nonresponder (Table 3).

Correlation Between SARS-CoV-2-specific T cells and Antibodies

Next, we analyzed the correlation between the T-cell and antibody response. The number of Spike-specific SFCs and level of S1 IgG antibodies correlated significantly in the overall cohort ($\rho = 0.41$, $P < 0.001$, respectively). Most of the controls and CKD G4/5 and dialysis patients had both an antibody and a T-cell response (76.1%, 70.4%, and 54.5%, respectively) with a weak but significant correlation (Figure 3A–C; $P = 0.009$, $P = 0.027$, $P = 0.003$, respectively). This was in contrast to KTRs, of whom only 27.9% had both an antibody and a T-cell response. Interestingly, 14.3% of KTRs showed a T-cell response without a detectable antibody response, and 27.9% had an antibody response without a detectable T-cell response (Figure 3D; $\rho = 0.35$, $P < 0.001$). Furthermore, we analyzed the characteristics of KTRs in each of the 4 response quadrants (Table 4). Age, lymphocyte counts, eGFR, time after transplantation, and immunosuppressive treatments were different between the groups. The groups with discordant responses (antibody+/T-cell– and antibody–/T-cell+) were quite heterogeneous. To assess which of these characteristics are predictors of response types, we therefore compared these between antibody and T-cell responders (+/+) and antibody and T-cell nonresponders (–/–) using multivariable logistic regression with a stepwise backward analysis. Mycophenolate mofetil (MMF) use, lower lymphocyte count, and lower eGFR remained significantly associated with a humoral and cellular nonresponder (OR 0.04 [0.01–0.20], $P < 0.001$; OR 4.26 [1.67–10.87], $P = 0.002$; OR 1.05 [1.01–1.09], $P = 0.019$).

SARS-CoV-2-specific IFN- γ T-cell response After Third Vaccination

In a subset of 20 KTRs, we also assessed T-cell response at 6 mo after second vaccination to assess the decay of the response and subsequently 28 d after the third vaccination to assess boosting of the response. Baseline characteristics are depicted in Table S2, SDC, <http://links.lww.com/TXD/A458>. Median Spike-specific SFCs increased from 10.9 (T1) (1.00–35.0) to 17.5 (T2) (1–132.5) SFCs/10 6 PBMCs 28 d after the second vaccination. At 6 mo after the second vaccination (T3), the median Spike-specific SFCs declined to 7.5

TABLE 1.**Baseline characteristics per study cohort**

	Control (n = 93)	CKD G4/5 (n = 81)	Dialysis (n = 77)	KTR (n = 141)
Female, n (%)	54 (58.1)	27 (33.3)	25 (32.5)	68 (48.6)
Caucasian, n (%)	88 (96.7)	71 (87.7)	67 (87.0)	128 (91.4)
Age, y	57.7 ± 13.6	59.4 ± 13.1	60.3 ± 14.8	56.4 ± 12.8
BMI, kg/m ²	27.5 ± 5.8	28.5 ± 4.8	27.3 ± 5.9	27 ± 5.2
SBP, mm Hg	147 ± 22	150 ± 25	140 ± 26	147 ± 21
DBP, mm Hg	85 ± 11	89 ± 12	79 ± 17	86 ± 11
Current smoking, n (%)	13 (14.0)	13 (16.0)	21 (27.3)	10 (7.2)
Current alcohol consumption, n (%)	52 (55.9)	30 (37.5)	17 (22.1)	54 (38.8)
Number of comorbidities	0 (0–1)	1 (1–2)	1 (1–2)	1 (1–2)
Comorbidities, n (%)				
Hypertension	21 (22.6)	67 (82.7)	52 (68.4)	111 (79.3)
Diabetes	7 (7.5)	24 (29.6)	17 (22.1)	30 (21.4)
History of coronary artery disease	4 (4.3)	17 (21.0)	14 (18.4)	20 (14.3)
Heart failure	1 (1.1)	7 (8.6)	7 (9.2)	7 (5.0)
Chronic lung disease	9 (9.7)	6 (7.4)	11 (14.5)	7 (5.0)
History of malignancy ^a	4 (4.3)	6 (7.4)	15 (19.7)	20 (14.3)
Autoimmune disease	3 (3.2)	2 (2.5)	3 (3.9)	6 (4.3)
Lymphocytes, 10 ⁹ /L	1.99 (1.6–2.5)	1.59 (1.3–2.0)	1.25 (0.9–1.6)	1.34 (0.9–1.9)
eGFR, mL/min/1.73 m ²	82.8 ± 20.9	18.2 ± 6.6	—	49.7 ± 18.2
Primary renal diagnosis, n (%)				
Primary glomerulonephritis	—	10 (13.9)	9 (13.2)	24 (19.4)
Pyelonephritis	—	1 (1.4)	0 (0.0)	2 (1.6)
Interstitial nephritis	—	4 (5.6)	2 (2.9)	7 (5.6)
Familial/hereditary renal diseases	—	12 (16.7)	13 (19.1)	31 (25.0)
Congenital diseases	—	3 (4.2)	1 (1.5)	7 (5.6)
Vascular diseases	—	15 (20.8)	15 (22.1)	12 (9.7)
Secondary glomerular/systemic disease	—	3 (4.2)	4 (5.9)	7 (5.6)
Diabetic kidney disease	—	5 (6.9)	12 (17.6)	6 (4.8)
Other	—	17 (23.6)	9 (13.2)	22 (17.7)
Unknown	—	2 (2.8)	3 (4.4)	6 (4.8)
Dialysis characteristics, n (%)				
Hemodialysis	—	—	50 (64.9)	—
Peritoneal dialysis	—	—	27 (35.1)	—
Time on dialysis, mo	—	—	29 (12.0–65.3)	—
Transplant characteristics				
First kidney transplant, n (%)	—	—	—	107 (76.4)
Time after last transplantation, years	—	—	—	6.5 (2.0–13.0)
Last transplant				
Living, n (%)	—	—	—	98 (70.0)
Preemptive, n (%)	—	—	—	54 (38.6)
Number of immunosuppressive agents	—	—	—	2.5 (2–3)
Immunosuppressive treatment, n (%)				
Steroids	—	—	—	105 (75.0)
Azathioprine	—	—	—	22 (15.7)
Mycophenolate mofetil	—	—	—	95 (67.9)
Calcineurin inhibitor	—	—	—	116 (82.9)
mTOR inhibitor	—	—	—	7 (5.0)
Other	—	—	—	2 (14.4)
Induction with rituximab last year, n (%)	—	—	—	2 (1.4)

Melanomas, excluding all other skin malignancies.

Variables are presented as mean ± SD or as median (interquartile range) in the case of nonnormal distribution.

BMI, body mass index; CKD, chronic kidney disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; KTR, kidney transplant recipient; mTOR, mammalian target of rapamycin; SBP, systolic blood pressure.

(1.0–54.2) SFCs/10⁶ PBMCs. Twenty-eight days after the third vaccination (T4), the median increased to 43.4 (2.0–82.9) SFCs/10⁶ PBMCs, although this did not reach formal statistical significance compared with 6 mo after the second vaccination ($P = 0.09$; Figure 4). With a responder cutoff of ≥50

SFCs/10⁶ PBMCs, at time point T2, 40% of the participants were responders, which declined to 30% at T3. At T4, 45% were responders. After the third vaccination, 14 of 21 participants showed a positive antibody response. Interestingly, after the third vaccination, no partial responders remained who

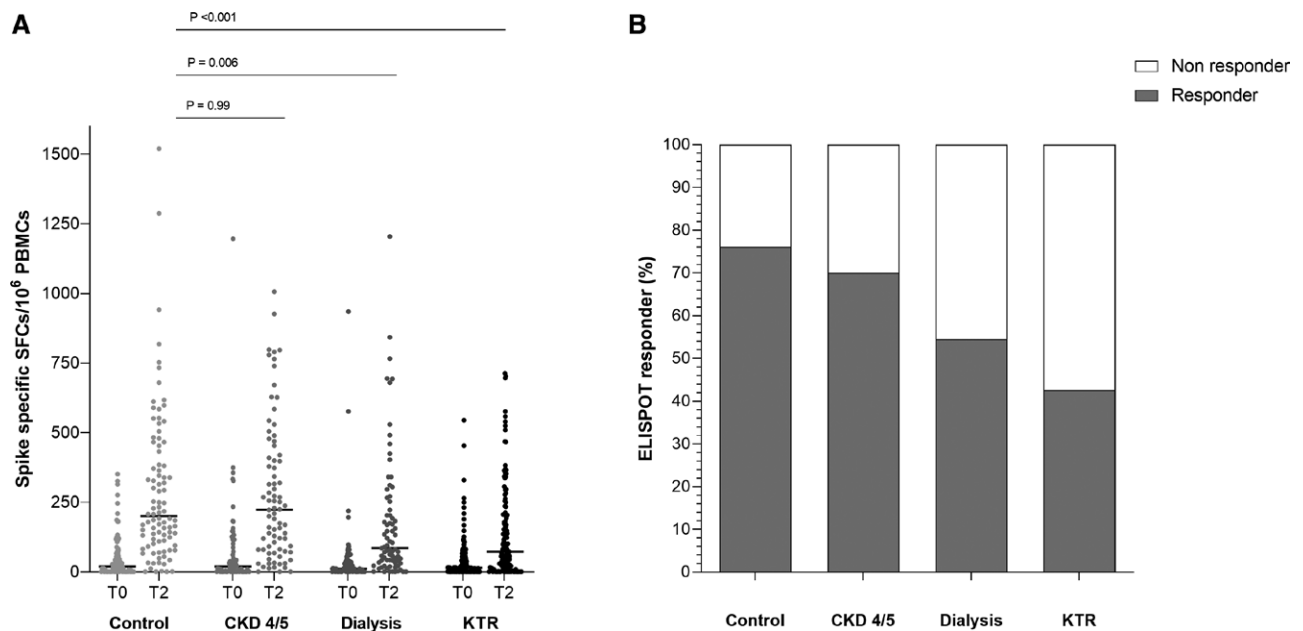


FIGURE 2. SARS-CoV-2 spike-specific T-cell response per study cohort at baseline (T0) and 28 d after second vaccination (T2). A, Dot plot of spike-specific spots per study cohort with the line representing median. P values were calculated using the Mann–Whitney U test and corrected for multiple testing by Bonferroni. B, Responders were defined as subjects with spike-specific spots $\geq 50/10^6$ PBMCs and at least 2-fold increase between T0 and T2; P values were calculated using chi-square ($P = 0.99$, $P = 0.01$, $P < 0.001$). CKD, chronic kidney disease; ELISPOT, enzyme-linked immune absorbent spot; KTR, kidney transplant recipient; PBMC, peripheral blood mononuclear cell; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; SFC, spot-forming cell; T0, baseline; T2, 28 d after vaccination.

had a T-cell response without an antibody response (Figure S1, SDC, <http://links.lww.com/TXD/A458>). The median T-cell response was significantly higher with 62.5 SFCs/10⁶ PBMCs (31.7–211.3) in the antibody responders versus 1.0 SFCs/10⁶ PBMCs (1.0–21.7) in the antibody nonresponders ($P = 0.006$).

DISCUSSION

In this study, we show that the cellular response in CKD G4/5 patients is comparable to that of controls at 28 d after the second vaccination with mRNA-1273. In contrast, dialysis patients and especially KTRs had significantly lower cellular response rates than controls. In KTRs, the use of calcineurin inhibitors was associated with cellular nonresponse. Cellular and humoral responses correlated in the various subgroups, and most of the CKD G4/5 and dialysis patients had both a humoral and cellular response. Conversely, the majority of KTRs only showed response for 1 of the 2 immunological endpoints 28 d after the second vaccination.

Cellular responses after COVID-19 vaccination measured with the ELISPOT method have not been reported previously in patients with CKD G4/5 compared with controls. A severely impaired kidney function has been associated with an impaired immunity. For instance, several studies have reported reduced immunogenicity of vaccination against hepatitis B, influenza, and *Streptococcus pneumoniae* in CKD G4/5 patients as compared with healthy controls.²⁰ These results are primarily based on humoral response. Here, we show that vaccination with mRNA-1273 seems to be as immunogenic in CKD G4/5 patients as in controls based on cellular response.

However, we did find a lower cellular response rate in dialysis patients than in controls. A potential explanation, besides an uremic milieu, could be that the dialysis

procedure is associated with diminished immune responsiveness.²¹ Nevertheless, the humoral response was not inferior compared with controls, as shown in our previous study (99.4%).³ It has been previously documented that the disturbance of acquired immunity is mainly related to T-lymphocyte and not B-lymphocyte functionality, which may explain the differences in humoral and cellular response found in this study.²² Other COVID-19 vaccine studies performed in dialysis patients found varying SARS-CoV-2-specific cellular response rates after vaccination, ranging from 58% to 100%.^{12,14,23,24} This wide range could be explained by differences in patient characteristics, such as the use of immunosuppressant agents, or be due to different COVID-19 vaccines. It could also be explained by the use of different cellular assays or different response rate definitions in the various studies.

The severely impaired cellular response rate in KTRs (42.6%) is in accordance with previously reported data varying from 29.8% to 57.8%.^{13,14,25–27} In the univariable analysis, a shorter time after transplantation and vaccination and the use of calcineurin inhibitors were associated with cellular nonresponse. In the multivariable analysis, only calcineurin inhibitor use remained significantly associated with cellular nonresponse. Interestingly, Cucchiari et al found diabetes, lymphopenia, and lower eGFR to be associated with cellular nonresponse. However, the cellular response was measured 2 wks after the second vaccination, which could have been too early to detect a full response.²⁵ In contrast, Jurdi et al did not find any association with the cellular response.²⁸ This may be due to their limited sample size. In our study, we measured the cellular response 28 d after vaccination, and we had a relatively large sample size, which makes our results more robust.

Calcineurin inhibitors have previously been described to affect COVID-19 vaccination T-cell response in patients using

TABLE 2.**Differences in subject characteristics between T-cell responders vs nonresponders per study cohort**

	Control		CKD G4/5		Dialysis		KTR	
	+	-	+	-	+	-	+	-
	(n = 70)	(n = 22)	(n = 57)	(n = 24)	(n = 42)	(n = 35)	(n = 59)	(n = 82)
Female, n (%)	45 (64.3)	9 (40.9)	18 (31.6)	9 (37.5)	13 (31.0)	12 (34.3)	30 (50.8)	38 (46.9)
Caucasian, n (%)	68 (97.1)	19 (95.0)	51 (89.5)	20 (83.3)	38 (90.5)	29 (82.9)	53 (89.8)	75 (92.6)
Age, y	57.5 ± 13.6	59.1 ± 13.7	59.9 ± 12.3	58.3 ± 15.0	59.8 ± 15.8	60.9 ± 13.8	55.5 ± 12.7	57.1 ± 12.9
BMI, kg/m ²	27.2 ± 5.7	27.8 ± 5.2	28.1 ± 4.2	29.3 ± 5.9	27.6 ± 6.1	26.8 ± 5.8	27.6 ± 5.4	26.6 ± 5.1
SBP, mm Hg	145 ± 22	153 ± 19	150 ± 25	150 ± 25	141 ± 28	138 ± 24	143 ± 20	149 ± 20
DBP, mm Hg	83 ± 12.5 <i>P</i> = 0.037	88 ± 8^a	86 ± 12	83 ± 14	83 ± 20	74 ± 13^b <i>P</i> = 0.036	86 ± 11	86 ± 11
Current smoking, n (%)	11 (15.7)	2 (9.1)	9 (15.8)	4 (16.7)	10 (23.8)	11 (31.4)	3 (5.2)	7 (8.6)
Current alcohol consumption, n (%)	38 (54.3)	13 (59.1)	25 (43.9)	5 (21.7)	9 (21.4)	8 (22.9)	19 (32.2)	35 (43.2)
Number of comorbidities	0 (0–1)	0 (0–1)	1 (1–2)	1.5 (1–2)	1 (1–2)	1 (1–2)	1 (1–2)	1 (1–2)
Comorbidities, n (%)								
Hypertension	16 (22.9)	5 (22.7)	47 (82.5)	20 (83.3)	31 (75.6)	21 (60.0)	49 (83.1)	62 (76.5)
Diabetes	2 (9.1)	5 (7.1)	16 (28.1)	8 (33.3)	9 (21.4)	8 (22.9)	13 (22.0)	17 (21.0)
History of coronary artery disease	3 (4.3)	1 (4.5)	11 (19.3)	6 (25.0)	8 (19.5)	6 (17.1)	7 (11.9)	13 (16.0)
Heart failure	1 (1.4)	0 (0.0)	4 (7.0)	3 (12.5)	4 (9.8)	3 (8.6)	3 (5.1)	4 (4.9)
Chronic lung disease	8 (11.4)	1 (4.5)	5 (8.8)	1 (4.2)	4 (9.8)	7 (20.0)	5 (8.5)	2 (2.5)
History of malignancy ^a	2 (2.9)	2 (9.1)	3 (5.3)	3 (12.5)	6 (14.6)	9 (25.7)	10 (16.9)	10 (12.3)
Autoimmune disease	2 (2.9)	1 (4.5)	1 (1.8)	1 (4.2)	0 (0.0)	3 (8.6)^b <i>P</i> = 0.044	2 (3.4)	4 (4.9)
Lymphocytes, 10 ⁹ /L	2.0 (1.6–2.5)	1.9 (1.5–2.2)	1.6 (1.2–1.8)	1.9 (1.4–2.4)^b <i>P</i> = 0.032	1.2 (0.9–1.6)	1.3 (0.9–1.6)	1.3 (0.9–2.1)	1.3 (0.9–1.9)
eGFR, ml/min/1.73 m ²	82.6 ± 22.0	82.5 ± 17.1	18.2 ± 6.9	18.2 ± 6.0	—	—	52.8 ± 20.2	47.4 ± 16.4
Primary renal diagnosis, n (%)								
Primary glomerulonephritis	—	—	7 (14.0)	3 (13.6)	4 (11.1)	5 (15.6)	7 (13.2)	17 (23.9)
Pyelonephritis	—	—	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.8)
Interstitial nephritis	—	—	3 (6.0)	1 (4.5)	1 (2.8)	1 (3.1)	3 (5.7)	4 (5.6)
Familial/hereditary renal diseases	—	—	9 (18.0)	3 (13.6)	10 (27.8)	3 (9.4)	17 (32.1)	14 (19.7)
Congenital diseases	—	—	3 (6.0)	0 (0.0)	1 (2.8)	0 (0.0)	4 (7.5)	3 (4.2)
Vascular diseases	—	—	9 (18.0)	6 (27.3)	7 (19.4)	8 (25.0)	6 (11.3)	6 (8.5)
Secondary glomerular/systemic disease	—	—	1 (2.0)	2 (9.1)	2 (5.6)	2 (6.3)	3 (5.7)	4 (5.6)
Diabetic kidney disease	—	—	3 (6.0)	2 (9.1)	5 (13.9)	7 (21.9)	3 (5.7)	3 (4.2)
Other	—	—	12 (24.0)	5 (22.7)	3 (8.3)	6 (18.8)	6 (11.3)	16 (22.5)
Unknown	—	—	2 (4.0)	0 (0.0)	3 (8.3)	0 (0.0)	4 (7.5)	2 (2.8)
Dialysis characteristics, n (%)								
Hemodialysis	—	—	—	—	28 (66.7)	22 (62.9)	—	—
Peritoneal dialysis	—	—	—	—	14 (33.3)	13 (37.1)	—	—
Time on dialysis, mo	—	—	—	—	29 (12–62)	29 (10–69)	—	—
Transplant characteristics								
First kidney transplant, n (%)	—	—	—	—	—	—	45 (76.3)	62 (76.5)
Time after last transplantation, y	—	—	—	—	—	—	9.0 (3.0–14)	5.0 (1.0–10)^b <i>P</i> = 0.03
Last transplant								
Living, n (%)	—	—	—	—	—	—	44 (74.6)	54 (66.7)
Preemptive, n (%)	—	—	—	—	—	—	24 (40.7)	30 (37.0)
No. immunosuppressive agents	—	—	—	—	—	—	2 (2–3)	3 (2–3)
Immunosuppressive treatment, n (%)								
Steroids	—	—	—	—	—	—	43 (72.9)	62 (76.5)
Azathioprine	—	—	—	—	—	—	9 (15.3)	13 (16.0)
Mycophenolate mofetil	—	—	—	—	—	—	38 (64.4)	57 (70.4)
Calcineurin inhibitor	—	—	—	—	—	—	43 (72.9)	73 (90.1)^b <i>P</i> = 0.008
mTOR inhibitor	—	—	—	—	—	—	7 (11.9)	0 (0.0)^b <i>P</i> = 0.001
Other	—	—	—	—	—	—	0 (0.0)	2 (2.5)
Induction with rituximab last year	—	—	—	—	—	—	2 (3.4)	0 (0.0)
Immunosuppressive regimen, n (%)								
CNI + MMF + steroids	—	—	—	—	—	—	20 (33.9)	39 (66.1)
CNI + MMF	—	—	—	—	—	—	10 (38.5)	16 (61.5)
CNI + steroids	—	—	—	—	—	—	7 (43.8)	9 (56.3)
Other	—	—	—	—	—	—	22 (55.0)	18 (45.0)

^aMelanomas, excluding all other skin malignancies.^b*P* < 0.05.Subjects were defined as responder with a level of SARS-CoV-2 spike-specific spots of ≥50 peripheral blood mononuclear cells/10⁶ 28 d after the second vaccination including a 2-fold change between the pre- and postvaccination time point.Variables are presented as mean ± SD or as median (interquartile range) in the case of nonnormal distribution. *P* values are calculated using an independent sample *t* test in case of normal distribution, Mann–Whitney *U* test in the case of nonnormal distribution, and chi-square test in the case of proportion.

+, responder; –, nonresponder; BMI, body mass index; CKD, chronic kidney disease; CNI, calcineurin inhibitor; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; KTR, kidney transplant recipient; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; SBP, systolic blood pressure.

TABLE 3.
Risk factors for being a T-cell responder vs nonresponder in kidney transplant recipients (n = 141)

	Univariable		Model 1		Model 2	
	OR (95% CI)	P	aOR (95% CI)	P	aOR (95% CI)	P
Age, y					1.01 (0.98, 1.03)	0.72
Female sex					0.86 (0.43, 1.71)	0.66
eGFR, mL/min/1.73 m ²	0.98 (0.97, 1.00)	0.09	0.98 (0.97, 1.00)	0.10		
Time after last transplantation, y	0.96 (0.92, 1.00)	<0.05	0.96 (0.92, 1.00)	0.06		
No. immunosuppressants	1.78 (0.97, 3.26)	0.06	1.85 (0.99, 3.43)	0.05		
Calcineurin inhibitor, yes vs no	3.40 (1.34, 8.59)	0.01	3.32 (1.30-8.46)	0.01	3.32 (1.30, 8.46)	0.01

ORs and *P* values were calculated using logistic regression analysis. The dependent variable is responder vs nonresponder (SARS-CoV-2 spike-specific spot-forming cells of $\geq 50/10^6$ peripheral blood mononuclear cells 28 d after second vaccination including a 2-fold increase between the 28 d postvaccination and the prevaccination time point vs $<50/10^6$ peripheral blood mononuclear cells and/or <2 -fold increase between pre- and postvaccination time point); independent variables are variables from Table 3 with a *P* value ≤ 0.10 .
 Univariable: Showing only variables from Table 3 with a *P* value ≤ 0.10 .

Model 1: Variables adjusted for age and sex.

Model 2: Variables that remain significantly associated in a stepwise backward analysis with age and sex as fixed variables.

aOR, adjusted odds ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

immunosuppressants for immune-mediated kidney disease.²⁹ Mechanistically, calcineurin inhibitors cause a reduction in the expression of nuclear factor of activated T cells-mediated proinflammatory genes, such as interleukin-2 and IFN- γ , and thereby may affect the induction of proper effector IFN- γ producing T cells.³⁰ Previously, we found that the use of MMF was associated with humoral nonresponse following vaccination with mRNA-1273. Mycophenolate inhibits the synthesis of guanine nucleotides and thus prevents DNA replication and subsequently cell proliferation, whereas calcineurin inhibitors prevent lymphocyte activation in specific T cells, which may explain the more pronounced effect on the T-cell response in our study.³¹

For optimal clinical protection after vaccination, both humoral and cellular responses are required. In this study, we found that up to 41.8% (59/141) of KTRs showed a partial response, with 27.7% (39/141) demonstrating either a humoral response but no cellular response and 14.2% (20/141) having a cellular response in the absence of a humoral response. Variables associated with nonresponse (both humoral and cellular) were MMF use, lower lymphocyte count, and lower eGFR. These variables were also associated with the humoral response alone, as we have previously published.³ This indicates that the cellular response is strongly related to the humoral response. However, when we consider the cellular response alone, the use of calcineurin inhibitors seems to be the determining factor for cellular nonresponse and may therefore explain the partial response in these patients. The clinical consequences of these findings with regard to protection against SARS-CoV-2 infection and COVID-19 severity are currently unknown. However, in our study, we did not have enough cases and follow-up time to answer this question. This should therefore be the subject of future studies.

Lower levels of vaccination-induced neutralizing antibodies have been detected with new SARS-CoV-2 variants, like Omicron.³² An explanation may be that mutations in the S protein include alterations in the receptor binding domain, which can lead to partial escape from neutralizing antibodies. Conversely, memory T cells were shown to respond equally well to peptide pools based on Omicron Spike-sequences as to the original Wuhan-based vaccine-type peptide pools.³³ This may be explained by the fact that T cells only need a few amino acids to recognize the virus,

which offers a much greater potential for cross-reactivity.¹⁰ Given that patients with low antibody levels are presumably at increased risk of infection with SARS-CoV-2, the cellular response may be crucial in these individuals for limiting viral infection and reducing the severity of COVID-19 symptoms.

The current vaccination schedule in KTRs consists of 3 vaccinations plus additional boosters to optimize protection against SARS-CoV-2 variants of concern. Therefore, we also analyzed the cellular response in 20 KTRs without an antibody response after the second vaccination. Fourteen of the 20 seroconverted after the third vaccination. In contrast, the cellular response did not significantly increase after the third compared with the second vaccination, which is opposed to the results from other literature.^{34,35} This may be due to our limited number of subjects included for this subanalysis or may be caused by selection bias because these subjects were all antibody nonresponders before receiving a third vaccination. However, Mitchell et al also did not find an increase in the cellular response.³⁶ Interestingly, the T-cell response was significantly higher in individuals who seroconverted after a third vaccination. This indicates that, if an increase in immune response can be detected after repeated vaccination, this will apply to both the humoral and cellular response. This was also reported by Kamar et al after a fourth dose.³⁷

The main strengths of this prospective study are that we (1) included a relatively large group of patients with immunological follow-up for measurement of cellular response, (2) included a control cohort, and (3) used the highly sensitive IFN- γ ELISpot assay to measure T-cell activity. This study also has some limitations. Although we included a relatively large group of CKD G4/5 and dialysis patients, the results may not be representative for the entire kidney patient population. We excluded patients that use immunosuppressive agents for their kidney disease to exclusively study the effect of impaired kidney function or dialysis on the immunogenicity of COVID-19 vaccination. Furthermore, although highly sensitive, the IFN- γ ELISPOT assay cannot make a distinction between the type of T-cell response or other IFN- γ producing white blood cells. Lastly, this study did not include kidney patients that received a fourth or fifth vaccine dose, which is nowadays

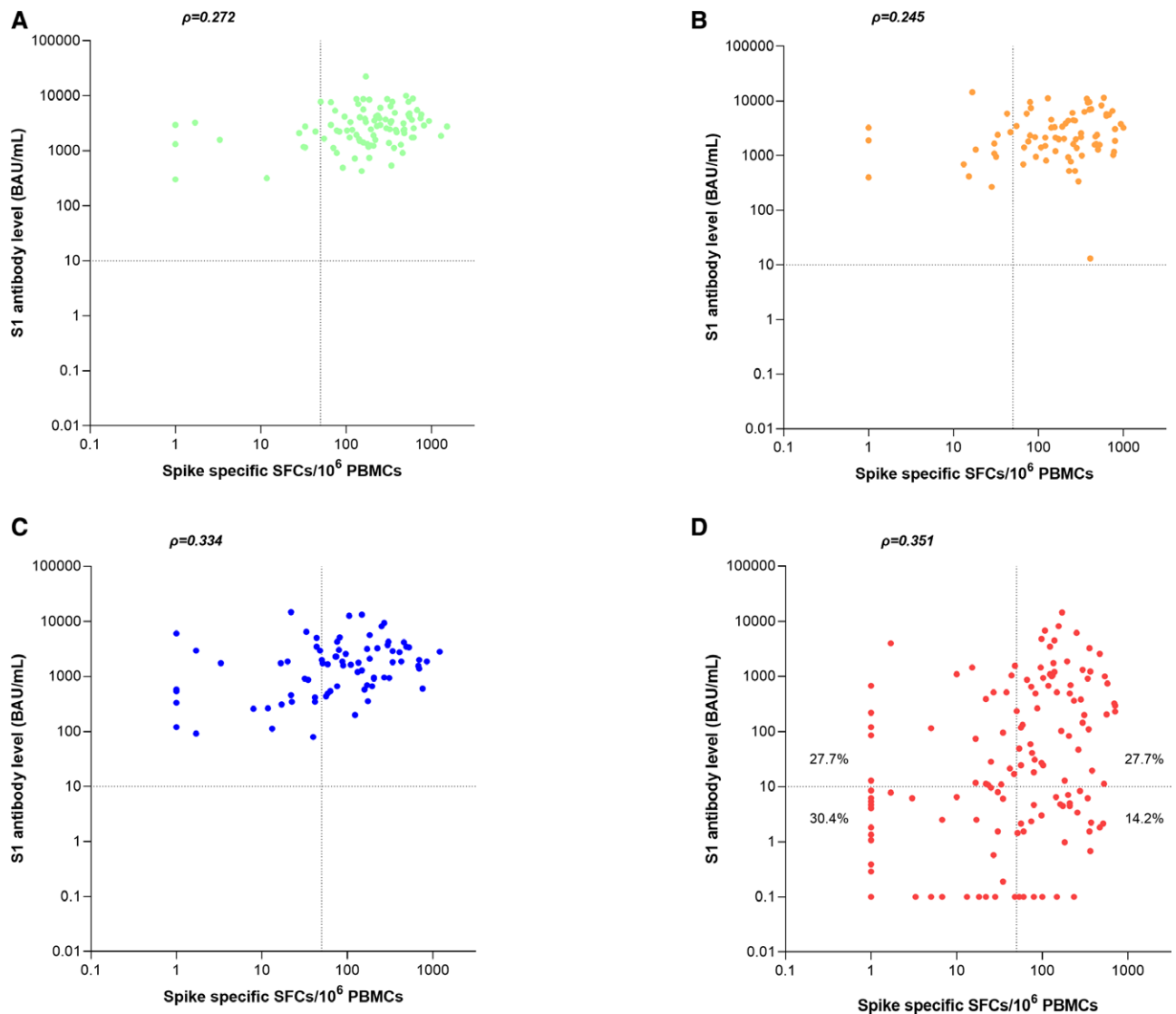


FIGURE 3. Scatter plot of the association between T-cell and antibody responses per study cohort at 28 d after second vaccination. Dotted vertical line indicates threshold for T-cell response (SFCs $\geq 50/10^6$ PBMCs) and dotted horizontal line the threshold for antibody response (S1 antibody level ≥ 10 BAU/mL). A, Scatter plot with Spearman correlation coefficient in the control cohort ($P = 0.009$). B, Scatter plot with Spearman correlation coefficient in the chronic kidney disease G4/5 cohort ($P = 0.027$). C, Scatter plot with Spearman correlation coefficient in the dialysis cohort ($P = 0.003$). D, Scatter plot with Spearman correlation coefficient in the kidney transplant recipient ($P < 0.001$). PBMC, peripheral blood mononuclear cell; SFC, spot-forming cell.

practice, and may therefore not be representative for immunogenicity against SARS-CoV-2 in patients at this moment. However, these data provide novel insights into immunogenicity after COVID-19 vaccination and can therefore aid in designing more effective vaccination strategies for other vaccinations or future emerging pathogens in this vulnerable patient group.

Our study provides insight into the cellular and humoral responses after COVID-19 vaccination in kidney patients, but it did not have enough power to analyze vaccine efficacy and therefore cannot provide conclusions about the protection against COVID-19. Future analyses are necessary to identify humoral and cellular immunological correlates of protection and to identify threshold values for clinical protection. Such information will be important to monitor COVID-19 immune status in at-risk populations and guide policy decisions on additional vaccinations.

In conclusion, we show that, besides an attenuated humoral response, dialysis patients and KTRs also have an inferior cellular response compared with controls after COVID-19 vaccination. This suggests that vaccination with mRNA-1273 is less effective in these patient groups, with humoral nonresponders also failing to mount an adequate cellular response. Of note, we also detected KTRs with a cellular response in whom we could not detect a humoral response. To what extent these T cells could offer protective immunity is unknown, and this study was not designed and did not have enough power to answer this question. However, as T cells have more potential for cross-reactivity against currently prevalent SARS-CoV-2 variants, the cellular response may be critical in preventing severe COVID-19 in patients with low antibody levels. Furthermore, these data are not only valuable for COVID-19 vaccines or mRNA platforms but could also be relevant for other vaccines or future emerging pathogens.

TABLE 4.**Baseline characteristics of kidney transplant recipients organized per quadrant in Figure 3D**

	A+ / T+ (n = 39)	A+ / T- (n = 39)	A- / T+ (n = 20)	A- / T- (n = 43)
Female, n (%)	18 (46.2)	16 (41.0)	12 (60.0)	22 (52.4)
Caucasian, n (%)	35 (89.7)	36 (92.3)	18 (90.0)	39 (92.9)
Age (y)	54.0 ± 13.4*	52.7 ± 14.8	58.6 ± 11.0	61.2 ± 9.3
BMI (kg/m ²)	27.9 ± 5.4	26.6 ± 5.5	26.9 ± 5.4	26.6 ± 4.8
SBP (mmHg)	141.5 ± 21.3	150.4 ± 22.8	146.4 ± 18.0	148.5 ± 18.0
DBP (mmHg)	85.8 ± 11.4	88.7 ± 12.4	86.9 ± 9.4	83.9 ± 8.7
Current smoking, n (%)	1 (2.6)	6 (15.4)	2 (10.0)	1 (2.4)
Current alcohol consumption, n (%)	13 (34.2)	16 (41.0)	6 (30.0)	19 (45.2)
Number of comorbidities	2 (1–2)	1 (1–2)	1 (1–2)	1 (1–2)
Comorbidities, n (%)				
Hypertension	29 (74.4)	29 (74.4)	20 (100.0)	33 (78.6)
Diabetes mellitus	9 (23.1)	5 (12.8)	4 (20.0)	12 (28.6)
History of coronary artery disease	5 (12.8)	6 (15.4)	2 (10.0)	7 (16.7)
Heart failure	2 (5.1)	2 (5.1)	1 (5.0)	2 (4.8)
Chronic lung disease	4 (10.3)	1 (2.6)	1 (5.0)	1 (2.4)
History of malignancy [†]	7 (17.9)	4 (10.3)	3 (15.0)	6 (14.3)
Auto-immune disease	2 (5.1)	3 (7.7)	0 (0.0)	1 (2.4)
Lymphocytes (10 ⁹ /L)	1.34 (1.0–2.4)*	1.56 (1.2–2.1)	1.17 (0.9–2.0)	0.97 (0.7–1.5)
eGFR (mL/min/1.73m ²)	56.0 ± 21.3*	52.6 ± 17.7	46.5 ± 16.5	42.6 ± 13.5
Primary renal diagnosis, n (%)				
Primary glomerulonephritis	3 (8.8)	10 (28.6)	4 (21.1)	7 (19.4)
Pyelonephritis	0 (0.0)	1 (2.9)	0 (0.0)	1 (2.8)
Interstitial nephritis	2 (5.9)	2 (5.7)	1 (5.3)	2 (5.6)
Familial/hereditary renal diseases	14 (41.2)	7 (20.0)	3 (15.8)	7 (19.4)
Congenital diseases	3 (8.8)	2 (5.7)	1 (5.3)	1 (2.8)
Vascular diseases	4 (11.8)	1 (2.9)	2 (10.5)	5 (13.9)
Secondary glomerular/systemic disease	3 (8.8)	1 (2.9)	0 (0.0)	3 (8.3)
Diabetic kidney disease	1 (2.9)	1 (2.9)	2 (10.5)	2 (5.6)
Other	3 (8.8)	9 (25.7)	3 (15.8)	7 (19.4)
Unknown	1 (2.9)	1 (2.9)	3 (15.8)	1 (2.8)
Transplant characteristics				
First kidney transplant, n (%)	31 (79.5)	31 (79.5)	14 (70.0)	31 (73.8)
Time after last transplantation (y)	11.0 (4.0–16.0)**	9.0 (5.0–17.0)	8.0 (2.3–11.0)	3.0 (0.0–6.3)
Last transplant				
Living, n (%)	29 (74.4)	27 (69.2)	15 (75.0)	27 (64.3)
Preemptive, n (%)	15 (38.5)	11 (28.2)	9 (45.0)	19 (45.2)
Number of immunosuppressive agents	2 (2–3)	3 (2–3)	3 (2–3)	3 (2–3)
Immunosuppressive treatment, n (%)				
Steroids	29 (74.4)	35 (89.7)	14 (70.0)	27 (64.3)
Azathioprine	9 (23.1)*	12 (30.8)	0 (0.0)	1 (2.4)
Mycophenolate mofetil	19 (48.7)**	19 (48.7)	19 (95.0)	38 (90.5)
Calcineurin inhibitor	27 (69.2)*	33 (84.6)	16 (80.0)	40 (95.2)
mTOR inhibitor	6 (15.4)*	0 (0.0)	1 (5.0)	0 (0.0)
Other	0 (0.0)	0 (0.0)	0 (0.0)	2 (4.8)
Induction with rituximab last year, n (%)	1 (2.6)	0 (0.0)	1 (5.0)	0 (0.0)

+ = responder; – = non-responder; A, antibody; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; mTOR, mammalian target of rapamycin; SBP, systolic blood pressure; T, T-cell.

Variables are presented as mean ± SD or as median (IQR) in case of nonnormal distribution.

[†]Including melanomas, excluding all other skin malignancies.

**P* < 0.05.

***P* < 0.001.

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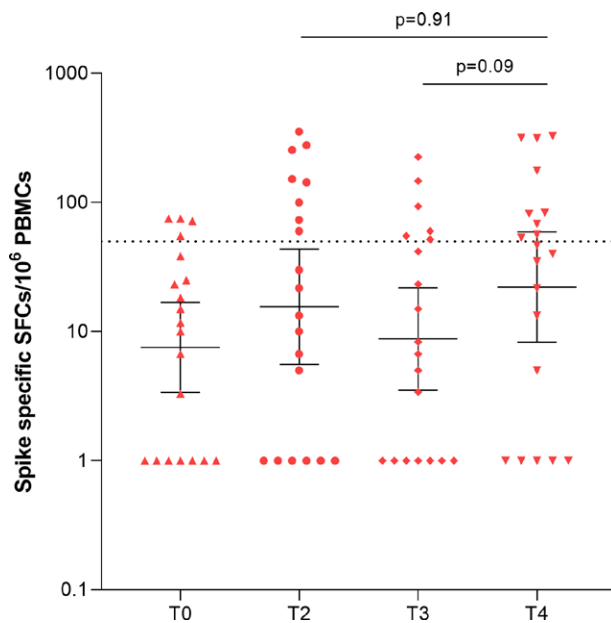


FIGURE 4. Dot plot of SARS-CoV-2 spike-specific T-cell response in kidney transplant recipients after first vaccination series and after third vaccination. *P* values were calculated using the Wilcoxon signed rank test. Dotted horizontal line indicates threshold for T-cell response (SFCs $\geq 50/10^6$ PBMCs). PBMC, peripheral blood mononuclear cell; SFC, spot-forming cell; T0, baseline; T2, 28 d after second vaccination; T3, 6 mo after second vaccination; T4, 28 d after third vaccination.

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