



Antibody and T Cell Response to SARS-CoV-2 Messenger RNA BNT162b2 Vaccine in Kidney Transplant Recipients and Hemodialysis Patients

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ABSTRACT

Background Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is associated with a high rate of mortality in patients with ESKD, and vaccination is hoped to prevent infection.

Methods Between January 18 and February 24, 2021, 225 kidney transplant recipients (KTRs) and 45 patients on hemodialysis (HDPs) received two injections of mRNA BNT162b2 vaccine. The postvaccinal humoral and cellular response was explored in the first 45 KTRs and ten HDPs.

Results After the second dose, eight HDPs (88.9%) and eight KTRs (17.8%) developed anti-spike SARS-CoV-2 antibodies ($P < 0.001$). Median titers of antibodies in responders were 1052 AU/ml (IQR, 515–2689) in HDPs and 671 AU/ml (IQR, 172–1523) in KTRs ($P = 0.40$). Nine HDPs (100%) and 26 KTRs (57.8%) showed a specific T cell response ($P = 0.06$) after the second injection. In responders, median numbers of spike-reactive T cells were 305 SFCs per 10^6 CD3+ T cells (IQR, 95–947) in HDPs and 212 SFCs per 10^6 CD3+ T cells (IQR, 61–330) in KTRs ($P = 0.40$). In KTRs, the immune response to BNT162b2 seemed influenced by the immunosuppressive regimen, particularly tacrolimus or belatacept.

Conclusion Immunization with BNT162b2 seems more efficient in HDPs, indicating that vaccination should be highly recommended in these patients awaiting a transplant. However, the current vaccinal strategy for KTRs may not provide effective protection against COVID-19 and will likely need to be improved.

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Pandemic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been particularly deleterious in kidney transplant recipients (KTRs)^{1–3} and in patients with ESKD⁴ because of the severity of the disease and the high rate of morbidity and mortality. Moreover, the kidney transplantation activity has been highly

affected by the pandemic.^{5,6} In order to protect these populations, SARS-CoV-2 vaccination is recommended through international guidelines.^{7,8} However, KTRs and patients on dialysis are considered low responders to vaccines⁹ and were not included in SARS-CoV-2 preauthorization vaccine clinical trials.

A low immunization after a first of an mRNA coronavirus disease 2019 (COVID-19) vaccine in solid organ transplant recipients has recently been reported.¹⁰ Evaluation of the humoral response to a vaccine readily evaluates its efficacy. However, in a population known to have lower seroconversion rates than the general nonimmunosuppressed population, the measurement of the cellular immune response could be particularly helpful and relevant.

We thus aimed in this study to explore the immunogenicity of mRNA BNT162b2 vaccine after the first and second doses not only by measuring vaccine-induced antibodies but also, by evaluating anti-SARS-CoV-2 spike-specific T cell response.

Between January 18 and February 3, 2021, 225 KTRs and 45 patients on hemodialysis received the first injection of the Pfizer SARS-CoV-2 mRNA BNT162b2 vaccine, and 3 weeks later, they received the second injection. Blood samples were collected from the first 45 KTRs (20%) and ten patients on hemodialysis

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(22.2%), and they were explored for both humoral and cellular immune response on the day of the second injection and 1 month later. Data were retrospectively analyzed. This retrospective study was submitted to the approbation of the Rouen Centre Institutional Review Board.

The anti-SARS-CoV-2 postvaccinal antibody response against the spike protein was assessed using the ARCHITECT IgG II Quant test (Abbott), with titers >50 arbitrary units (AU) per milliliter being considered as positive (detection range: 6.8–40,000 AU/ml; positive agreement, 99.4%; negative agreement, 99.6%).

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation of blood samples and used immediately. PBMCs (in concentrations adjusted to 2×10^5 CD3+ T cells per well) were plated in anti-IFN γ -coated ELISPOT 96-well plates in the presence of overlapping 15-mer peptide pools spanning the sequence of SARS-CoV-2 spike protein S (pool S1 spanning the N-terminal part of the protein including the S1-subunit and pool S2 spanning the C-terminal part) as well as N, M, ORF3A, and ORF7A in order to detect a potential exposition to SARS-CoV-2 (JPT, Strassberg, Germany). Negative and positive control stimulation, respectively, medium only and CEFX (a pool of 176 known peptides from various infectious agents (JPT)) were included in the assay. After an overnight culture, cells were washed, and captured IFN γ was revealed using a colorimetric assay (UCytech, Utrecht, The Netherlands). Spots were counted with an automated ELISPOT reader (AID, Strassberg, Germany). For each stimulation condition, the average spot number observed in wells without antigen was subtracted. Results were expressed as spot-forming cells (SFCs) per 10^6 CD3+ T cells. For each assay, a specific response was considered positive if the SFC number was superior to three SDs of spot numbers observed in wells without antigens (ranging between nine and 20 SFCs per 10^6 CD3+ T cells).¹¹

Quantitative data are presented as mean (SD) or median (interquartile range [IQR]). Qualitative data are

presented as percentages. The nonparametric Wilcoxon and Mann-Whitney tests were used to compare characteristics between groups with StatView version 5.0 (SAS Institute).

Baseline characteristics of the 45 KTRs are described in Table 1. No patient developed a SARS-CoV-2 infection for up to 1 month after the second injection.

Regarding patients on hemodialysis, mean age was 71.2 ± 16.4 years. Their median duration of chronic dialysis was 3.14 years (0.6–12.6). One patient on hemodialysis experienced a SARS-CoV-2 infection 3 days after the second injection of vaccine. She was maintained at home until recovery. She was excluded from the analysis after the second dose.

Three weeks after the first injection, only one patient on hemodialysis (11.1%) and one KTR (2.2%) developed anti-SARS-CoV-2 antibodies ($P=0.19$) (Figure 1A). Antibody titers in responders were 178.9 AU/ml in patients on hemodialysis and 311 AU/ml in KTRs. One month after the second injection, eight patients on hemodialysis (88.9%) and eight KTRs (17.8%) developed anti-SARS-CoV-2 antibodies ($P<0.001$). Median antibody titers in responders were 1052 AU/ml (IQR, 515–2689) in patients on hemodialysis and 671 AU/ml (IQR, 172–1523) in KTRs ($P=0.40$).

The single patient tested positive for SARS-CoV-2, developed after the first significant antibody titer (titer: 161 AU/ml), that dramatically increased after the second injection and COVID-19 (titer: 53,737.6 AU/ml).

Three weeks after the first injection, five patients on hemodialysis (55.6%) and 11 KTRs (24.4%) displayed a significant number of spike-reactive T cells ($P=0.06$) (Figure 1B). In responders, median numbers of specific T cells were 208 SFCs per 10^6 CD3+ T cells (IQR, 65–315) in patients on hemodialysis and 45 SFCs per 10^6 CD3+ T cells (IQR, 35–55) in KTRs ($P=0.02$). No response to N, M, ORF3A, and ORF7A was evidenced in KTRs and patients on hemodialysis, excluding a potential exposition to SARS-CoV-2.

One month after the second injection, a specific T cell response was detected in

Significance Statement

Antibody and T cell responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mRNA vaccines are poorly reported in kidney transplant recipients (KTRs) and patients on hemodialysis (HDPs). The authors investigated the response to BNT162b2 vaccine in 45 KTRs and ten HDPs. After the second dose, 88.9% of HDPs and only 17.8% of KTRs developed anti-SARS-CoV-2 antibodies. A specific T cell response was induced in 100% of HDPs and 57.8% of KTRs. The immune response seemed influenced by the immunosuppressive regimen in KTRs, particularly tacrolimus and belatacept. These results could help to better define the strategy of vaccination in this immunocompromised population.

nine patients on hemodialysis (100%) and 26 KTRs (57.8%; $P=0.06$). In responders, median numbers of spike-reactive T cells were 305 SFCs per 10^6 CD3+ T cells (IQR, 95–947) in patients on hemodialysis and 212 SFCs per 10^6 CD3+ T cells (IQR, 61–330) in KTRs ($P=0.40$).

In the patient who tested positive for SARS-CoV-2, spike-specific T cell numbers were 155 SFCs per 10^6 CD3+ 3 weeks after the first injection and 3245 SFCs per 10^6 CD3+ after the second injection and SARS-CoV-2 infection. In this case, T cell responses to N, M, ORF3A, and ORF7A were detected.

Baseline characteristics of the 45 KTRs according to the baseline immunosuppressive regimen are described in Table 2.

One month after the second injection, two KTRs (8.3%) developed anti-SARS-CoV-2 antibodies in group 1, zero KTRs developed anti-SARS-CoV-2 antibodies in group 2, and six KTRs (54.5%) developed anti-SARS-CoV-2 antibodies in group 3 (group 1 versus group 2, $P=0.34$; group 2 versus group 3, $P=0.005$; group 1 versus group 3, $P=0.002$) (Figure 2A).

One month after the second injection, 12 KTRs (50%) in group 1, four KTRs (40%) in group 2, and ten KTRs (90.9%) in group 3 displayed a specific T cell response (group 1 versus group 2, $P=0.40$; group 2 versus group 3, $P=0.01$; group 1 versus group 3,

Table 1. Baseline characteristics of KTRs explored

Baseline Characteristics	KTRs, n=45
Age, yr	63.5±16.3
Sex (M/W), n	23/22
Diabetes mellitus, n (%)	10 (22.2)
Hypertension, n (%)	36 (80)
BMI, kg/m ²	26.2±4.7
Time from transplantation, yr	
Median (range)	6.9 (0.22–30.2)
Immunized KTR, n (%)	12 (26.7)
Median PRA class I	6
Median PRA class II	14.5
Previous history of rejection, n (%)	1 (2.2)
eGFR, ml/min per 1.73 m ²	43.3±15.7
P/C ratio, g/g	0.26±0.06
Lymphocytes count, per mm ³	
CD3+	892±476
CD4+	447±263
CD8+	397±297
Induction therapy for KT, n (%)	
ATG	18 (40)
Anti-R-IL2	27 (60)
IS regimen, n (%)	
Tacrolimus	24 (53.3)
Cyclosporin	8 (17.8)
MMF	37 (82.2)
AZA	4 (8.9)
Everolimus	3 (6.7)
Belatacept	10 (22.2)
Steroids	21 (46.7)

M, men; W, women; BMI, body mass index; PRA, panel reactive antibodies; P/C, proteinuria/creatininuria; KT, kidney transplantation; ATG, antithymoglobulin; R-IL2, IL-2 receptor; IS, immunosuppressive; MMF, mycophenolate mofetil; AZA, azathioprine.

$P=0.02$). (Figure 2B). Median numbers of SARS-CoV-2-reactive T cells, after the second dose, were 160 SFCs per 10^6 CD3+ T cells (IQR, 46–303) in group 1, 42.5 SFCs per 10^6 CD3+ T cells (IQR, 35–69) in group 2, and 298 SFCs per 10^6 CD3+ T cells (IQR, 240–475) in group 3 (group 1 versus group 2, $P=0.05$; group 2 versus group 3, $P=0.005$; group 1 versus group 3, $P=0.01$).

In univariate analysis, predictive factors for a positive antibody response were the duration of kidney transplantation ($P=0.003$) and a cyclosporin-based immunosuppressive regimen ($P<0.001$). No factor predictive of a significant T cell response was identified. T cell counts were not associated with the detection or the magnitude of the antibody but were

associated with T cell response to the vaccine in univariate analysis ($P=0.01$ for CD3, $P=0.05$ for CD4, and $P=0.03$ for CD8).

We report here the anti-SARS-CoV-2 antibody and T cell responses after two doses of the mRNA vaccine BNT162b2 in a cohort of KTRs and patients on hemodialysis. We demonstrate that an antibody response is scarcely induced in immunocompromised KTRs after a first vaccine dose and in only 17.8% of them after the second dose. Induction of an antispikes T cell-specific response occurs more frequently in 51.1% of KTRs after the second injection. By contrast, in patients on hemodialysis, specific humoral and cellular responses are observed in 88.9% and 100%, respectively, of patients after the second dose. These results contrast with the robust and early induced immunity observed during mRNA vaccine trials, showing 100% antispikes seroconversion after vaccination with mRNA-1273¹² or BNT162b2.¹³ Narasimhan *et al.*¹⁴ observed after the first vaccine dose in a naïve population, using the same serologic assay, median IgG titers of 2217 AU/ml (95% confidence interval, 0 to 44,182) that following the booster dose, dramatically increased 8.2-fold to 18,272 AU/ml (at 98% confidence interval, 11,724 to 21,750; $P<0.001$). Specific IgG titers found in our patients on hemodialysis and KTRs were significantly lower. Using a similar IFN γ ELISPOT assay, Angyal *et al.*¹⁵(preprint)

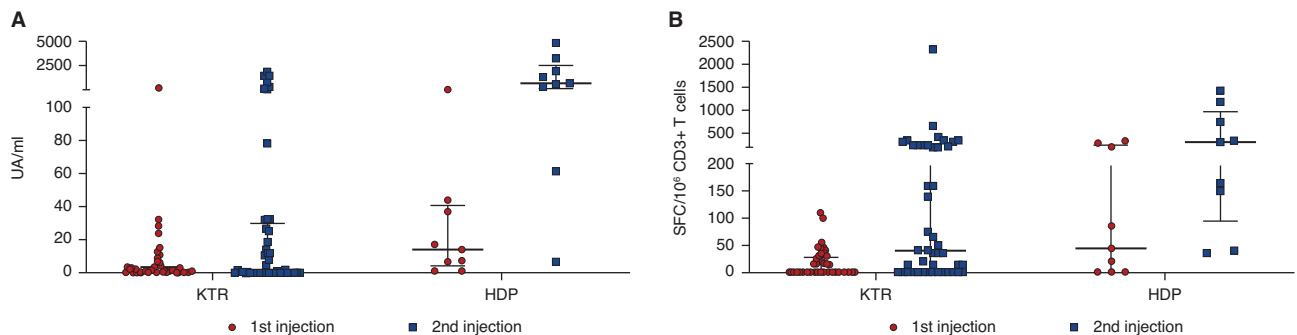


Figure 1. (A) SARS-CoV-2 antispikes antibody response and (B) SARS-CoV-2-reactive IFN γ -producing T cells in KTRs and patients on hemodialysis (HDPs) following the first and second injections of the SARS-CoV-2 mRNA BNT162b2 vaccine. Titers of S IgG are shown in the samplings of 45 KTRs and nine HDPs. Medians and IQRs are shown. Numbers of T cells (expressed as SFCs per 10^6 CD3+ T cells) reactive to overlapping peptide pools spanning SARS-CoV-2 structural protein S (pools S1 and S2) in 45 KTR and nine HDPs are shown. Medians and IQRs are shown. UA, arbitrary units.

Table 2. Baseline characteristics of KTRs explored according to the immunosuppressive regimen

Baseline Characteristics	Group 1: Tacrolimus-Based IS Regimen, n=24	P Value ^a	Group 2: Belatacept-Based IS Regimen, n=10	P Value ^b	Group 3: Nontacrolimus- and Nonbelatacept-Based IS Regimen, n=11	P Value ^c
Age, yr	60.2±17.4	0.21	68.1±13.7	0.82	66.6±15.5	0.29
Sex (M/W), n	12/12	0.59	6/4	0.51	5/6	
Time from transplantation, yr	6.1±4.9	0.92	6.3±4.7	0.001	17.1±7.9	<0.001
eGFR, ml/min per 1.73 m ²	45.6±13.7	0.005	30.4±11.6	0.007	50.1±17.4	0.41
Induction therapy for KT, n (%)						
ATG	11 (45.8)	0.16	2 (20)	0.21	5 (45.5)	
Anti-R-IL2	13 (54.2)		8 (80)		6 (54.5)	
IS regimen, n (%)						
Tacrolimus	24 (100)	<0.001	0	<0.001	0	<0.001
C0 tacrolimus	6.1±1.8	0.007	—	0.54	—	<0.001
Cyclosporin	0	0.78	0	0.69	8 (72.7)	0.05
C0 cyclosporin	—	0.14	—	0.48	67.2±15.3	0.26
MMF	23 (95.8)	0.12	6 (60)	0.60	8 (72.7)	0.56
MMF median of dosage, mg	1000	<0.01	625	<0.001	1000	0.03
AZA	1 (4.2)	0.13	2 (20)	0.12	1 (9.1)	0.77
Everolimus	0		1 (10)		2 (18.2)	
Belatacept	0		10 (100)		0	
Steroids	10 (41.7)		7 (70)		4 (36.4)	

IS, immunosuppressive; M, men; W, women; KT, kidney transplantation; ATG, antithymoglobuline; R-IL2, IL-2 receptor; C0, through level; MMF, mycophenolate mofetil; AZA, azathioprine.

^aComparison between groups 1 and 2.

^bComparison between groups 2 and 3.

^cComparison between groups 1 and 3.

recently reported (in SARS-CoV-2-naïve health care workers) median numbers of spike-specific T cells of 58 SFUs per 10⁶ PBMCs (IQR, 29–146) after a single dose of BNT162b2 and 165 SFUs per 10⁶ PBMCs (IQR, 101–277) after two doses. These values are in the same range as those observed in our study in KTRs and patients on hemodialysis, especially after the second dose. Thus, postvaccinal T cell immunity in KTRs and patients on

hemodialysis seems comparable with that of healthy naïve subjects.

Data regarding vaccination in dialysis are very scarce. Seroconversion rates after influenza vaccine administration in such patients vary in the literature, ranging from 33% to 80%.^{16–18} Regarding the protection from SARS-CoV-2 of patients on dialysis, among 31 waitlisted patients with ESKD, 87% of them mounted an antibody response after a first vaccine dose (*P*=0.05).¹⁹

Grupper *et al.*²⁰ reported very recently that most of 56 patients on hemodialysis (96%) developed specific antibodies following full BNT162b2 vaccination but with significantly lower titers than controls. Our data are in line with these results and extend them by showing effective induction of both humoral and T cell responses after two doses of the BNT162b2 vaccine.

In KTRs, vaccinal responses are expected to be impaired, particularly

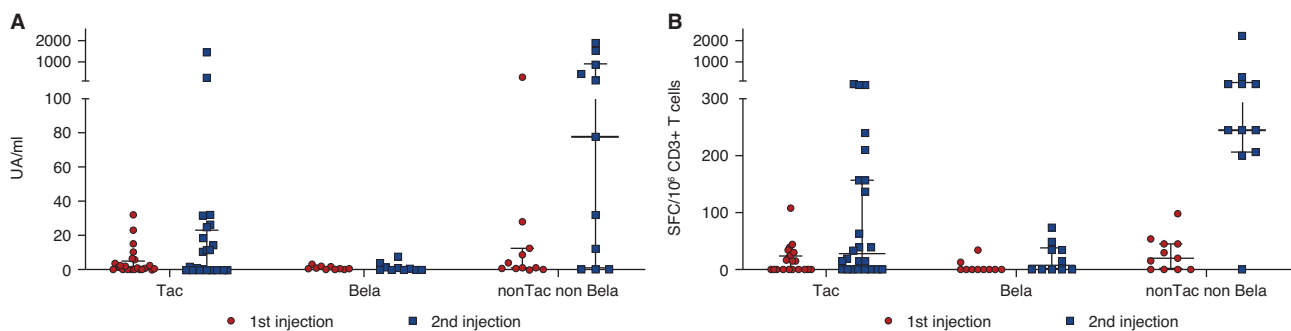


Figure 2. (A) SARS-CoV-2 antispike antibody response and (B) SARS-CoV-2-reactive IFN γ -producing T cells in KTRs following the first and second injections of the SARS-CoV-2 mRNA BNT162b2 vaccine according to the immunosuppressive regimen. Numbers of T cells (expressed as SFCs per 10⁶ CD3+ T cells) reactive to overlapping peptide pools spanning SARS-CoV-2 structural protein S (pools S1 and S2) in the samplings of 45 KTRs are shown. Median and IQR are shown. Bela, belatacept; Tac, tacrolimus. UA, arbitrary units.

early post-transplantation, after treatment for rejection or rituximab therapy.^{21–23} KTRs of 65 years and older on ≥ 2 g daily mycophenolate mofetil generally have reduced humoral responses to influenza vaccines.²⁴ Regarding the efficacy of the SARS-CoV-2 mRNA vaccine in KTRs, Boyarsky *et al.*¹⁰ reported that 82.6% of transplant recipients ($n=436$) did not mount significant anti-spike antibody titers after a first dose of mRNA vaccine. In the same vein, Benotmane *et al.*²⁵ showed that only 10.8% of KTRs had a positive serology 28 days after the first injection of the mRNA-1273 vaccine. The median IgG titer was 224 AU/ml (IQR, 76 – 496 AU/ml). Similarly, Yi *et al.*¹⁹ reported a seroconversion rate of only 6.2% of KTRs after the first mRNA vaccine dose. Our results are thus in line with these studies, showing in addition that after the second vaccine dose, the seroconversion rate is only modestly increased (17.8%). Furthermore, they show that despite a low seroconversion rate, an anti-spike-specific T cell response is triggered in half of the patients after two vaccine doses. Presence of anti-spike T cells in absence of specific antibodies could provide some level of protection from SARS-CoV-2 infection by limiting the extent of viral replication, as reported in the context of CMV infection in KTRs.^{26,27}

Our results suggest that the immune response to the BNT262b2 vaccine is essentially influenced by the intensity of the immunosuppressive regimen. Belatacept-treated patients were indeed the worst responders, developing no antibodies and no or only a few specific T cells. Belatacept is in fact suspected to be associated with an increase in the incidence of opportunistic infections^{28,29} and CMV disease.³⁰ Tacrolimus-treated patients also responded weakly to vaccination, although significant T cell numbers were induced in some of them. It should be noted that the majority of our patients were on mycophenolate mofetil, which may have contributed to impairing post-vaccine antibody responses.³¹

In conclusion, the mRNA BNT162b2 vaccine seems efficient in patients on hemodialysis, indicating that vaccination

should be highly recommended in these patients. By contrast, the low seroconversion rate observed in KTRs is worrying. In seronegative patients displaying significant numbers of anti-spike T cells, a third dose of vaccine might trigger a humoral response. However, in patients failing to generate any response, should we prefer standard adjuvanted vaccines or adenovirus-based vaccines? Alternatively, should immunizing household members and close contacts be the priority? Postvaccination COVID-19 incidence data in KTRs should provide answers to these questions.

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