Humoral Response of Renal Transplant Recipients to the BNT162b2 SARS-CoV-2 mRNA Vaccine Using Both RBD IgG and Neutralizing Antibodies

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Background. Data about SARS-CoV-2 vaccines efficacy in renal transplant recipients (RTR) are lacking. **Methods.** To reveal predictors for humoral response to BNT162b2 vaccine among RTR, patients were divided into positive (N = 42) and negative (N = 78) response groups based on receptor-binding domain (RBD) immunoglobulin G (IgG) ≥1.1 and neutralizing antibodies (NA) ≥16 dilution versus RBD IgG <1.1 or NA <16, respectively. NA were detected using a SARS-CoV-2 pseudovirus. **Results.** NA were detected in only 42 of 120 (35%) of RTR versus 197 of 202 (97.5%) immunocompetent controls ($P < 0.001$). NA geometric mean titers in RTR were significantly lower versus the control group {83.7 (95% confidence interval [CI], 50.5-138.8) versus 482 (95% CI, 411-566), $P < 0.001$}. In a multivariable analysis, mycophenolic acid (MPA) dose and hemoglobin level were found to be independent predictors for antibody response in RTR. A positive response rate of 27% versus 63% was observed in patients on and off MPA, respectively. An increase in MPA dose by 1 mg/kg weight reduced the odds for a positive response by 17% (odds ratio = 0.83; 95% CI, 0.75-0.92; $P < 0.001$). Geometric mean titers for RBD IgG were significantly reduced as MPA daily dose increased. Hemoglobin blood level <13 g/dL reduced the antibody response by 63% ($P = 0.04$). Pain at the injection site after the second vaccine dose was significantly higher in the responders versus nonresponders (20.5% versus 5.5%, $P = 0.01$). **Conclusions.** Only 35% of RTR develop NA to the BNT162b2 mRNA vaccine. MPA is a major suppressor of antibody response in RTR.

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INTRODUCTION

Transplant recipients are at an increased risk of complications from COVID-19 because of their chronic immunosuppression and other comorbidities,[1-8] but the exact role of immunosuppression in COVID-19 modulation is unclear and whether immunosuppression has beneficial impact on outcomes is still subject to debate. Chronic immunosuppressive treatment could decrease the severity of the cytokine storm, while withdrawal or a significant reduction of immunosuppression could exacerbate inflammation, aggravate the clinical picture, and lead to long-term effects including precipitation of de novo donor-specific antibodies and/or subclinical rejection.[9] In contrast, continuation of immunosuppressive treatment could decrease the ability to mount an antibody response to COVID-19.[10]

Tremendous efforts on vaccine development yielded effective vaccines utilizing mRNA, nonreplicating viral vector, or protein-based vaccines that do not pose a known risk to immunosuppressed patients as opposed to live attenuated vaccines. The American Society of Transplantation recommends that all transplant candidates and their household members should receive a SARS-CoV-2 vaccination, ideally >2 wk before transplantation or 1–6 mo after transplantation.[11] The Israeli Society of Transplantation has approved the vaccination of solid organ transplant (SOT) recipients, except those treated recently with anti-CD20 (rituximab) and anti-thymocyte globulin as early as 1 mo posttransplant. Results of the Pfizer-BioNTech vaccine clinical trials have shown that the vaccine exhibits 95% efficacy in preventing symptomatic

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laboratory-confirmed COVID-19, but the trials excluded immunocompromised patients.[12]

The COVID-19 vaccination campaign in Israel was initiated on December 19, 2020, with BNT162b2 mRNA vaccine being the only vaccine administered. In this study, we analyzed the receptor-binding domain (RBD) immunoglobulin G (IgG) and neutralizing antibodies (NA) responses to the BNT162b2 vaccine in 120 renal transplant recipients (RTR) with the aim to reveal predictors for the humoral response and to focus specifically on the type and strength of immunosuppressive therapy as a potential inhibitor of an appropriate antibody response to the vaccine. We also characterized adverse events (AEs) following the vaccination in our RTR population.

MATERIALS AND METHODS

Study Population

One hundred twenty stable RTR who had received 2 doses of the BNT162b2 vaccine were tested for antibodies 2–4 wk following the second vaccine dose. Patients with a positive SARS-CoV-2 polymerase chain reaction test before or after the first vaccination and during the first week after the second vaccination were excluded from the study. Vaccination was avoided during the first month following transplantation and during active treatment for rejection. For RTR treated with anti-thymocyte globulin and/or rituximab around the time of the transplantation, vaccination was postponed to ≥3 mo posttransplant. On the day of antibody testing, blood was drawn for complete blood count, blood chemistry, and tacrolimus or cyclosporine trough levels. A control group included 202 immunocompetent healthcare workers who were also tested for antibodies 2–4 wk following the second dose of the BNT162b2 vaccine. Written informed consent was obtained from all participants. The protocol and informed consent were approved by our Institutional Review Board (7982-20-SMC).

Immunosuppression

In our RTR clinic, maintenance immunosuppression includes a calcineurin inhibitor (usually tacrolimus), an anti-metabolite, usually a mycophenolate-based drug (mainly mycophenolic acid [MPA]), and prednisone. In patients with a low immunologic risk for rejection, an early steroid withdrawal protocol is implemented, with steroid discontinuation between the fifth and eighth days posttransplant. These patients are maintained on 2 drugs, usually tacrolimus and MPA. Conversion to mammalian target of rapamycin inhibitors (sirolimus or everolimus) is performed according to the patient's risk of malignancy and intolerance to calcineurin inhibitors.

Primary Outcome

A positive response to the BNT162b2 vaccine was defined as RBD IgG ≥1.1 and the presence of NA capable of reducing viral replication by 50% at a 16-fold dilution or above.

Data Extraction and Study Assessments

The following information was extracted from electronic patient records: age, gender, cause of end-stage renal disease, dialysis pretransplant (yes/no), time on dialysis pretransplant, transplant number, donor type, transplant date and relevant medical history, specifically a history of hypertension (HTN), congestive heart failure (CHF), ischemic heart disease, or pretransplant diabetes.

The following biochemical parameters that were recorded on the day of antibody testing were retrieved in an automated fashion from MDClone, a data acquisition system at Sheba Medical Center: serum creatinine, tacrolimus or cyclosporine trough blood levels, total white blood cell count, absolute lymphocyte and neutrophil counts, hemoglobin, glucose, globulins, albumin, platelet count, and C-reactive protein. The following additional clinical and biochemical information was also retrieved from MDClone: average systolic and diastolic blood pressures in the 3 mo before the antibody testing, weight and body mass index on the day of the antibody testing, average tacrolimus trough blood levels in the 2 wk and 1 mo before antibody testing, and average HbA_{1C} level in the 6 mo before antibody testing. Total daily dose on the testing day for the following medications was automatically obtained from MDClone: tacrolimus, cyclosporine, prednisone, azathioprine, rapamycin, everolimus, and MPA or mycophenolate (for 23 patients, total daily mycophenolate dose was converted to the equivalent MPA dose by dividing mycophenolate dose by 1.388).

AEs obtained using a specific questionnaire included local reactions (pain at injection site, redness, and swelling) and systemic reactions (fever, fatigue, headache, myalgia, chills, nausea/vomiting, and paresthesia) within 30 d after each dose. Patients were instructed to report any suspected AE and were actively screened for any other systemic and local complaints.

Antibody Detection Assays

Samples from vaccinated RTR and controls were evaluated with an ELISA (ELISA) that detects IgG antibodies against the RBD of SARS-CoV-2. Briefly, a 96 well microtiter Polysorb plate (Nunc, Thermo, Denmark) coated overnight with 1 μg/mL of RBD antigen was blocked with 5% skimmed milk at 25°C for 60 min and human serum samples (diluted 1:100 with 3% skimmed milk) were added to antigen-coated wells. Following incubation at 25°C for 120 min and incubation for 60 min after the addition of horseradish peroxidase-conjugated anti-human IgG horseradish peroxidase conjugate (Jackson ImmunoResearch, PA; Code: 109-035-129) (diluted 1:15 000) TMB substrate was added followed by stop solution (1M HCl) and the OD of each well was measured at 450 nm. Cutoff values for a positive result were set as the mean +3 SD of negative control sera (n = 100). ELISA index value was defined as the ratio between sample and cutoff ODs. Based on our previous studies[13,14] titers ≥1.1 were defined as positive. A SARS-CoV-2 pseudo-virus neutralization assay was performed using a propagation-competent vesicular stomatitis virus spike, which was kindly provided by Gert Zimmer, University of Bern, Switzerland. Following titration, 100 focus forming units of pseudo-SARS-2 were incubated with a 2-fold serial dilution of heat-inactivated (56°C for 30 min) serum. Following incubation, the virus/serum mixture was transferred to Vero E6 cells and incubated for 90 min at 37°C. Following an additional 24 h of incubation, a 50% reduction in the plaque titer was calculated by counting green fluorescent foci under a fluorescence microscope (EVOS M5000; Invitrogen). Sera not capable of reducing viral replication by 50% at a 1

in 8 dilutions or below were considered non-neutralizing. During the validation of the neutralizing assay at our lab, we found that RBD IgG negative samples are non-neutralizing. Therefore, only samples that were positive for RBD-IgG were tested for NA.

Statistical Analysis

Descriptive statistics were expressed as percentages for categorical data or mean ± SD for continuous variables. Differences in baseline characteristics between the groups were tested using $\chi 2$ for the categorical variables or t-test for the continuous variables.

Multivariable logistic regression analysis was used to identify factors associated with the vaccine-induced antibody response in the entire cohort (RTR and immunocompetent controls). To analyze the association between the antibody response and immunosuppressive therapy for other clinical and laboratory variables, a multivariable logistic regression analysis was constructed with a positive antibody response as the dependent variable, while adjusting for potential confounders. The variables used in the multivariate analysis were those with a $P < 0.15$ in the univariate analysis and those of clinical and biological relevance. Results are presented as odds ratio (OR), 95% confidence intervals (CIs), and *P*. The correlation between IgG and log-transformed NA was analyzed using Spearman's correlation by 2-tailed parametric t-test means with 95% CIs.

All data analyses were performed with the SAS 9.4 software (Cary, NC). Scatter plots of log-transformed IgG and NA were obtained using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA). A $P < 0.05$ was considered statistically significant.

RESULTS

Cohort Characteristics

The first 120 RTR who received the BNT162b2 mRNA vaccine comprised our study cohort. Mean age was 59.7 ± 13 y (range, 26–84 y); 94 (80%) were males; and mean body mass index was 27 ± 5 kg/m^2. Mean time from transplant was 5.8 ± 6.3 y. Of the RTR, 80% had received a living donor transplant; 60.8% had undergone hemodialysis pretransplant for a mean of 2.4 ± 2.4 y; and 77.5%, 37.5%, 10.8%, and 6.7% had HTN, diabetes, ischemic heart disease, and CHF, respectively (Table 1). Of the patients, 92.5% were treated with a calcineurin inhibitor (85.8% with tacrolimus and 6.7% with cyclosporine), 77.5% with MPA, and 79.2% with prednisone. Most patients, 58 (48.3%), were treated with the combination tacrolimus–MPA–prednisone immunosuppression regimen, while the protocol for 21 (17.5%) patients consisted only of tacrolimus and MPA (Table 2).

Mean time from the second vaccine to antibody testing was 26.7 ± 13.3 d. Fifty-two (43.3%) recipients had RBD IgG ≥1.1. Ten of these 52 recipients tested positive for RBD IgG but, with a low mean RBD IgG of 1.74, did not develop NA and were therefore considered as nonresponders. Based on the 2 criteria—RBD IgG and NA—our RTR cohort included 42 patients (35%) in the positive response group (RBD IgG ≥ 1.1 and NA ≥ 16) and 78 (65%) in the negative response group (RBD IgG < 1.1 or NA < 16). We found a significant correlation ($r = 0.934$) between RBD IgG and NA (Figure 1).

The characteristics of the control group of 202 immunocompetent healthcare workers are shown in Table 3.

Response to BNT162b2 Vaccine in RTR Versus Immunocompetent Vaccinated Population

RBD IgG were detected in 199 (98.5%) of controls compared to 52 (43.4%) of RTR ($P < 0.001$). RBD IgG geometric mean titers in the control group was 6.02 (95% CI, 5.7-6.42) compared to 0.93 (95% CI, 0.76-1.15) in the RTR group ($P < 0.001$). NA were detected in 197 of 202 (97.5%) of the controls versus 42 of 120 (35%) of RTR ($P < 0.001$). NA geometric mean titer was significantly higher in the control than the RTR group (482.4 [95% CI, 410.9-566] and 83.7 [95% CI, 50.52-138.8], respectively; $P < 0.001$; Table 3). In a multivariable logistic regression analysis adjusted for age, gender, and days after the second vaccine, the estimated OR for a positive humoral response to the BNT162b2 vaccine was significantly reduced in the RTR patients compared to the immunocompetent control population (OR = 0.01, $P < 0.001$; Table 4).

Univariate Comparison of Positive Versus Negative Response Groups

Rate of living versus deceased donors was significantly higher in the positive response group ($P = 0.03$). Comorbidities were less prevalent in patients who responded to the vaccine, namely, only 66.7% of this group had HTN compared to 83.3% of those with a negative response ($P = 0.04$). A medical history of CHF was also less prevalent in the responders as opposed to the nonresponders ($P = 0.03$). For all other demographic, clinical, and laboratory variables, the differences between the groups were not significant (Table 1).

A significantly lower use of MPA was demonstrated for patients with a positive antibody response (59.5% for responders versus 87.2% for nonresponders). Overall, 63% of patients not receiving MPA mounted a positive RBD IgG response compared to only 36%, 25.3%, and 25% of patients receiving 360, 720, and 1440 mg MPA daily, respectively (Figure 2A). The total daily dose and daily dose per kg weight were also significantly lower for the responders versus nonresponders ($P = 0.001$ and 0.0002, respectively). In addition, RBD IgG levels decreased significantly with an increase in total daily MPA dose (from 0 to 360 mg, $P = 0.04$; from 0 to 740 mg, $P < 0.0001$) (Figure 2B). Patients who responded to the vaccine were less likely to be treated with the triple immunosuppressive regimen containing MPA ($P = 0.04$) and more likely to be treated with the 2-drug regimen of tacrolimus and prednisone ($P = 0.002$). The difference in tacrolimus daily dose per kilogram weight between the groups approached significance with a lower dose in the positive response group ($P = 0.05$); however, tacrolimus trough blood levels were similar in both groups. The administration of prednisone, cyclosporine, and other immunosuppressants was not associated with a reduced antibody response (Table 2).

Multivariable Logistic Regression for Positive Antibody Response

Multivariable logistic regression analysis found that for every 1 mg/kg weight increase in total daily MPA dose, the

TABLE 1.

Demographic, clinical, and biochemical characteristics of RTRs stratified by antibody response

Variable	Total cohort (N = 120)	Negative (N = 78)	Positive (N = 42)	*P*
RTR characteristics				
Age, mean ± SD (y)	59.7 ± 13	60.6 ± 13.22	58.17 ± 12.71	0.33
Female sex, n (%)	24 (20)	14 (17.9)	10 (23.8)	0.44
Transplant to antibody testing date, mean ± SD (y)	5.8 ± 6.3	6.0 ± 6.5	5.5 ± 6.0	0.73
second vaccine to antibody testing date, mean ± SD (d)	26.72 ± 13.32	26.46 ± 13.26	27.19 ± 13.56	0.69
ESRD cause, n (%)				
ADPKD	20 (16.7)	14 (17.9)	6 (14.3)	0.94
Diabetic nephropathy	19 (15.8)	14 (17.9)	5 (11.9)	
Glomerulonephritis	33 (27.5)	20 (25.6)	13 (31)	
Nephrosclerosis	19 (15.8)	12 (15.4)	7 (16.7)	
Other	16 (13.3)	10 (12.8)	6 (14.3)	
Unknown	13 (10.8)	8 (10.3)	5 (11.9)	
Dialysis pretransplant n (%)				
Yes	73 (60.8)	51 (65.4)	22 (52.4)	0.36
No	44 (36.7)	25 (32.1)	19 (45.2)	
Unknown	3 (2.5)	2 (2.6)	1 (2.4)	
Time on dialysis, mean ± SD (y)	2.4 ± 2.4	2.36 ± 2.41	2.56 ± 2.45	0.76
Transplant number, n (%)				
1	109 (90.8)	70 (89.7)	39 (92.9)	0.85
2	7 (5.8)	5 (6.4)	2 (4.8)	
3	4 (3.3)	3 (3.8)	1 (2.4)	
Donor type, n (%)				
Living	96 (80)	60 (76.9)	36 (85.7)	**0.03**[a]
Deceased	22 (18.3)	18 (23.1)	4 (9.5)	
Unknown	2 (1.7)	0 (0)	2 (4.8)	
Medical history				
Hypertension, n (%)	93 (77.5)	65 (83.3)	28 (66.7)	**0.04**[a]
SBP 3 mo average, mean ± SD (mm Hg)	134 ± 17.8	135.1 ± 19.6	132.3 ± 13.8	0.49
DBP 3 mo average, mean ± SD (mm Hg)	76.48 ± 8.81	76.26 ± 9.63	76.9 ± 7.12	0.75
Ischemic heart disease, n (%)	13 (10.8)	10 (12.8)	3 (7.1)	0.34
Congestive heart failure, n (%)	8 (6.7)	8 (10.3)	0 (0)	**0.03**[a]
Diabetes, n (%)	45 (37.5)	29 (37.2)	16 (38.1)	0.92
HbA_{1C} 6 mo average, mean ± SD (%)	6.36 ± 1.23	6.36 ± 1.3	6.35 ± 1.15	0.98
Weight, mean ± SD (kg)	81.62 ± 16.36	80.24 ± 17.15	84.18 ± 14.63	0.21
BMI, mean ± SD (kg/m^2)	27.59 ± 4.99	27.13 ± 5.04	28.5 ± 4.84	0.20
Laboratory results on antibody testing day, mean ± SD				
White blood cell (K/μL)	7.47 ± 2.25	7.47 ± 2.53	7.47 ± 1.63	1.00
Lymphocyte absolute (K/μL)	2.04 ± 1.45	2.14 ± 1.76	1.86 ± 0.51	0.34
Neutrophils absolute (K/μL)	4.50 ± 1.70	4.41 ± 1.79	4.65 ± 1.51	0.48
Neutrophil/lymphocyte ratio	2.73 ± 1.89	2.71 ± 2.04	2.76 ± 1.59	0.90
Hemoglobin (g/dL)	13.33 ± 1.43	13.18 ± 1.53	13.6 ± 1.17	0.10
Platelets (K/μL)	192.1 ± 65.9	187.8 ± 72.7	199.9 ± 50.6	0.34
Creatinine (mg/dL)	1.24 ± 0.43	1.27 ± 0.43	1.18 ± 0.44	0.30
eGFR (CKD-EPI)[b]	65.78 ± 19.43	63.9 ± 19.26	69.28 ± 19.49	0.15
Glucose (mg/dL)	119.7 ± 35.1	118.7 ± 37.5	121.6 ± 30.3	0.67
Albumin (g/dL)	4.07 ± 0.31	4.03 ± 0.32	4.14 ± 0.27	0.06
Globulins (g/dL)	2.57 ± 0.36	2.54 ± 0.36	2.62 ± 0.36	0.25
C-reactive protein (mg/L)	6.48 ± 14.49	7.86 ± 17.77	3.98 ± 3.41	0.17

[a] $P < 0.05$.

[b] The eGFR was calculated according to the following CKD-EPI formula: eGFR = 141 × min (Scr/k, 1)α × max(Scr/k, 1) − 1.209 × 0.993 Age × 1.018 [if female] × 1.159 [if black] (where Scr, standardized serum creatinine; k = 0.7 if female, 0.9 if male; α = −0.329 if female, −0.411 if male; min = the minimum of Scr/k of 1; max = the maximum of Scr/k or 1).

ADPKD, autosomal dominant polycystic kidney disease; BMI, body mass index; CKD-EPI, chronic kidney disease epidemiology collaboration; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HbA_{1C}, hemoglobin A_{1C}; RTR, renal transplant recipient; SBP, systolic blood pressure.

TABLE 2.

Renal transplant recipient immunosuppression treatment on testing day stratified by antibody response

Immunosuppressive therapy	Total cohort (N = 120)	Negative (N = 78)	Positive (N = 42)	*P*
Tacrolimus, n (%)	103 (85.8)	67 (85.9)	36 (85.7)	0.98
Tacrolimus daily dose on antibody testing day, mean ± SD (mg)	2.69 ± 2.58	2.97 ± 2.91	2.17 ± 1.72	0.10
Tacrolimus daily dose per weight on antibody testing day, mean ± SD (mg/kg)	0.03 ± 0.04	0.04 ± 0.04	0.03 ± 0.02	0.05
Tacrolimus trough level on antibody testing day, mean ± SD (µg/L)	7.10 ± 1.93	7.29 ± 1.89	6.75 ± 1.99	0.23
Tacrolimus trough level 2 wk average, mean ± SD (µg/L)	7.11 ± 1.79	7.33 ± 1.77	6.73 ± 1.78	0.13
Tacrolimus trough level 1 mo average, mean ± SD, µg/L	7.02 ± 1.81	7.19 ± 1.8	6.7 ± 1.83	0.22
MPA, n (%)	93 (77.5)	68 (87.2)	25 (59.5)	0.0005[a]
MPA daily dose on serology date, mean ± SD (mg)	537.0 ± 336.5	609.2 ± 291.9	402.9 ± 374.6	0.001[b]
MPA daily dose per weight on serology date, mean ± SD (mg/kg)	6.75 ± 4.32	7.81 ± 3.80	4.78 ± 4.56	0.0002[a]
Prednisone, n (%)	95 (79.2)	61 (78.2)	34 (81)	0.72
Prednisone daily dose on serology date, mean ± SD (mg)	4.17 ± 2.37	4.13 ± 2.45	4.23 ± 2.24	0.84
Prednisone daily dose per weight on serology date, mean ± SD (mg/kg)	0.05 ± 0.04	0.06 ± 0.04	0.05 ± 0.03	0.58
Cyclosporine, n (%)	8 (6.7)	5 (6.4)	3 (7.1)	0.88
Cyclosporine trough level, mean ± SD (µg/L)	111.4 ± 52.5	112 ± 58.4	110.3 ± 53	0.97
Azathioprine, n (%)	3 (2.5)	1 (1.3)	2 (4.8)	0.24
mTORi (everolimus), n (%)	6 (5)	3 (3.8)	3 (7.1)	0.43
mTORi (sirolimus), n (%)	1 (0.8)	1 (1.3)	0 (0)	0.46
Immunosuppressive regimen, n (%)				
Tacrolimus + MPA+ prednisone	58 (48.3)	43 (55.1)	15 (35.7)	0.04[b]
Tacrolimus + MPA	21 (17.5)	15 (19.2)	6 (14.3)	0.49
Tacrolimus + prednisone	20 (16.7)	7 (9)	13 (31)	0.002[b]
Cyclosporine + MPA+ prednisone	8 (6.7)	5 (6.4)	3 (7.1)	0.88
Tacrolimus + azathioprine	2 (1.7)	1 (1.3)	1 (2.4)	0.65
Tacrolimus + azathioprine + prednisone	1 (0.8)	0 (0)	1 (2.4)	0.17
mTORi (everolimus or sirolimus)	7 (5.8)	4 (5.1)	3 (7.1)	0.65

[a]$P < 0.001$.
[b]$P < 0.05$.
MPA, mycophenolic acid; mTORi, mammalian target of rapamycin inhibitor.

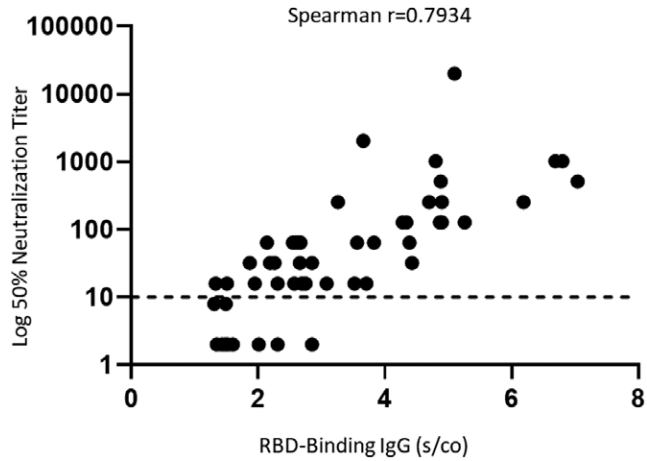

FIGURE 1. Correlation between RBD IgG and neutralizing antibodies in renal transplant recipients with a positive RBD IgG (N = 52). Each dot represents a combined IgG-RBD and neutralizing antibodies result for 1 participant. IgG, immunoglobulin G; RBD, receptor-binding domain; s/co, signal-to-cutoff ratio.

likelihood of a positive response decreased by 17% (OR = 0.83; 95% CI, 0.75-0.92; $P < 0.001$). Hemoglobin level below 13 g/dL was also found to be an independent predictor for antibody response (OR = 0.37; 95% CI, 0.14-0.96; $P = 0.04$; Table 5).

Adverse Events

AEs were recorded for 27.7% of the RTR group, 18.8% and 19.6% after the first and second doses, respectively. Local AEs (all pain at the injection site) developed in 18 (16.1%) recipients following the first and/or the second

TABLE 3.

Univariate analysis for RTR vs immunocompetent control vaccinated population

	RTR (N = 120)	Control (N = 202)	*P*
Gender, n (%)			
F	24 (20)	141 (69.8)	<0.0001
M	96 (80)	61 (30.2)	
Age, mean ± SD (y)	59.7 ± 13	57.04 ± 13.55	0.08
Days from the second vaccine to antibody testing, mean ± SD	26.7 ± 13.3	23.97 ± 5.6	0.033
Positive RBD IgG, n (%)	52 (43.3)	199 (98.5)	<0.0001
Positive NA, n (%)	42 (35)	197 (97.5)	<0.0001
IgG-RBD, GMT (95% CI)	0.93 (0.76-1.15)	6.02 (5.66-6.42)	<0.0001
NA, GMT (95% CI)	83.7 (50.52-138.8)	482.3 (410.9-566)	<0.0001

CI, confidence interval; GMT, geometrical mean titer; IgG, immunoglobulin G; NA, neutralizing antibodies; RBD, receptor-binding domain; RTR, renal transplant recipient.

TABLE 4.

Multivariable logistic regression analysis for RTR vs immunocompetent control vaccinated population

Effect	Odds ratio	95% CI	*P*
Gender—F vs M	1.5	0.63-3.57	0.3548
Age, <65 vs >65 y	1.88	0.9-3.91	0.0917
Days after second vaccine	1.01	0.99-1.04	0.3428
RTR group vs control group	0.01	0-0.04	**<0.0001**[a]

[a]$P < 0.001$.
CI, confidence interval; F, female; M, male; RTR, renal transplant recipient.

vaccine dose. Systemic AEs appeared in 19 (17%) of RTR and included fatigue, headache, and myalgia. Pain at the injection site was more common after the second vaccine dose in the positive vaccine responders (20.5% versus 5.5%, $P = 0.01$). No other differences in the prevalence of local or systemic AEs were found between the responders and the nonresponders (Tables 6 and 7). No rejection episodes were observed, and renal allograft function remained stable at a mean follow-up of 60 d following the second vaccine dose. Allergic responses were not documented.

DISCUSSION

We found that the rate and intensity of the humoral response to the BNT162b2 vaccine in RTR were significantly lower compared to the response in immunocompetent control subjects. RTR with a positive antibody response were characterized by an increased likelihood of a living donor and a lower prevalence of HTN and CHF. MPA treatment and the total daily dose of MPA were significantly lower in RTR with a positive as opposed to a negative antibody response. A multivariable model adjusted for age, sex, and time from the second vaccine dose revealed that the total daily MPA dose and hemoglobin level were independently associated with the antibody response. Vaccination of RTR with the BNT162b2 vaccine was associated with a low rate of AEs, with the most prevalent AE being pain at the injection site. Despite the low rate of AEs, a significant difference between the groups in the prevalence of pain at the injection site following the second vaccine dose was observed.

To the best of our knowledge, this is the first study to report the rate and predictors of the humoral response to the BNT162b2 vaccine using NA in RTR. Despite the high correlation between RBD IgG and NA, the inclusion of NA as a response criterion reduced the response rate from

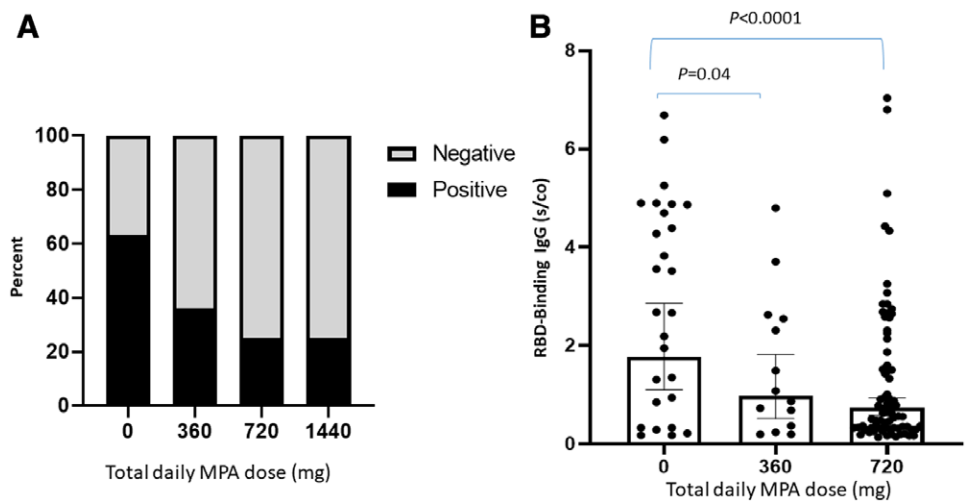

FIGURE 2. Effects of MPA daily dose on antibody response. A, Proportion of patients with positive antibody response following administration of the BNT162b2 vaccine stratified by total daily MPA dose. B, Geometrical mean of RBD IgG antibody levels stratified by total daily MPA dose. IgG, immunoglobulin G; MPA, mycophenolic acid; RBD, receptor-binding domain; s/co, signal-to-cutoff ratio.

TABLE 5.

Univariate and multivariate stepwise logistic regression analysis for positive antibody response in renal transplant recipients.

	Univariate logistic regression		Stepwise logistic regression	
Effect	**Odds ratio (95% CI)**	***P***	**Odds ratio (95% CI)**	***P***
Age >65 vs <65 y	0.64 (0.29-1.43)	0.28	0.62 (0.25-1.49)	0.28
Gender F vs M	1.43 (0.57-3.57)	0.45	1.53 (0.5-4.7)	0.45
Time from transplant to day of antibody testing	1.0 (1.0-1.0)	0.68	—	—
Time from second vaccine to day of antibody testing	1.0 (0.98-1.03)	0.77	—	—
Donor type living vs deceased	1.8 (0.65-4.95)	0.25	—	—
eGFR for every increase in 1 mL/min	1.01 (0.99-1.04)	0.15	—	—
Hemoglobin <13 vs ≥13 g/dL	0.55 (0.24-1.22)	0.14	0.37 (0.14-0.96)	**0.04**[a]
Albumin per 1 g/dL increase	3.56 (0.92-13.75)	0.06	—	
Globulins per 1 mg/dL increase	1.87 (0.65-5.39)	0.24	—	—
Total daily MPA dose per kg for every increase in 1 mg/kg	0.84 (0.77-0.93)	0.0004	0.83 (0.75-0.92)	**0.0004**[b]
Tacrolimus, MPA, prednisone regimen	0.45 (0.21-0.98)	0.04	—	—
Tacrolimus, MPA regimen	0.7 (0.25-1.96)	0.5	—	—
Tacrolimus, prednisone regimen	4.55 (1.65-12.55)	0.003	—	—
Hypertension	0.4 (0.17-0.96)	0.04	—	—

Variables included in the multivariable analysis were those with $P < 0.15$ in the univariate analysis as well as age, gender, time from the second vaccine to day of antibody testing, body mass index, eGFR, and globulins.
[a]$P < 0.05$.
[b]$P < 0.001$.
CI, confidence interval; eGFR, estimated glomerular filtration rate; F, female; M, male; MPA, mycophenolic acid.

TABLE 6.

Any AEs reported post first and/or second BNT162b2 vaccine stratified by antibody response

AEs	**Total cohort, n (%) (N = 112)**	**Negative, n (%) (N = 73)**	**Positive, n (%) (N = 39)**	***P***
Local AE				
Any local AE after first vaccine	15 (13.4)	7 (9.6)	8 (20.5)	0.11
Any local AE after second vaccine	12 (10.7)	4 (5.5)	8 (20.5)	**0.01**[a]
Any local AE	18 (16.1)	8 (11)	10 (25.6)	**0.04**[a]
Systemic AEs				
Any systemic AE after first vaccine	10 (8.9)	7 (9.6)	3 (7.7)	0.74
Any systemic AE after second vaccine	14 (12.5)	8 (11)	6 (15.4)	0.45
Any systemic AE	19 (17)	13 (17.8)	6 (15.4)	0.74
Any AEs (local or systemic)				
Any AE after first vaccine	21 (18.8)	11 (15.1)	10 (25.6)	0.17
Any AE after second vaccine	22 (19.6)	10 (13.7)	12 (30.8)	**0.03**[a]
Any AE	31 (27.7)	18 (24.7)	13 (33.3)	0.33

[a]$P < 0.05$.
AE, adverse event.

43% to 35%. NA show antibody functionality—not only binding but also neutralization and protection. A recent study suggested that being seropositive (by NA) to SARS-CoV-2 through natural infection protects the survivor robustly from asymptomatic and symptomatic reinfection.[15] Thus, the use of NA is of the utmost importance to increase the accuracy of the humoral response assessment by reducing the number of false-positive results, which could potentially make patients less cautious, putting them at increased risk for infection exposure.

A robust early immune response was observed in mRNA-1273 and BNT162b2 vaccine trials[16,17] in which immunosuppressed individuals were not included. Since the publication of those trials, a recent letter has reported that antibodies were detected in only 76 of 436 (17%) of SOT recipients after the first mRNA vaccine dose.[18] An antibody response to the first dose of the Moderna mRNA-1273 vaccine was detected in 26/242 RTR (10.8%).[19] Humoral and T cell responses to the BNT 162b2 mRNA vaccine were even lower post the first and second injections in 101 RTR treated with belatacept.[20] In a recent study, the rate of anti-spike IgG (ELISA) to the BNT162b2 vaccine was 37.5%,[21] but NA were not determined. A similarly reduced antibody response was also reported for a cohort of 70 SOT recipients with confirmed COVID-19 infection, with only 51% of patients developing an antibody response.[10]

The above findings are consistent with prior studies showing decreased antibody production following an infection or vaccination in SOT recipients. The

TABLE 7.

Local and systemic AEs reported after the first and second BNT162b2 vaccine stratified by antibody response

	Total cohort, n (%) (N = 112)		Negative, n (%) (N = 73)		Positive, n (%) (N = 39)		*P*	
AEs	First vaccine	Second vaccine	First vaccine	Second vaccine	First vaccine	Second vaccine	First vaccine	Second vaccine
Local AEs								
Pain at injection site	15 (13.4)	12 (10.4)	7 (9.6)	4 (5.5)	8 (20.5)	8 (20.5)	0.106	**0.01**[a]
Swelling	0	0						
Redness	0	0						
Systemic AEs								
Fever	0	0						
Fatigue	7 (6.25)	11 (9.8)	3 (4.1)	7 (9.6)	4 (7.7)	4 (10.3)	0.4224	0.91
Headache	5 (4.5)	6 (5.4)	3 (4.1)	2 (2.7)	2 (5.1)	4 (10.3)	0.8036	0.09
Myalgia	2 (1.8)	4 (3.6)	1 (1.4)	2 (2.7)	1 (2.6)	2 (5.1)	0.6494	0.52
Chills	0	0						
Nausea/vomiting	0	0						
Paresthesia	0	0						

[a]$P < 0.05$.
AE, adverse event.

seroconversion rate after influenza infection was approximately 65% in SOT recipients compared to 82%–85% in immunocompetent individuals.[22] Response to a standard or high dose of influenza vaccine revealed an antibody response in only 56%–79% of 161 SOT recipients, a number significantly lower than that in the general population.[23] A poor antibody response was also reported for hepatitis A, B, and pneumococcal vaccines.[24-26] Other studies in SOT recipients have yielded inconsistent data, with reports of influenza vaccine efficacy being adequate,[27,28] decreased,[29] or reduced only in patients prescribed cyclosporine[30] or mycophenolate mofetil.[31,32] A seroconversion rate of anti-hepatitis A virus IgG of 71.8% was reported in RTR prescribed mainly cyclosporine and azathioprine.[33] A significantly lower seroconversion rate after hepatitis A vaccination was found in RTR treated with a high-intensity tacrolimus-containing regimen.[34]

The variability observed in the antibody response is related mainly to the type and intensity of the immunosuppressive therapy, which impairs cellular and humoral mediated immunity. In our cohort, MPA use was associated with a poor antibody response (26.9% versus 63%). This finding strengthens prior data showing a suppressed humoral immune response in MPA-treated patients.[31,32,35] MPA inhibits de novo purine biosynthesis preferentially in T and B lymphocytes, thereby suppressing cell-mediated immune responses and antibody formation.[36-38] In our cohort, lower levels of blood globulins were detected in RTR with a blunted RBD IgG response to the vaccine (2.54 ± 0.34 versus 2.64 ± 0.38 in negative and positive response groups based only on RBD IgG, respectively; $P = 0.05$). The lower level of total globulins observed in the nonresponders (using only the RBD IgG criterion) is most probably MPA induced, a notion that is supported by reduced anti-thymocyte globulin titer in patients treated with MPA.[39] Furthermore in our study RBD IgG intensity was found to be significantly reduced with an increase in total daily MPA dose as shown in Figure 2B. Another variable that was associated with poor antibody response was the hemoglobin level that may be a surrogate for the overall health and renal allograft function among RTR. It may, however, have a beneficial effect on the immune response, a notion that should be explored.

We found the BNT162b2 mRNA vaccine to be safe. The incidence of AEs was low compared to that in the general population, as reported in the Pfizer phase 2/3 trial. This low rate is compatible with the reduced immune response observed in our study. Interestingly, the rate of local AEs was higher among those who developed an antibody response, which may reflect immune system activation postvaccine exposure.

Certain limitations should be taken into consideration in interpreting our results. The study is not an efficacy trial (there is no control group), but NA have a reasonable correlation with protection from SARS-CoV-2. NA testing was not done in those that were RBD IgG negative. It is possible that some patients could have non-RBD antibody or IgA/IgM antibody that is still neutralizing. The implications of our findings are limited by the small number of patients and the short follow-up period after vaccination. Antibodies may wane over time, and the half-life of the neutralizing response cannot be predicted. Furthermore, cellular immunity was not assessed.

The odds for a positive BNT162b2 vaccine response among RTR as opposed to immunocompetent individuals were reduced by 99%. This emphasizes the importance of vaccinating any close contacts of immunosuppressed individuals to reduce virus transmission, which may prove to be more protective than vaccination itself in this population. Our results suggest a strong correlation between immunosuppression and seroconversion, as vaccination of MPA-treated RTR is likely to be ineffective. Vaccination before transplantation should therefore be recommended. Despite the strong association observed between type and strength of immunosuppressive therapy and the humoral response, we do not recommend any change in immunosuppressive therapy before vaccination to enhance serological response, as this approach may trigger rejection and de novo appearance of donor-specific antibodies.

Further studies on both B- and T-cell responses are needed to better define the protective immunity provided by BNT162b2 vaccine in RTR. As antigens of mRNA vaccines are synthesized in the cell cytosol where they can be processed and bound to MHC-class I molecules on the cell surface for recognition by CD8 T cells, the cellular response may be more powerful and durable. Humoral response to mRNA vaccines was significantly impaired in belatacept treated kidney transplant recipients versus those not taking belatacept,[40] but the rate of a specific T cell response in belatacept patients was higher than the humoral response.[20] Sixty-five percent of RTR developed either humoral or cellular response to mRNA-1273 (Moderna) vaccine.[41] In contrast to these reports cellular response was significantly reduced in RTR compared to controls and dialysis patients as Spike-specific CD8+ T cell responses were almost undetectable in transplanted patients.[42] Finally, strategies to improve the rate and the strength of vaccine response in RTR, such as administration of a higher dose or a booster dose, should be explored.

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