Risk Factors Associated With an Impaired Antibody Response in Kidney Transplant Recipients Following 2 Doses of the SARS-CoV-2 mRNA Vaccine

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) vaccine has provided an optimistic outlook to the otherwise devastating toll of the COVID-19 pandemic. With promising initial outcomes following vaccine administration in regards to safety and disease prevention in the general population,1-3 there has been a strong push to vaccinate vulnerable patient populations, such as solid organ transplant recipients (SOTRs).4 Although there are substantial efforts evaluating antibody response from the vaccine in the general population,5-8 only limited reports on vaccine efficacy in SOTRs exist. Kidney transplant recipients (KTRs) seem especially vulnerable, as researchers have observed a decline and loss of anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies by 6 mo after SARS-CoV-2 infection.9 Additionally, KTRs exhibit a diminished antibody response to other vaccines, such as influenza A virus subtype H1N1 and influenza.10,11 It is unclear if the poor antibody response is due to the immunosuppressed state. We previously published our initial experience of KTRs who received 1 dose of the mRNA vaccine.12 In that report, we showed that only 6.2% of our kidney transplant cohort demonstrated an antibody response compared with 87% of those on the kidney transplant waitlist. This is comparable to other reports evaluating antibody response in SOTRs following 1
vaccine dose. In an effort to further evaluate the immune response of the mRNA vaccines in transplant patients, we examined the overall antibody response rate in KTRs following 2 doses of the SARS-CoV-2 mRNA vaccine and sought to identify factors associated with anti-SARS-CoV-2 antibody response.

**MATERIALS AND METHODS**

This was an institutional review board approved (IRB0507-0053) retrospective review of KTRs who received 2 doses of either the Pfizer-BioNTech or Moderna mRNA-1273 vaccine at the Houston Methodist J.C. Walter Jr Transplant Center in Houston, TX, from January 2, 2021, to April 1, 2021. Patients received the specific vaccine brand based on availability, and the doses were administered per manufacturer guidelines. Anti-SARS-CoV-2 labs were obtained before each vaccine dose and at least 2 wk following administration of the second vaccine. Patient demographics (age, gender, and race), maintenance immunosuppression, induction agent, history of T-cell depleting therapy (ie, antithymocyte globulin) within 6 mo, history of rejection, and time between vaccine dose to transplant and labs were collected. Those with a positive COVID-19 polymerase chain reaction test, anti-SARS-CoV-2 antibodies at the time of their first vaccine dose, or evidence of anti-SARS-CoV-2 nucleocapsid antibodies were excluded from analysis. Per institutional protocols, patients who were within 1 mo of transplant were excluded from receiving the vaccine. Antibody response or reactivity was defined as the presence of either anti-SARS-CoV-2 immunoglobulin (Ig) IgG or total antibody or anti-SARS-CoV-2 Spike total Ig ≥1:50.

**Clinical Assays**

Anti-SARS-CoV-2 antibody testing used clinically validated assays and was performed in a Clinical Laboratory Improvement Amendments-certified laboratory at Houston Methodist Hospital. Qualitative anti-SARS-CoV-2 Spike total Ig and Anti-SARS-CoV-2 IgG-specific assays (Ortho Clinical Diagnostics, Markham, ON, Canada) were performed on the VITROS 3600 automated immunoassay analyzer according to the manufacturer’s protocol. Anti-SARS-CoV-2 Spike Ig titer measurements were as follows: ≤1:50, 1:50–1:150, 1:150–1:450, and >1:1350, with reactivity defined as titers ≥1:50 as previously reported at our institution. A lab-developed semi-quantitative test to detect anti-SARS-CoV-2 Spike protein IgG-specific ELISA test was performed on a Tecan Freedom EVO instrument as previously described. Anti-SARS-CoV-2 nucleocapsid IgG was tested using the Elecsys anti-SARS-CoV-2 serological assay (Roche Diagnostics, Indianapolis, IN) on a Cobas E602 instrument.

**Institutional Immunosuppression Protocol**

KTRs received an immunosuppression regimen per our institutional protocol. Patients considered at high risk of acute rejection (African Americans, transplant, and highly sensitized recipients) received a 3-d course of rabbit antithymocyte globulin (Thymoglobulin; Genzyme, Cambridge, MA) at a dose of 1.5 mg/kg/d, beginning on the day of transplantation. Patients ≥70 years old were excluded from this group. All other subjects received 20 mg/kg of Basiliximab (Novartis, East Hanover, NJ) on the day of transplantation and on the third day posttransplant. Maintenance immunosuppression consisted of tacrolimus, mycophenolate mofetil, and prednisone. The dose of tacrolimus was adjusted to maintain a trough level of 8 to 10 ng/mL for the first 3-mo postransplantation, tapered to 5 to 8 ng/mL thereafter. Mycophenolate mofetil was given at a dose of 1000 mg twice daily. Methylprednisolone (250 mg) was given on the day of transplantation, tapered to 25 mg by 5 d posttransplant, and then to 5 to 10 mg by 6 mo postransplantation. Patients who had biopsy-proven acute cellular rejection as defined by the Banff criteria also received a 5-d course of rabbit antithymocyte globulin per institutional protocol.

**Statistical Analysis**

Patient characteristics were reported as frequencies and proportions for categorical variables and as median and interquartile range (IQR) for continuous variables. Differences between groups were compared using the χ² or Fisher exact tests for categorical variables and Wilcoxon rank-sum test for the continuous variables. The optimal thresholds of age (68 years of age) and time from transplant (6 mo) in discriminating the antibody response were determined by the Youden index.

A generalized linear model (GLM) was used to determine factors associated with having a reactive antibody response to the COVID-19 vaccine. Variables for the multivariable models were selected based on the clinical importance and also by the least absolute shrinkage and selection operator (Lasso) method using the cross-validation selection option. Variables used in the univariable analysis, after being checked for biological plausibility and collinearity, were assessed by the LASSO program, which suggested good models that included the variables with the highest probability of being a risk factor. Potential risk factors were also discussed with senior clinicians to ensure the biological plausibility of the selected covariates. To avoid overfitting, variables which were significant in the univariate analysis but insignificant in the multivariable analysis were not selected in the final model if their exclusion did not affect the diagnostic performance of the final model (such as prednisone and mammalian target of rapamycin inhibitors). Induction type was included in the final model based on its clinical importance. Variables included in the final GLM model were age (< or ≥68 y), time from transplant to vaccination (in years), T-cell depleting therapy within 6 mo, and immunosuppression therapies (mycophenolate, prednisone, and mammalian target of rapamycin inhibitors). All analyses were performed on Stata version 17.0 (StataCorp LLC, College Station, TX). A P value of <0.05 was considered statistically significant.

**RESULTS**

**Demographics**

As of April 2021, 105 KTRs received 2 doses of either the Pfizer-BioNTech or Moderna mRNA-1273 vaccine and had antibody titers obtained at least 2 wk following the second vaccine dose at our institution. The median age of this cohort was 57 years (IQR, 46–65), with 61.9% (65 of 105) identified as male. The majority of these patients were Caucasian (62.9%, 66 of 105), followed by African American (17%, 18 of 105), Hispanic (10.5%, 11 of 105), and Asian (9.5%, 10 of 105). Only 13% (14 of 105) received T-cell depleting therapy within 6 mo before vaccine administration. Nineteen patients had rejection before vaccine, whereas 6 patients had rejection following vaccine administration. This data is summarized in Table 1.
| Demographics of recipients with and without reactivity to the mRNA COVID-19 vaccine | Total, N = 105 | Nonreactive (n = 67) | Reactive (n = 38) | P       |
|--------------------------------------|---------------|----------------------|-------------------|---------|
| Age (y), median (IQR)                | 57.0 (46.0–64.0) | 56.0 (46.0–64.0) | 57.5 (45.0–68.0) | 0.57    |
| Age (y)                              | 0.052         |                      |                   |         |
| <68                                  | 85 (81.0)     | 58 (86.6)            | 27 (71.1)         |         |
| ≥68                                  | 20 (19.0)     | 9 (13.4)             | 11 (28.9)         |         |
| Gender                               | 0.52          |                      |                   |         |
| Female                               | 40 (38.1)     | 24 (35.8)            | 16 (42.1)         |         |
| Male                                 | 65 (61.9)     | 43 (64.2)            | 22 (57.9)         |         |
| Ethnicity                            | 0.10          |                      |                   |         |
| White                                | 66 (62.9)     | 37 (55.2)            | 29 (76.3)         |         |
| Black                                | 18 (17.1)     | 15 (22.4)            | 3 (7.9)           |         |
| Hispanic                             | 11 (10.5)     | 9 (13.4)             | 2 (5.3)           |         |
| Asian                                | 10 (9.5)      | 6 (9.0)              | 4 (10.5)          |         |
| Time from transplant to vaccination (y), median (IQR) | 1.0 (0.0–3.0) | 1.0 (0.0–2.0) | 2.0 (1.0–7.0) | 0.002   |
| Time from transplant to vaccination (y) | 0.01          |                      |                   |         |
| <6 mo                                | 37 (35.2)     | 30 (44.8)            | 7 (18.4)          |         |
| ≥6 mo                                | 68 (64.8)     | 37 (55.2)            | 31 (81.6)         |         |
| Vaccine                              | 0.48          |                      |                   |         |
| Moderna mRNA-1273                    | 45 (42.9)     | 27 (40.3)            | 18 (47.4)         |         |
| Pfizer-BioNTech                      | 60 (57.1)     | 40 (59.7)            | 20 (52.6)         |         |
| Days between vaccine 1 and vaccine 2, median (IQR) | 26.0 (21.0–28.0) | 26.0 (21.0–28.0) | 25.5 (21.0–28.0) | 0.97    |
| Days between vaccine 2 and last lab date, median (IQR) | 91.0 (45.0–110.0) | 89.0 (46.0–106.0) | 93.5 (42.0–122.0) | 0.38    |
| History of rejection                  | 0.48          |                      |                   |         |
| No                                   | 79 (76.0)     | 52 (78.8)            | 27 (71.1)         |         |
| Yes                                  | 25 (24.0)     | 14 (21.2)            | 11 (28.9)         |         |
| Rejection before or after vaccination (n = 25) | 0.55          |                      |                   |         |
| Before                               | 19 (76.0)     | 10 (71.4)            | 9 (61.8)          |         |
| After                                | 6 (24.0)      | 4 (28.6)             | 2 (18.2)          |         |
| Time from transplant to rejection (y), median (IQR) (n = 25) | 0.8 (0.4–1.5) | 0.8 (0.4–1.5) | 0.8 (0.1–2.1) | 0.70    |
| Immunosuppression therapy and induction |                     |                      |                   |         |
| T-cell depleting therapy, ≤6 mo      | 0.02          |                      |                   |         |
| No                                   | 91 (86.7)     | 54 (80.6)            | 37 (97.4)         |         |
| Yes                                  | 14 (13.3)     | 13 (19.4)            | 1 (2.6)           |         |
| Tacrolimus                           | 1.00          |                      |                   |         |
| No                                   | 11 (10.5)     | 7 (10.4)             | 4 (10.5)          |         |
| Yes                                  | 94 (89.5)     | 60 (89.6)            | 34 (89.5)         |         |
| Mycophenolate                        | 0.003         |                      |                   |         |
| No                                   | 20 (19.0)     | 7 (10.4)             | 13 (34.2)         |         |
| Yes                                  | 85 (81.0)     | 60 (89.6)            | 25 (65.8)         |         |
| Prednisone                           | 0.02          |                      |                   |         |
| No                                   | 6 (5.7)       | 1 (1.5)              | 5 (13.2)          |         |
| Yes                                  | 99 (94.3)     | 66 (98.5)            | 33 (86.8)         |         |
| Azathioprine                         | 0.02          |                      |                   |         |
| No                                   | 101 (96.2)    | 67 (100.0)           | 34 (89.5)         |         |
| Yes                                  | 4 (3.8)       | 0 (0.0)              | 4 (10.5)          |         |
| Cyclosporine                         | 1.00          |                      |                   |         |
| No                                   | 97 (92.4)     | 62 (92.5)            | 35 (92.1)         |         |
| Yes                                  | 8 (7.6)       | 5 (7.5)              | 3 (7.9)           |         |
| Belatacept                           | 0.53          |                      |                   |         |
| No                                   | 103 (98.1)    | 65 (97.0)            | 38 (100.0)        |         |
| Yes                                  | 2 (1.9)       | 2 (3.0)              | 0 (0.0)           |         |
| mTOR inhibitors                      | 0.13          |                      |                   |         |
| No                                   | 97 (92.4)     | 64 (95.5)            | 33 (86.8)         |         |
| Yes                                  | 8 (7.6)       | 3 (4.5)              | 5 (13.2)          |         |
| Induction receipt                    | 1.00          |                      |                   |         |
| No                                   | 9 (8.6)       | 6 (9.0)              | 3 (7.9)           |         |
| Yes                                  | 96 (91.4)     | 61 (91.0)            | 35 (92.1)         |         |
| Induction type                       | 0.97          |                      |                   |         |
| None                                 | 9 (8.6)       | 6 (9.0)              | 3 (7.9)           |         |
| Thymoglobulin                        | 70 (66.7)     | 44 (65.7)            | 26 (68.4)         |         |
| Simulect                             | 24 (22.9)     | 16 (23.9)            | 8 (21.1)          |         |
| Campath                              | 2 (1.9)       | 1 (1.5)              | 1 (2.6)           |         |

Values are in number (%) unless otherwise specified.
COVID-19, coronavirus disease 19; IQR, interquartile range; mTOR, mammalian target of rapamycin.
**Vaccine Response and Associated Factors**

The median time between kidney transplant and the first vaccine dose was 1 year (IQR, 0–3) and 57% (60 of 105) of patients received the Pfizer-BioNTech vaccine. The median time between vaccine doses was 26 days (IQR, 21–28), consistent with manufacturer recommendations, and the median follow-up after the second vaccine dose was 91 days (IQR, 45–110).

Only 36.2% (38 of 105) of KTRs exhibited an antibody response. Of these, 61% (22 of 38) had an anti-SARS-CoV-2 Spike Ig titer ≥1:50. Median time from transplant response. Of these, 61% (22 of 38) had a reactive antibody response to the vaccine. Although this observation is higher than the 6.2% to 17% antibody response rate following 1 vaccine dose,12,13 our observation is significantly lower than the estimated 95% antibody response rate in the general population.21 An important difference between KTRs and the general population is that KTRs are immunosuppressed, and factors associated with antibody response in KTRs appear to be linked to the immunosuppressed state. In a multivariate analysis, recipients ≥68 years old received T-cell depleting therapy within 6 mo before vaccination, 9 were due to rejection, and 5 were due to induction. Rejection and induction type were not found to be statistically significant factors for vaccine-associated antibody response.

**DISCUSSION**

Our findings showed that of the 105 KTRs who received 2 doses of the SARS-CoV-2 mRNA vaccine at our institution, only 36.2% (n = 38) had a reactive antibody response to the vaccine. Although this observation is higher than the 6.2% to 17% antibody response rate following 1 vaccine dose,12,13 our observation is significantly lower than the estimated 95% antibody response rate in the general population.21 An important difference between KTRs and the general population is that KTRs are immunosuppressed, and factors associated with antibody response in KTRs appear to be linked to the immunosuppressed state. In a multivariate analysis, recipients ≥68 years old were less likely to receive T-cell depleting therapy at the time of transplant and potentially have a lower level of maintenance immunosuppression. Per our institutional protocol, KTRs ≥70 years old do not receive T-cell depleting therapy for induction because of concerns for infection. Additionally, older KTRs are less likely to have allograft rejection,22 thus prompting a lower level of maintenance immunosuppression (ie, lower calcineurin inhibitor levels, half the antimitabolite dose ± prednisone) than the younger cohort.16 In our study, only 1 patient ≥68 years old received T-cell depleting therapy within 6 mo of receiving the vaccine. This finding may reflect the older KTR cohort’s ability to exhibit an antibody response to the vaccine because we also observed that KTRs receiving T-cell depleting therapy within 6 mo of vaccination were less likely to exhibit an antibody response.

Similar to the older KTRs, patients with a longer time from transplant were more likely to exhibit an antibody response as they were further from the time of their induction treatment and usually maintained on lower immunosuppression.18 All patients in the antibody-reactive group were beyond 2 years from transplant.
| Characteristic                                      | Univariable |                      | P   |                      | P     |
|----------------------------------------------------|-------------|----------------------|-----|----------------------|-------|
|                                                    | RR (95% I)  | RR (95% I)           |     |                      |       |
| **Age (y), median (IQR)**                          | 1.01 (0.98–1.03) |                      | 0.65 |                      |       |
| **Gender**                                         |             |                      |     |                      |       |
| Female (Reference)                                 |             |                      | –   |                      | –     |
| Male                                               | 0.85 (0.51-1.41) |                      | 0.52 |                      | –     |
| **Ethnicity**                                      |             |                      |     |                      |       |
| White (Reference)                                  |             |                      | –   |                      | –     |
| Black                                              | 0.38 (0.13-1.10) |                      | 0.08 |                      | –     |
| Hispanic                                           | 0.41 (0.11-1.49) |                      | 0.18 |                      | –     |
| Asian                                              | 0.91 (0.41-2.04) |                      | 0.82 |                      | –     |
| **Time from transplant to vaccination (y), median (IQR)** | 1.08 (1.04–1.12) |                      | <0.001 |                      | 1.07 (1.00–1.15) | 0.045 |
| **Vaccine**                                        |             |                      |     |                      |       |
| Moderna mRNA-1273 (Reference)                      |             |                      | –   |                      | –     |
| Pfizer-BioNTech                                    | 0.83 (0.50-1.38) |                      | 0.48 |                      | –     |
| **History of rejection**                           |             |                      |     |                      |       |
| No (Reference)                                     |             |                      | –   |                      | –     |
| Yes                                                | 1.29 (0.75-2.20) |                      | 0.36 |                      | –     |
| **Rejection before or after vaccination (n = 25)**  |             |                      |     |                      |       |
| Before (Reference)                                 |             |                      | –   |                      | –     |
| After                                              | 0.70 (0.21-2.40) |                      | 0.58 |                      | –     |
| **Immunosuppression therapy and induction**        |             |                      |     |                      |       |
| T-cell depleting therapy, ≤6 mo                    |             |                      |     |                      |       |
| No (Reference)                                     |             |                      | –   |                      | –     |
| Yes                                                | 0.18 (0.03-1.18) |                      | 0.07 |                      | 0.27 (0.04–2.04) | 0.20 |
| Tacrolimus                                         |             |                      |     |                      |       |
| No (Reference)                                     |             |                      | –   |                      | –     |
| Yes                                                | 0.99 (0.44-2.27) |                      | 0.99 |                      | –     |
| Mycophenolate                                      |             |                      |     |                      |       |
| No (Reference)                                     |             |                      | –   |                      | –     |
| Yes                                                | 0.46 (0.29-0.72) |                      | 0.001 |                      | 0.42 (0.21–0.87) | 0.02 |
| Prednisone                                         |             |                      |     |                      |       |
| No (Reference)                                     |             |                      | –   |                      | –     |
| Yes                                                | 0.40 (0.25-0.63) |                      | <0.001 |                      | –     |
| Cyclosporine                                       |             |                      |     |                      |       |
| No (Reference)                                     |             |                      | –   |                      | –     |
| Yes                                                | 1.04 (0.41-2.64) |                      | 0.94 |                      | –     |
| mTOR inhibitors                                    |             |                      |     |                      |       |
| No (Reference)                                     |             |                      | –   |                      | –     |
| Yes                                                | 1.84 (1.00-3.36) |                      | 0.048 |                      | –     |
| Induction                                          |             |                      |     |                      |       |
| No (Reference)                                     |             |                      | –   |                      | –     |
| Yes                                                | 1.09 (0.42-2.86) |                      | 0.86 |                      | –     |
| Induction type                                      |             |                      |     |                      |       |
| None (Reference)                                   |             |                      | –   |                      | –     |
| Thymoglobulin                                       | 1.11 (0.42-2.95) |                      | 0.83 |                      | 2.24 (0.59–8.52) | 0.24 |
| Simulect                                           | 1.00 (0.34-2.95) |                      | 1.00 |                      | 1.65 (0.43-6.35) | 0.47 |
| Campath                                            | 1.50 (0.28-7.93) |                      | 0.63 |                      | 4.91 (0.44-55.09) | 0.20 |

Values are in number (%) unless otherwise specified.
IQR, interquartile range; mTOR, mammalian target of rapamycin; RR, relative risk.
Recent studies examining antibody response to the COVID-19 vaccine in SOTRs have reported similar data to our own. In addition to providing one of the largest studies to date, we have also offered additional insight into associated factors related to antibody response in KTRs. Rusk et al\textsuperscript{14} presented 1 SORT who did not exhibit an antibody response following 2 doses of the COVID-19 vaccine. Boyarsky et al\textsuperscript{13} followed up with their initial series by evaluating their SOTRs after 2 vaccine doses and identified a similarly low antibody response rate to the vaccine.\textsuperscript{21} Specific to KTRs, Korth et al\textsuperscript{24} identified significantly lower immunogenicity with 2 doses of the Pfizer-BioNTech vaccine than with healthy controls. Likewise, our study did not suggest a difference in immunogenicity based on mRNA vaccine type. These early reports all identify low immunogenicity among SOTRs after 2 doses of the SARS-CoV-2 mRNA vaccine.

Our findings are similar to the immunogenic response rate for the influenza vaccine in SOTRs. When dosed for the general population, the influenza vaccine had a suboptimal response rate of about 15% to 70%.\textsuperscript{23,26} Studies utilizing higher-dose vaccines showed improved antibody response in these patients,\textsuperscript{27,28} and the current recommendations are for transplant recipients to receive the high-dose influenza vaccine. This experience can provide guidance for our evolving management of transplant patients receiving the COVID-19 vaccine.

Although we have identified several factors associated with antibody response in KTRs to the COVID-19 vaccine, there are a few limitations to our study. First, we had a relatively small sample size when variable groups were stratified. Second, our study was observational, as there was no randomization or control group. Third, we only studied the SARS-CoV-2 mRNA vaccines because of limited availability and restrictions of other COVID-19 vaccines. Last, the vaccine may induce important T-cell response in this population that we could not measure. Thus, despite a lack of antibody response to the SARS-CoV-2 mRNA vaccine, it remains possible that KTRs may convey some immunologic defense against SARS-CoV-2.

With increasing COVID-19 infections in the community, there is an opportunity to better understand the efficacy of the SARS-CoV-2 mRNA vaccine in KTRs in terms of infection rate and antibody response. There are new reports of break through infections following vaccination,\textsuperscript{29,30} with Wadei et al\textsuperscript{30} observing 7 COVID-19 positive SOTRs who received 1 or 2 doses of the mRNA vaccine. In this small cohort, none of the patients developed antibodies following vaccine administration. More data will be needed to guide our management in this vulnerable patient population.

In the growing field of research investigating SARS-CoV-2 vaccine efficacy in transplant patients, we have presented important data evaluating the antibody response in KTRs after 2 doses of the SARS-CoV-2 mRNA vaccine and suggest that the degree of immunosuppression likely contributes to the lack of antibody response. As the majority of COVID-19 positive cases in SOTRs at our institution are in KTRs, we chose to analyze this high-risk cohort given our routine use of induction agents (including T-cell depleting therapy) and relatively high level of maintenance immune suppression. Future studies will include evaluation of other COVID-19 vaccine types, outcomes of additional booster vaccines and vaccine dose adjustment, and identification of potential biomarkers of response.

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