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Evaluation of the Correlation Between Responders and Non-Responders to the Second Coronavirus Disease Vaccination In Kidney Transplant Recipients: A Retrospective Single-Center Cohort Study

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ABSTRACT

Background. The immune response to COVID-19 vaccination in kidney transplant (KTx) recipients is significantly lower than that in healthy controls. We evaluated immune responses after the COVID-19 vaccine and their possible relationship with other cofactors in KTx recipients.

Methods. This retrospective single-center cohort study included 29 KTx recipients 2-8 weeks after receiving 2 doses of the Pfizer-BioNTech SARS-CoV-2 messenger RNA vaccine. Anti-SARS-CoV-2 spike (S) immunoglobulin (Ig)-G levels were evaluated to define cofactors influencing the immune response between the responder (anti-SARS-CoV-2 IgG level ≥0.8 U/mL) (n = 16) and nonresponder groups (anti-SARS-CoV-2 IgG level <0.8 U/mL) (n = 13). The kinetics of antibodies between 2 and 6 months after the second vaccination was also compared between the groups.

Results. KTx recipients with IgG levels ≥0.8 U/mL were younger (54 [interquartile range {IQR}, 46.5-61] years vs 65 [IQR, 55-71.5] years; P = .01), had been transplanted for a longer median time (1588 [IQR, 1382-4751] days vs 1034 [IQR, 548.5-1833] days; P = .02), and were more often treated with a lower mycophenolate mofetil dosage (765.6 ± 119.6 vs 1077 ± 76.9 mg; P = .04) than KTx recipients with IgG levels <0.8 U/mL. There was no significant difference in antibody titers between time periods after the second dose in the responder group. At the 6-month follow-up, a serologic response against the SARS-CoV-2 S was observed in 44.4% of KTx recipients in the nonresponder group.

Conclusions. More than 50% of KTx recipients developed a higher antibody response after the second dose of COVID-19 vaccination.

As reported elsewhere, the immune response to COVID-19 vaccination in patients with chronic kidney disease receiving renal replacement therapy was significantly lower than that in healthy controls, particularly in kidney transplant (KTx) recipients [1]. Several studies have indicated that chronic kidney disease is the most common comorbidity in severe COVID-19 cases [2,3]. Furthermore, patients undergoing renal replacement therapy with KTx have shown the highest morbidity and mortality rates [2]; thus, vaccination is the most important way to prevent infection. However, reports of anti-SARS-CoV-2 spike (S) antibodies after a second vaccination in KTx patients are scarce [4-6].

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Herein, we aimed to evaluate immune responses after 2 doses of the COVID-19 messenger (m)-RNA vaccine and their possible relationship with other cofactors in KTx recipients.

MATERIALS AND METHODS

Ethics Statements

All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the Ethics Committee of Kameda Medical Center (approval number: 21-025) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The requirement for written informed consent was waived because of the retrospective nature of this study.

Study Design and Participants

This retrospective single-center cohort study was performed at Kameda Medical Center to evaluate the correlation of immune response against SARS-CoV-2 S in KTx recipients 2-8 weeks after receiving 2 doses of the Pfizer-BioNTech SARS-CoV-2 mRNA vaccine (BNT162b2) at a recommended interval of 21 days. In this study, we added new cases to a previous report [1], and KTx recipients were divided into 2 groups based on the level of antibodies against SARS-CoV-2 S proteins: the responder group (anti-SARS-CoV-2 IgG level \( \geq 0.8 \) U/mL) and nonresponder group (anti-SARS-CoV-2 IgG level <0.8 U/mL). Then, we evaluated the correlation of immune response to COVID-19 vaccination between the 2 groups. To further analyze the kinetics of antibodies after 6 months in KTx recipients, we additionally assessed and compared antibody titers at 2 and 6 months after the second vaccination in both groups.

Owing to Japan’s vaccine delivery systems, group vaccination was conducted mostly with 2 doses of the Comirnaty COVID-19 vaccine (BioNTech-Pfizer BNT162b2). All participants received the first and second doses of COVID-19 mRNA vaccines between March 18, 2021 and October 1, 2021. Sample collection for antibody titer follow-up continued until March 30, 2022. Patient data on kidney function and immunosuppression were collected from patients’ medical records at the time of sample collection.

Humoral Response Assessment

Serum samples were tested for SARS-CoV-2 antibodies (IgG levels) using the Elecsys Anti-SARS-CoV-2 S RUO test system (Roche Diagnostics, Basel, Switzerland). Antibody titers >0.8 U/mL were considered as positive immune responses to vaccination [1,7-9].

Outcomes

The primary outcomes evaluated in this study included quantitative humoral responses to the second dose of the COVID-19 mRNA vaccine. Anti-SARS-CoV-2 S IgG levels were evaluated to define cofactors influencing the immune response between the responder and nonresponder groups. In both groups, a comparison was also performed to analyze the kinetics of antibodies between 2 and 6 months after the second vaccination.

Statistical Analysis

Categorical variables were analyzed using chi-square or Fisher exact tests and are expressed as counts and percentages. Continuous variables were first tested for normal distribution using the Kolmogorov-Smirnov test. If normally distributed, continuous data were analyzed using the t test and are expressed as means ± standard deviations; if not, the Mann-Whitney test was used, and values are expressed as medians and interquartile ranges (IQRs). All data were analyzed using GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA). P value < 0.05 was considered statistically significant.

RESULTS

Patients’ Characteristics

A total of 29 patients were included: 16 (55.1%) had detectable anti-SARS-CoV-2 IgG antibodies (responder group) (Table 1), whereas 13 (44.8%) did not have detectable antibodies (nonresponder group) (Table 2). Most KTx recipients were taking uniform immunosuppressive therapy including a calcineurin inhibitor in 28 of 29 patients, mycophenolate mofetil (MMF) or mizoribine in 27 of 29, and glucocorticoids in 29 of 29.

Only 1 patient in the responder group had a history of polyclonal antibody response. Following COVID-19 vaccination, differences between responders and nonresponders were analyzed. Table 3 presents and compares the demographic and laboratory data between the 2 study groups. The demographics and clinical characteristics, including kidney function, of the responder and nonresponder groups were similar (Tables 1 and 2). However, despite the small sample size, KTx recipients with IgG levels \( \geq 0.8 \) U/mL were younger (54 [IQR, 46.5-61] vs 65 [IQR, 54-71.5] years; \( P = .01 \)), had been transplanted for a longer median time (1588 [IQR, 1382-4751] vs 1034 [IQR, 548.5-1833] days; \( P = .02 \)), and were more often treated with a lower MMF dosage (765.6 ± 119.6 vs 1077 ± 76.9 mg; \( P = .04 \)) than KTx recipients with IgG levels <0.8 U/mL (Table 3).

Regarding immunosuppressive maintenance therapy, in the responder group, although all 3 maintenance immunosuppressants (calcineurin inhibitor, MMF, and glucocorticoid) tended to be used at lower doses, only MMF showed a significant difference. Furthermore, in the responder group, 3 patients (cases 2, 9, and 11) ceased using MMF owing to adverse clinical events, and 1 of them (case 2) was switched to mizoribine treatment.

Concerning the use of rituximab in the nonresponder group, 9 of 13 patients used rituximab (Table 2). In contrast, in the responder group, 9 of 16 patients used rituximab (Table 1), which showed that treatment with rituximab was not significantly associated with nonresponders (Table 3). We also observed a correlation between age and rituximab use in the responder group; 3 of 9 recipients (cases 5, 7, and 11) were in their 30s. Thus, young age could be related to acquisition of high antibody levels despite the use of rituximab.
### Table 1. Baseline Characteristics of the Responders

| Case No. | Age, y | Sex | BMI, kg/m² | Cause of ESKD | Hypertension | Diabetes Mellitus | Days from KTx to Sample Taken | ABO Incompatibility | Use of Rituximab | Serum Creatinine, mg/dL | eGFR, mL/min/1.73 m² | Tacrolimus, mg | Mycophenolate Mofetil, mg | Methylprednisolone, mg | Others |
|----------|--------|-----|------------|---------------|--------------|------------------|-----------------------------|---------------------|----------------|---------------------|-------------------|----------------|----------------------|------------------|--------|
| 1        | 62     | M   | 22.7       | DMN           | Yes          | Yes              | 579                         | Compatible          | No               | 0.98               | 60.4              | 5.5            | 1000                 | 2                |         |
| 2        | 52     | M   | 17.4       | DMN           | Yes          | Yes              | 1377                        | Compatible          | No               | 2.26               | 25.6              | 2              | 0                    | 2                |         |
| 3        | 64     | M   | 24.9       | IgAN          | Yes          | Yes              | 2484                        | Incompatible        | Yes              | 1.82               | 30.5              | 0.5            | 500                  | 2                |         |
| 4        | 56     | F   | 32.2       | IgAN          | Yes          | Yes              | 1265                        | Incompatible        | Yes              | 1.18               | 37.7              | 2              | 1000                 | 2                |         |
| 5        | 38     | F   | 30.3       | ORG           | Yes          | Yes              | 642                         | Compatible          | Yes              | 0.91               | 56.0              | 3              | 0                    | 1                |         |
| 6        | 51     | M   | 27.0       | DMN           | Yes          | Yes              | 1629                        | Compatible          | Yes              | 1.08               | 57.7              | 2              | 1000                 | 2                |         |
| 7        | 39     | M   | 19.0       | IgAN          | No           | Yes              | 4976                        | Compatible          | Yes              | 0.97               | 70.1              | 2              | 0                    | 4                |         |
| 8        | 63     | M   | 21.6       | IgAN          | Yes          | No               | 4077                        | Compatible          | No               | 1.75               | 32.0              | 3              | 500                  | 2.5              |         |
| 9        | 67     | F   | 21.7       | IgAN          | Yes          | No               | 1534                        | Incompatible        | No               | 2.23               | 17.8              | 0              | 500                  | 4                |         |
| 10       | 57     | F   | 21.5       | Renal allograft dysfunction | Yes          | Yes              | 2937                        | Compatible          | No               | 0.83               | 54.8              | 3              | 1000                 | 2                |         |

BMI, body mass index; CAKUT, congenital anomalies of the kidney and urinary tract; DMN, diabetic nephropathy; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; F, female; IgAN, immunoglobulin A nephropathy; KTx, kidney transplant; M, male; no., number; ORG, obesity-related glomerulopathy.

### Table 2. Baseline Characteristics of Nonresponders

| Case No. | Age, y | Sex | BMI, kg/m² | Cause of ESKD | Hypertension | Diabetes Mellitus | Days from KTx to Sample Taken | ABO Incompatibility | Use of Rituximab | Serum Creatinine, mg/dL | eGFR, mL/min/1.73 m² | Tacrolimus, mg | Mycophenolate Mofetil, mg | Methylprednisolone, mg | Others |
|----------|--------|-----|------------|---------------|--------------|------------------|-----------------------------|---------------------|----------------|---------------------|-------------------|----------------|----------------------|------------------|--------|
| 1        | 76     | M   | 20.2       | DMN           | Yes          | Yes              | 2134                        | Complatable          | Yes             | 1.42               | 38.1              | 4.5            | 1000                 | 2                |         |
| 2        | 65     | F   | 22.0       | Lupus nephritis | No           | No               | 796                         | Complatable          | Yes             | 0.66               | 68.1              | 2              | 1000                 | 3                |         |
| 3        | 42     | M   | 19.8       | IgAN          | Yes          | No               | 1531                        | Complatable          | Yes             | 1.40               | 45.9              | 1.5            | 1000                 | 4                |         |
| 4        | 74     | M   | 23.0       | CGN           | Yes          | Yes              | 1034                        | Incompatible        | Yes             | 1.67               | 32.1              | 1.5            | 1000                 | 2                |         |
| 5        | 67     | M   | 20.2       | FSGS          | Yes          | Yes              | 859                         | Incompatible        | Yes             | 1.14               | 50.2              | 4              | 1500                 | 4                |         |
| 6        | 45     | M   | 28.2       | Nephrosclerosis | No           | Yes              | 1251                        | Complatable          | No              | 1.80               | 34.2              | 4              | 1000                 | 2                |         |
| 7        | 81     | M   | 25.6       | Unknown       | Yes          | No               | 2985                        | Incompatible        | Yes             | 1.10               | 49.0              | 2              | 500                  | 2                |         |
| 8        | 55     | F   | 27.7       | DMN           | Yes          | Yes              | 1461                        | Incompatible        | Yes             | 0.91               | 50.3              | 1              | 1000                 | 2                |         |
| 9        | 69     | F   | 33.2       | IgAN          | Yes          | Yes              | 413                         | Complatable          | No              | 1.39               | 29.6              | 3.5            | 1000                 | 4                |         |
| 10       | 55     | M   | 24.0       | Unknown       | No           | Yes              | 684                         | Complatable          | Yes             | 1.40               | 42.2              | 4              | 1000                 | 2                |         |
| 11       | 61     | M   | 26.4       | Goodpasture syndrome | Yes          | Yes              | 3359                        | Complatable          | Yes             | 1.48               | 38.8              | 0              | 1500                 | 3                |         |

BMI, body mass index; CGN, chronic glomerulonephritis; DMN, diabetic nephropathy; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; F, female; IgAN, immunoglobulin A nephropathy; KTx, kidney transplant; M, male; no., number.
Categorical variables were analyzed with chi-squared or Fisher exact tests and expressed as counts and percentages. Continuous variables were first tested for normal distribution using Kolmogorov-Smirnov test. If normally distributed, continuous data were analyzed using t test and values were expressed as the median and interquartile range (IQR).

### DISCUSSION

Almost all patients on hemodialysis and peritoneal dialysis and healthy controls produce antibodies against the SARS-CoV-2 S proteins [1]; however, antibody levels were significantly lower in KTx recipients than in healthy controls [1]. The seroconversion rates in this study resemble those described in previous studies [4–6]. These reports are mainly from the United States and Europe; thus, the current study may provide valuable information about Asian data.

Several studies have indicated that risk factors for inadequate antibody response in KTx recipients were older age, less time after transplant, number of immunosuppressants used, and type of immunosuppressant (antimetabolite MMF or co-stimulation blocker belatacept) [10–12]. In comparison with them, despite the small number of cases, a similar trend was observed in the present study; the median antibody levels were considerably low in nonresponders compared with responders, with a shorter time since transplantation, older age, and use of higher MMF doses. These findings suggest that the capacity to produce antibodies is impaired early after KTx, and this is probably related to the amount of immunosuppression administered, independent of the recipient’s age [10].

In our study, there was no correlation between anti-SARS-CoV-2 antibodies and rituximab use. However, another study showed that in rituximab-treated patients, anti-SARS-CoV-2 antibody titers and B cell proportions after rituximab treatment (B lymphocyte depletion) are directly correlated, and for seroconversion, only a small amount of B lymphocytes (<1%) is needed [13]. Furthermore, the association between impaired immune response and rituximab use was also observed in a study of 216 KTx recipients, suggesting a possible need for a change in immunosuppressive therapy ahead of vaccination [14].

The third vaccination has been available in Japan since February 2022; thus, we additionally assessed antibody titers at 6 months just before receiving the third dose. Several studies have reported that waning of the humoral response after a second dose of COVID-19 vaccine has been observed in healthy controls and patients on hemodialysis [9,15,16]. However, to our knowledge, data on the kinetics of antibodies at 6 months after the second dose of vaccination in KTx recipients do not currently exist, and it is important to recommend a third vaccination. In this study, at the 6-month follow-up, a serologic response against the SARS-CoV-2 S proteins was observed in

| General | Responders, n = 16 (55.1%) | Nonresponders, n = 13 (44.8%) | P Value |
|---------|--------------------------|--------------------------|--------|
| Age, y, median (IQR) | 54 (46.5-61) | 65 (55-71.5) | 0.01 |
| Male, n (%) | 11 (68.7) | 9 (69.2) | 0.97 |
| BMI, kg/m², mean ± SD | 24.5 ± 4.6 | 24.6 ± 4.1 | 0.93 |
| Hypertension, n (%) | 16 (100) | 11 (84.6) | 0.10 |
| Diabetes mellitus, n (%) | 9 (56.2) | 5 (38.4) | 0.34 |
| History of COVID-19, n (%) | 1 (0.06) | 0 (0) | >0.9999 |
| Transplantation | | | |
| Time since transplant, d, median (IQR) | 1588 (1382-4751) | 1034 (548.5-1833) | 0.02 |
| Distribution of Antibodies against SARS-CoV-2 Spike Antigen | | | |
| 6 Months after Vaccination | | | |
| There was no significant difference in antibody titers between 2 and 6 months after the second dose in the responder group (Fig 1A). In contrast, at the 6-month follow-up, a serologic response against the SARS-CoV-2 S protein was observed in 44.4% of KTx recipients in the nonresponder group. The median antibody titer increased from 0.4 (IQR, 0.4-0.4) U/mL at 2 months after the second vaccination to 0.4 (IQR, 0.4-0.4) U/mL at 6 months after the second vaccination (P = .01) (Fig 1B).

**Table 3. Comparison of the Evaluated Demographic and Clinical Characteristics in the Responder and Nonresponder Groups**

BMI, body mass index; eGFR, estimated glomerular filtration rate; IQR, interquartile range; SD, standard deviation.
44.4% of the KTx recipients in the nonresponder group. This antibody seroconversion indicates possible association with asymptomatic or subclinical infection [17]; however, all patients tested negative for SARS-CoV-2 nucleocapsid (N) protein, which indicates subclinical infection. However, reverse transcription-PCR (RT-PCR) confirmation was not performed in our study. We speculate that antibody formation may have been slower than usual owing to certain factors associated with immunosuppression-related impairments in the immune response. Although the patients tested negative on antibody titer testing at 2 months, sufficient amounts of antibodies were subsequently produced; seroconversion could therefore be confirmed at 6 months.

A recent study found that a third dose of the COVID-19 mRNA vaccine induced a serologic response in 49% of KTx recipients who did not respond after 2 doses [18]; therefore, for those patients who are still negative for antibodies, we expect that third-dose booster vaccination may lead to enhanced humoral immune responses.

The present study limitations include the small sample size of KTx recipients and lack of cellular immune response data. Further studies are necessary to clarify the kinetics of antibodies and to provide a better estimate of antibody response in responders and nonresponders.

CONCLUSIONS
This study found that, according to the levels of anti-SARS-CoV-2 antibodies, >50% of KTx recipients developed a higher antibody response after the second dose of COVID-19 mRNA vaccine. Factors that may significantly affect the adequacy of response to the COVID-19 mRNA vaccine in these patients are younger age, longer time after KTx, and use of a lower dosage of MMF for maintenance immunosuppression.

Meanwhile, in the nonresponder group, at the 6-month follow-up, a serologic response against the SARS-CoV-2 S proteins was observed in KTx recipients who did not respond after the second dose. Nevertheless, since more than half of the nonresponders still did not develop anti-SARS-CoV-2 antibodies, an additional booster dose of the COVID-19 mRNA vaccine after 6 months may be needed to enhance humoral response, particularly in those with lower antibody titers after 2 doses.

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