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RESEARCH ARTICLE

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# A prospective humoral immune monitoring study of kidney transplant recipients receiving three doses of SARS-CoV-2 mRNA vaccine

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## Abstract

Kidney transplant recipients (KTRs), like other solid organ transplant recipients display a suboptimal response to mRNA vaccines, with only about half achieving seroconversion after two doses. However, the effectiveness of a booster dose, particularly in generating neutralizing antibodies (NAbs), remains poorly understood, as most studies have mainly focused on non-neutralizing antibodies. Here, we have longitudinally assessed the humoral response to the SARS-CoV-2 mRNA vaccine in 40 KTRs over a year, examining changes in both anti-spike IgG and NAbs following a booster dose administered about 5 months post-second dose. We found a significant humoral response increase 5 months post-booster, a stark contrast to the attenuated response observed after the second dose. Of note, nearly a quarter of participants did not achieve protective plasma levels even after the booster dose. We also found that the higher estimated glomerular filtration rate (eGFR) correlated with a more robust humoral response postvaccination. Altogether, these findings underscore the effectiveness of the booster dose in enhancing durable humoral immunity in KTRs, as evidenced by the protective level of NAbs found in 65% of the patients 5 months post- booster, especially those with higher eGFR rates.

KEYWORDS

booster vaccination, estimated glomerular filtration rate (eGFR), kidney transplant recipients (KTRs), neutralizing antibodies, SARS-CoV-2

Vincenzo Cantaluppi and Marisa Gariglio equally contributed as senior author.

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## 1 | INTRODUCTION

Kidney transplant recipients (KTRs), like other solid organ transplant (SOT) recipients, are at an increased risk of severe progression of COVID-19, often with high mortality rates.[1–3] Consequently, SOT recipients were prioritized early in SARS-CoV-2 vaccination campaigns.

Previous studies have shown that mRNA vaccines exhibit poor immunogenicity in adult SOT recipients, especially KTRs, with only about 50% of them seroconverting after two doses.[4–7] This suboptimal vaccine response has been linked to a high incidence of breakthrough infections, some severe, leading to the regulatory endorsement of additional booster doses. These boosters have resulted in increased levels of SARS-CoV-2 neutralizing antibodies (NAbs) in these patients, consistent with patterns seen in the general population.[8–11] However, the extent of immunity, especially in term of duration of the neutralizing activity of the humoral response achieved through booster vaccination in SOT recipients remains to be better elucidated and further longitudinal analyses are required to establish immune correlates of protection.

In this context, NAbs are considered the most reliable correlate of protection against SARS-CoV-2 infections. They play a pivotal role in counteracting infectious diseases due to their ability to target both circulating viruses and infected cells via antibody-mediated effector mechanisms.[12]

In this prospective study, we have evaluated the longitudinal humoral response to the SARS-CoV-2 mRNA vaccine in a cohort of 40 KTRs over 1-year period, including clinical and laboratory correlations. Our findings reveal a significant increase in humoral response 5 months post-booster, in marked contrast to the reduced response observed after the second dose. Notably, we found that the estimated glomerular filtration rate (eGFR) had a substantial impact on the humoral response postvaccination, whereas the immunosuppressive regimen did not significantly affect it.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients, samples, and data collection

This prospective observational study, approved by the Ethics Committee of Maggiore della Carità Hospital in accordance with the Biobank of the University of Piemonte Orientale-UPO (CE 078/2022, KT-UPO-B-Kidney Transplant UPO Biobank), involved 40 KTRs treated at the Nephrology and Kidney Transplantation Unit. The eligible participants were adults (aged ≥18 years) who had undergone kidney transplantation and had received full vaccination against SARS-CoV-2 with the mRNA-based Pfizer/BNT162b2 vaccine according to the Italian national vaccination guidelines at the time of the study. Young patients (aged <18 years), patients who had received multiple organ transplants, and those who did not complete the vaccination program were not included in the study.

Biological samples were collected between March 2021 and February 2022 before each vaccine injection (Figure 1A). All participants provided written informed consent. Patient samples and associated data were pseudonymized and recorded on the REDCap (https://www.project-redcap.org/) web application in compliance with current GDPR and Italian legislation on the protection of sensitive data and privacy.

The study investigators extracted data on patient characteristics from electronic medical records and clinical charts.

### 2.2 | Quantitative determination of anti-SARS-CoV-2 IgG antibodies

For the quantitative determination of IgG against the full-length SARS-CoV-2 spike protein, the LIAISON SARS-CoV-2 TrimericS IgG Kit (DiaSorin, Saluggia, Italy) was employed. The immunoassay was used following the manufacturer's instructions. Samples falling below 33.8 BAU/ml were considered negative.

### 2.3 | SARS-CoV-2-specific neutralizing antibody assay

The assay to determine the neutralizing capability of antibodies against SARS-CoV-2 was carried out as previously described.[13–16] Vero E6, Vero E6-TMPRSS2 cells and the replication-competent vesicular stomatitis virus rVSV-eGFP-SARS-CoV-2-$S_{\Delta 21}$ [17] were kindly provided by John Hiscott (Pasteur Institute Rome, Italy) and Sean P.J. Whelan (Washington University School of Medicine, USA), respectively. Images were acquired using Cytation 5 (Agilent, Santa Clara, CA) and were further processed using the Gen5 software (Agilent, Santa Clara, CA) to calculate the ID50, which represents the concentration of antibodies required to inhibit 50% of viral infection in the assay system.

### 2.4 | Statistical analysis

Data conforming to a normal distribution were presented as mean and standard deviation (SD), whereas data non-normally distributed were presented as median and interquartile range (IQR). Categorical variables were summarized as counts and percentages. The Wilcoxon signed rank test was used to compare the anti-SARS-CoV-2 IgG antibodies and NAb titers at different time points. The Mann Whitney test was performed to analyze the differences of titers between groups. Spearman correlation was applied to assess the relationship between IgG and NAb levels. Binary logistic regression was performed to evaluate the association between eGFR levels and IgG and NAb levels. The odds ratios (OR) and the 95% confidence interval (95% CI) were calculated adjusting for possible confounders, including age, gender, and time post-transplantation. A $p$-value < 0.05 was considered statistically significant, indicating the presence of a

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meaningful difference or association. STATA v.16 was used for statistical analyses (StataCorp 2019. Stata Statistical Software: Release 16. StataCorp LLC, College Station, TX).

## 3 | RESULTS

### 3.1 | Patient characteristics

In March 2021, we enrolled 40 KTRs, comprising 10 females and 30 males. As shown in Table 1, the median age at the time of enrollment was 58.3-year-old (IQR 49–71). At baseline, the median eGFR level, as determined by CKD-EPI formula, was 43.5 ml/min/1.73 $m^2$ (IQR 31–58). The median duration from transplantation to vaccination was 4.3 years (IQR 1.9–10.0).

During the 1-year observation period from March 2021 to February 2022, all the subjects received four doses of the mRNA-based Pfizer/BNT162b2 vaccine. Blood samples were collected at each time point just before vaccine injection (Figure 1A). The second dose (T1) was administered 21 ± 3 days after the first one (T0), the booster dose at 162 ± 4 days after the initial 2-dose regimen (T2), and the fourth at 151 ± 8 days after the booster (T3).

Among the KTRs, 22 experienced an RT-PCR-confirmed SARS-CoV-2 infection: 4 (18%) before any vaccine dose and 18 (82%) after the booster (T2). Six of them (27.3%) received monoclonal antibodies against SARS-CoV-2 S protein during their acute infection phase in accordance with standard of care in the specific period. While 16 of the 22 infected patients (72.7%) required hospitalization, none needed intensive care unit (ICU) admission. Patients' baseline demographic and clinical characteristics are summarized in Table 1.

**TABLE 1** Patients' demographic and clinical characteristics.

| Characteristics | N (%) Patients ($n$ = 40) |
|---|---|
| **Age, median (IQR) years** | 58.3 (49–71) |
| **Time after last transplantation, median (IQR) years** | 5.2 (2.8–11) |
| **Gender** | |
| Male | 30 (75.0) |
| Female | 10 (25.0) |
| **Presence of comorbidities** | |
| Arterial hypertension | 39 (97.5) |
| Diabetes | 6 (15.0) |
| Cardiovascular diseases | 15 (37.5) |
| **End-stage renal disease (ESRD)** | |
| Glomerulonephritis/vasculitis | 12 (30.0) |
| Genetically based diseases | 11 (27.5) |
| Urologic diseases | 5 (12.5) |
| Diabetes | 2 (5.0) |
| Nephroangiosclerosis/hypertension | 1 (2.5) |
| Unknown | 9 (22.5) |
| **eGFR, median (IQR)** | 43.5 (31–58) |
| eGFR < 30 | 9 (22.5) |
| eGFR ≥ 30 | 31 (77.5) |
| **Therapy** | |
| mTOR inhibitors | 1 (2.5) |
| CNIs - MMF/MPA | 5 (12.5) |
| CNIs - Steroids | 11 (27.5) |
| CNIs - mTOR inhibitors | 1 (2.5) |
| CNIs - Steroids - mTOR inhibitors | 5 (12.5) |
| CNIs - Steroids - MMF/MPA | 17 (42.5) |
| **SARS-CoV-2 infection** | |
| Before prior two doses | 4 (10.0) |
| Post-booster dose | 18 (45.0) |
| **COVID-19 therapy** | |
| Anti-spike monoclonal Abs | 6 (27.3) |
| Molnupiravir | 5 (22.7) |
| **Vaccine regimen (four doses)** | |
| Pfizer/BNT162b2 | 40 (100) |

Abbreviations: CNIs, Calcineurin inhibitors; MMF, Mycophenolate mofetil; MPA, Mycophenolic acid.

### 3.2 | Seroconversion rate and magnitude of the IgG and neutralizing antibody response to SARS-CoV-2

As the goal of our study was to assess the humoral response after the booster dose (T3) compared to the response elicited by the first and second dose at T1 and T2, we measured the serum levels of anti-spike (s) IgG along with the SARS-CoV-2 neutralizing activity of sera at each time point (Figure 1A). More specifically, we compared the values at T3, taken approximately 5 months after the booster dose and just before the fourth vaccine dose, with those obtained at T1 and T2, which were harvested just before the administration of the second and booster doses, respectively (Figure 1A).

At T3, the overall seroconversion rate for anti-s IgG in our study cohort was 87.5% (35/40), as judged by the manufacturer's cut-off (33.8 BAU/ml) when using its LIAISON SARS-CoV-2 TrimericS IgG Kit. Furthermore, at the same time point, 70% (28/40) of subjects had IgG values exceeding 264 BAU/ml, a threshold predicting 80% vaccine efficacy against symptomatic infection.[18]

As expected, comparing the anti-s IgG antibody titers at 21 days after the first vaccine dose (T1) to the baseline (T0), when the seroconversion rate from prior natural infection was 12.5% (5/40), revealed a significant increase (T0 median: 4.8 BAU/ml [IQR: 4.8–4.8] vs. T1 (median: 130 BAU/ml [IQR: 6.65–1480]; $p < 0.0001$) (Figure 1B; blue: low eGFR; red: high eGFR). However, retesting after 5 months from the administration of the second vaccine dose

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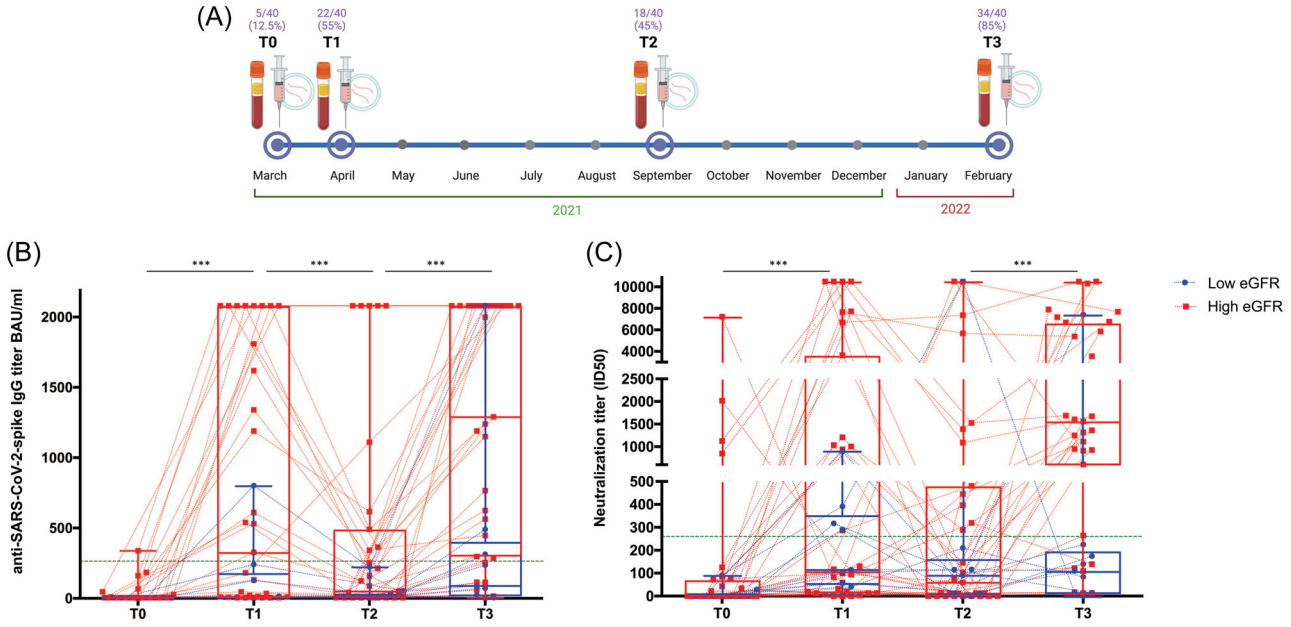


**FIGURE 1** Humoral response in kidney transplant recipients. (A) Timeline of the vaccination schedule and sample collection points (T0: first dose; T1: second dose; T2: third dose; T3: fourth dose). The purple numbers represent the count of seropositive patients (anti-SARS-CoV-2-spike IgG > 33.8 BAU/ml). (B–C) Longitudinal analysis of the anti-SARS-CoV-2-spike IgG (B) or ID50 neutralization (C) titers in the cohort of KTRs ($n = 40$) at different time points. The colored dots and box plots indicate eGFR levels and quartile and median values of the two groups (blue: eGFR < 30 ml/min/1.73 $m^2$; red: eGFR ≥ 30 ml/min/1.73 $m^2$). The horizontal green dotted line in (B) marks the IgG level predictive of 80% vaccine efficacy against symptomatic SARS-CoV-2 infection (264 BAU/ml), while in (C) it indicates the 50% protective neutralization level against SARS-CoV-2 infection (ID50 = 260). ***$p < 0.0001$.

(T2) showed a significant reduction in anti-s IgG titers (median: 18.9 BAU/ml [IQR: 4.8–297.5]; $p < 0.0001$), very likely due to the waning humoral response from the two-dose regimen as previously observed.[19] By contrast, a retest 5 months after the booster dose revealed a significant increase in anti-s IgG titers (median: 958 BAU/ml [IQR: 175.5–2080; $p < 0.0001$). Consequently, the percentage of patients with anti-s IgG titers above the protection cut-off value (264 BAU/ml) rose from 25% (10/40) at T2 to 70% (28/40) at T3.

Given the importance of determining the neutralizing effects of anti-SARS-CoV-2-spike Abs to understand the protective effects of the immune response, we measured NAb levels by testing the sera against rVSV-SARS-CoV-2-$S_{\Delta 21}$ infection of Vero E6-TMPRSS2 cells, as previously described.[13–16] NAb quantification revealed that the median effective dose (ID50) neutralization titers correlated with the anti-s IgG titers only at T3 (Spearman's correlation $r = 0.79$, $p < 0.0001$), with lower correlations at T0 ($r = 0.22$; $p = 0.17$), T1 ($r = 0.28$; $p = 0.08$), and T2 ($r = 0.23$; $p = 0.15$).

The median ID50 neutralization titer values fluctuated similarly to the anti-s IgG levels: at T0, it was 0 (IQR: 0–39.0), at T1 it was 110.5 (IQR: 20.7–1021.3), at T2 it dropped to 67.1 (IQR: 0–421.4), and at T3 it increased significantly to 1181.5 (IQR: 130.8–5630.2). Using the mathematical modeling approach developed by Davenport and co-workers,[20] which provides a quantitative prediction of the link between NAb levels and clinical protection, we estimated the 50% protective neutralization level against SARS-CoV-2 infection in our cohort to be 260, calculated as 20.2% of the T2 mean level (mean ID50 = 1287). This value perfectly matched the cut-off (256.6) established in a previous study from our group, which analyzed a cohort of hematologic malignancy patients with a confirmed SARS-CoV-2 infection within 6 months before testing.[15]

Five months after the booster dose at T3, 65% (26/40) of patients had a neutralization titer above 260, a significant increase compared to the 32.5% (13/40) at T2 ($p < 0.0001$, Figure 1C; blue: low eGFR; red: high eGFR).

Of note, both anti-s IgG values and NAb levels showed a similar trend, dropping at T2 and increasing at T3. In particular, anti-s IgG values decreased at T2 in 22/40 (55%) KTRs (median difference between T2 and T1 values: −5.4 BAU/ml [IQR: −496.9–0]), whereas they increased at T3 in 32/40 (80%) KTRs (median difference between T3 and T2: 291.25 BAU/ml [IQR: 37.8–1297]). Correspondingly, NAb levels dropped at T2 (median difference between T2 and T1 values: −33.4 [IQR: −849.5 to −43.6]) in 28/40 (70%) and increased at T3 (median difference between T3 and T2 values: 596.1 [IQR: 3.2–2871.6]) in 31/40 (77.5%) KTRs. Despite this enhancement, a substantial proportion of KTRs at T3 (almost a quarter of them) still displayed suboptimal humoral responses. Specifically, 12/40 (30%) patients had anti-s IgG values below the cut-off level of 264 BAU/ml, and 14/40 (35%) had ID50 neutralizing titers lower than 260. In addition, six patients (15%) never mounted an immunological response to either anti-s IgG or NAb at any time point.

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### 3.3 | Association between clinical factors and anti-SARS-CoV-2 humoral response

We next analyzed how various factors, such as age, gender, comorbidities, time from transplantation, end-stage renal disease (ESRD), eGFR values, and immunosuppressive treatments, correlated with the humoral responses. Statistically significant correlations were only obtained when the patients were stratified according to their eGFR rate into two groups: low ($n = 9$) and high ($n = 31$), using 30 ml/min/1.73 $m^2$ as the cut-off. We chose this cut-off because it allowed us to distinguish individuals with a good preserved renal functional reserve versus those at higher risk of further decline. By contrast, in KTRs who experienced SARS-CoV-2 breakthrough infections, we did not observe a trend of low eGFR rates. The median eGFR rate in these patients was 36.5 ml/min/1.73 $m^2$ [IQR: 30–55], suggesting that renal function as indicated by eGFR did not significantly impact the incidence of breakthrough infections in our study group.

At T1, the median anti-s IgG values in the low eGFR group was significantly lower compared to that observed in the high eGFR group (9.5 [IQR: 4.8–128] vs. 327 [IQR: 9.4–2080] BAU/ml; $p = 0.038$). No significant differences were seen at T2, in good agreement with the general decline in immune response. The trend observed at T1 persisted at T3 (89.4 [IQR: 25.4–314] vs. 1290 [IQR: 298–2080] BAU/ml; $p = 0.004$), at which time point logistic regression analysis showed a significant association between patients belonging to the low eGFR group and anti-s IgG titers below the cut-off value of 264 BAU/ml (crude OR: 8.3, 95% CI: 1.6–43.3, $p = 0.012$; adjusted OR by age, gender, and time post-transplantation (13.2, 95% CI: 1.6–106.3, $p = 0.016$).

Concerning ID50 neutralization titers, no significant variations were found between the two groups at T1 and T2. In contrast, at T3, the median ID50 value in the high eGFR group was significantly higher (1560.7 [IQR: 610.41–6697.53]) compared to that of the low eGFR group (110.4 [IQR: 13.8–174.8] $p = 0.004$). Consistently, a significant association was found between high eGFR levels and neutralization titers higher than the protective cut-off of 260 at T3 ($p = 0.002$ and $p = 0.005$; adjusted by age, gender, and time post-transplantation).

Noteworthy, of the 40 patients in our study, 18 contracted SARS-CoV-2 infection after the administration of the second vaccine dose. As expected, these individuals displayed median titers for both anti-s IgG and NAbs below the respective protection cut-off against SARS-CoV-2 infections (4.8 BAU/ml [IQR: 4.8–15.4] and 37.1 [IQR: 0–210.3], respectively) at T2. At T3, the median titers for anti-s IgG were 2040 (IQR: 115–2080) in infected patients versus 469.5 (IQR: 236–1290) BAU/ml in uninfected ones, and for NAbs, 1616.9 (IQR: 139.5–7182.2) versus 437.5 (IQR: 110.4–1607.1), respectively (Figure S1; blue: No COVID; orange: COVID post-T2; red: COVID pre-T0). Moreover, KTRs who had SARS-CoV-2 infection before receiving any vaccine dose (n = 4) displayed anti-s IgG values and NAb titers exceeding the respective cut-offs at all time points, with consistently high readings across T0, T1, T2, and T3. Specifically, for anti-s IgG, the medians were 162 (IQR: 46.9–184) at T0, 2080 (IQR: 1710–2080) at T1, 2080 (IQR: 1146.5–2080) at T2, and 2080 (IQR: 2040–2080) at T3. The median NAb values were 626.4 (IQR: 63.2–1573.1) at T0, 657.4 (IQR: 59.4–5854.3) at T1, 552.3 (IQR: 6.6–5791.9) at T2, and 1616.9 [IQR: 1255.8–4680.9] at T3.

In our cohort, only 22 patients were on MMF/MPA regimen. Comparing these patients with those not on MMF/MPA, we observed no statistically significant differences in anti-s IgG values at any time points. The median values at T0 were 4.8 (IQR: 4.8–10.3) for the MMF/MPA group and 4.8 (IQR: 4.8–4.8) for non-MMF/MPA group. At T1, the medians were 89.5 (IQR: 4.8–1340) and 184.5 (IQR: 8.5–1620), respectively. At T2, they were 18.8 (IQR: 4.8–616) and 29.1 (IQR: 4.8–160), and at T3, 665 (IQR: 115–2080) and 1170 (IQR: 236–2080).

Similarly, there were no statistically significant differences in NAb titers between the two groups, except at T2 ($p = 0.02$), where both groups had median values below the protective cut-off. The MMF/MPA group had medians of 0.2 (IQR: 0–72.7) at T0, 110.5 (IQR: 32.8–900.69) at T1, 162.7 (IQR: 12.9–1388.6) at T2, and 1101 (IQR: 174.8–6697.5) at T3. The non-MMF/MPA group had corresponding medians of 0 (IQR: 0–29.2), 109.28 (IQR: 12.7–1034.4), 12.40 (IQR: 0–116.8; and 1215.2 (IQR: 109.4–2615.6).

## 4 | DISCUSSION

Our study aimed to evaluate the humoral response (anti-spike IgG and NAbs) following the administration of the SARS-CoV-2 mRNA vaccine booster dose (T3) in comparison with the responses triggered by the initial and second doses at T1 and T2. Our findings are in keeping with several reports indicating that continued vaccination boosts the number of individuals who respond positively and enhances their protection against adverse outcomes.[21–24] Indeed, this study reveals that the mRNA SARS-CoV-2 booster dose substantially enhances the humoral response in KTRs. Noteworthy, 65% of patients showed NAb titers above the protective cut-off at T3, and 70% of them exhibited protective anti-s IgG titers 5 months post-booster. This is particularly important as the humoral response assessed 5 months after the booster was markedly higher than that observed 5 months following the second dose.[19] This finding emphasizes the prolonged efficacy of the booster dose over the initial two-dose regimen. However, 15% of patients failed to develop an immunological response at any assessed time points. As previously reported, even after receiving multiple doses (up to seven), a significant portion of the population still remains at high risk of infection, showing no measurable humoral response to the vaccine.[25] For these patients, additional booster doses and/or preexposure prophylaxis with monoclonal antibodies may be required.

Our findings are in line with previous studies linking renal impairment, as indicated by low eGFR rates, to a compromised immune response in KTRs. In particular, these studies have consistently shown that KTRs with better pre-vaccination renal function can achieve higher antibody titers against SARS-CoV-2.[26,27] In our study, a significant disparity was noted in the percentage of

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patients displaying NAb activities above the 50% protective neutralization threshold between groups with high and low eGFR group (80% vs. 11%, respectively). However, KTRs experiencing SARS-CoV-2 breakthrough infections did not exhibit low eGFR rates, as these were within a normal range (median: 36.5 ml/min/1.73 $m^2$ [IQR: 30−55]). KTRs with low eGFR levels represent an effective model of chronic kidney disease (CKD), which is frequently associated with increased mortality due to cardiovascular events and severe infections. These clinical findings correlate with immune system alterations, including a state of immunoactivation characterized by the presence of low-grade chronic inflammation and a concomitant state of immunodepression with reduced T and B cell responses, further exacerbated by the immunosuppressive effects of anti-rejection drugs.[28]

All participants in our study adhered to the immunosuppressive regimen standard at our center, with only 22 out of 40 patients receiving MMF/MPA. Contrary to previous reports showing that an MMF/MPA-free regimen significantly correlates with enhanced seroconversion after SARS-CoV-2 mRNA vaccination,[29,30] MMF/MPA administration did not influence the humoral response extent in our cohort (Table 1). Conversely, high eGFR rates were associated with protective NAb titers against SARS-CoV-2. Patients who experienced breakthrough infections after vaccination typically displayed humoral responses below the protective threshold.

Multiple studies, including our own previous work, have reported improved vaccination responses following natural SARS-CoV-2 infection, both in terms of anti-SARS-CoV-2 IgG levels and antibody neutralizing activities.[16,31,32] Magicova et al.,[32] observed a marked difference in seroconversion rates between KTRs with prior SARS-CoV-2 infection and those without. Likewise, our findings show that KTRs who contracted SARS-CoV-2 during the observation period ($n = 22$) had more robust humoral responses compared to those of uninfected subjects ($n = 18$), even though the difference was not statistically significant.

Altogether, our findings underscore the robust immunogenic effect of the booster dose especially in those patients who displayed good renal function, as indicated by higher eGFR rates at the time of vaccination. In addition, it calls for extra caution in the care of fragile KTRs with low eGFR levels, regardless of the time elapsed since their transplant. Considering their reduced NAb production, these patients should be prioritized for receiving booster vaccine doses or monoclonal antibodies targeting new SARS-CoV-2 variants.

While this study did not assess T cell-mediated immunity, its strength lies in the longitudinal analysis of both anti-s IgG titers and the neutralizing activity at multiple time points over a 1-year observation period. This comprehensive approach, to our knowledge, is unprecedented in KTR research. Future research should include T cell immunity assessments to gain a more complete understanding of the immune response in KT patients following vaccination. In addition, we observed no clear correlations between the immunosuppressive regimen and the immune response, very likely due to the small number of patients in each subgroup, which limited the ability to make significant comparisons.

## AUTHOR CONTRIBUTIONS

Participated in research design: Vincenzo Cantaluppi, Marisa Gariglio, Cinzia Borgogna; Participated in the writing of the paper: Cinzia Borgogna, Vincenzo Cantaluppi, Marisa Gariglio; Participated in the performance of the research: Greta Rosso, Gabriele Guglielmetti, Marco Quaglia, Irene Lo Cigno, Valeria Caneparo, Stefano Raviola; Participated in data analysis: Daniela Ferrante, Cinzia Borgogna.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data sharing requests for access to data should be addressed to the corresponding author (MG). The individual participant data collected will be available, after pseudonymization. All proposals requesting data access will need to specify how the data will be used, and all proposals will need the approval of the investigator team before data release. Data will be shared through the online platform REDCap (https://www.project-redcap.org/). The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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