the podocytes.[S4] Because IFN pathways have an important role in the pathogenesis of CG, notably in patients homozygous for *APOL1* high-risk variants, their stimulation by SARS-CoV-2 immunization could be a potential *second-hit* triggering CG development, especially among genetic susceptible patients.

Finally, we believe that risks of glomerular injury development should not prevent the use of SARS-CoV-2 vaccines. SARS-CoV-2 immunization must not be avoided based on the fear of triggering or worsening a glomerulopathy.

## DISCLOSURE

All the authors declared no competing interests.

## ETHICAL ASPECTS

This case report was approved by the local ethical committee of the Faculty of Medicine of University of São Paulo under the number CAAE 17279219.8.0000.0068. Written informed consent was obtained from the patients.

## SUPPLEMENTARY MATERIAL

Supplementary File (PDF)
**Supplementary References.**

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# Natural SARS-CoV-2 infection results in higher neutralization response against variants of concern compared with 2-dose BNT162b2 vaccination in kidney transplant recipients

**To the editor:** Seroconversion rates in kidney transplant recipients (KTRs) after 2-dose BNT162b2 (Pfizer–BioNTech) mRNA vaccination are in the range of 3%–59% and thus are significantly lower compared with the >90% achieved in healthy controls.[1] In convalescent coronavirus disease 2019 (COVID-19) patients, antibody levels decline only slightly after 6–8 months, whereas vaccine-induced immunity appears to decrease more rapidly.[2–4] Recent data suggest that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern (VoCs) partially escape humoral immune responses induced by natural infection with a nonescaping variant or vaccination.[5] However, little is known about protection against VoCs in KTRs who recovered from COVID-19 or were immunized with 2 doses of BNT162b2.

We compared humoral immunity in 18 KTRs hospitalized for COVID-19 infection with immunity in 25 KTRs with seroconversion after 2-dose BNT162b2 vaccination. Nucleocapsid antibodies were measured after the second vaccination in vaccinated patients or at hospitalization in COVID-19–infected patients to exclude prior SARS-CoV-2 infection. Baseline characteristics, including immunosuppressive regimens, are given in Supplementary Table S1. COVID-19 disease severity ranged from moderate to critical, with 2 COVID-19–related deaths (Supplementary Table S2). Immunosuppressive antimetabolite medication was stopped in all COVID-19 patients, and 9 of 18 (50%) patients received corticosteroids only (Supplementary Table S3). Eight patients had infection with the original SARS-CoV-2 strain, 8 patients with the VoC B.1.1.7 (alpha), and 2 patients with B.1.351 (beta). Serum was collected at a median (interquartile range [IQR]) of 72 (67–77) days after hospitalization, or 62 (54–64) days after prime vaccination for COVID-19–infected or vaccinated KTRs, respectively. We determined anti–wild-type SARS-CoV-2 spike S1 IgG, neutralizing surrogate antibodies, and performed a bead-based multiplex analysis of various SARS-CoV-2 target epitopes in 16 convalescent KTRs available for follow-up and in all 25 vaccinated KTRs. In addition, neutralizing antibodies to wild-type, B.1.1.7 (alpha), B.1.351 (beta), and B.1.617.2 (delta) were measured using a full virus assay (Supplementary Methods).

Our data show that there is no significant difference between convalescent or vaccinated KTRs for commercially available tests, such as anti-S1 IgG, neutralizing antibodies determined by a surrogate virus neutralization assay, or anti–receptor-binding domain antibodies (Figure 1a–c). In a bead-based analysis of antibodies against different SARS-CoV-2
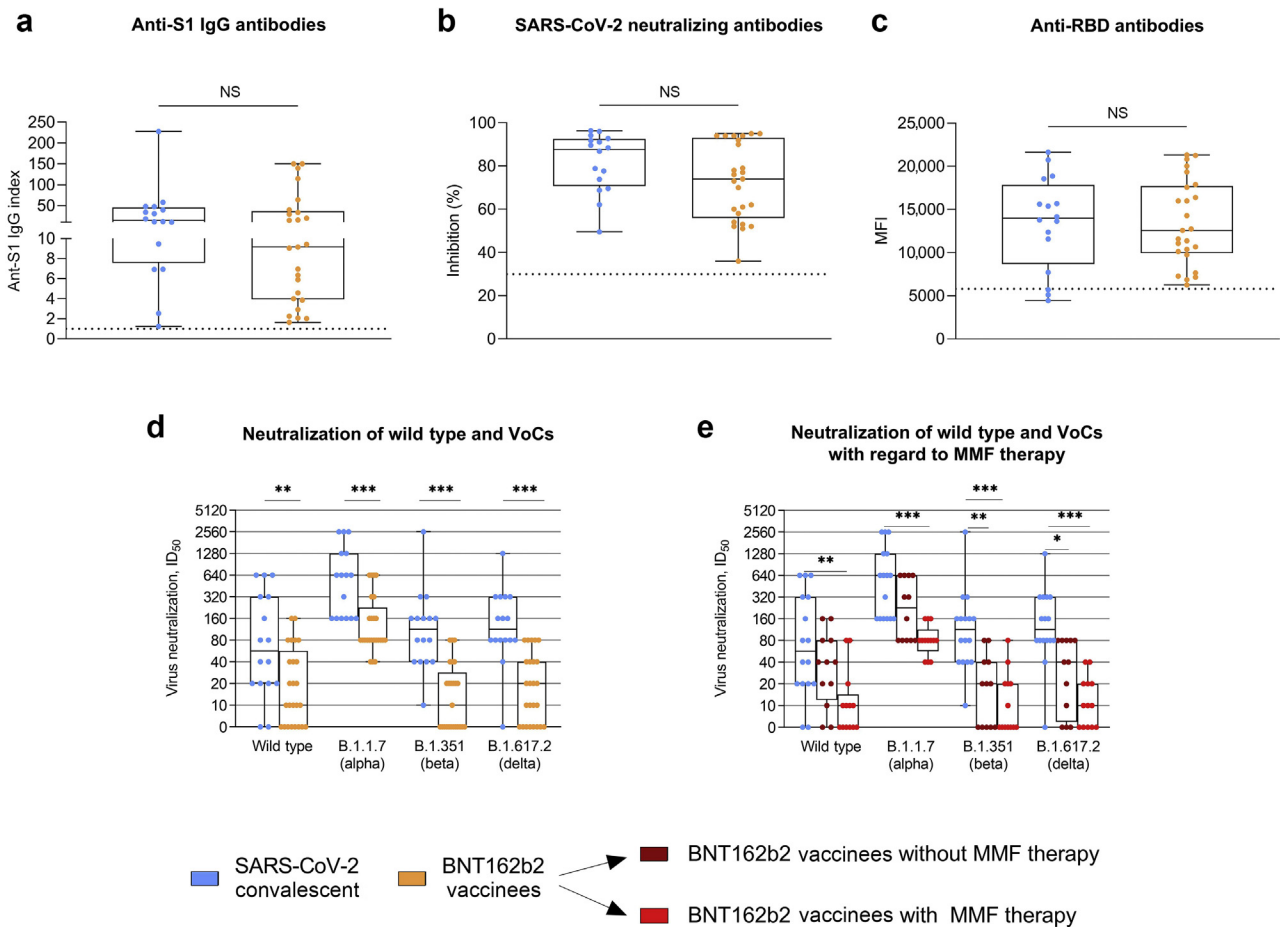
**Figure 1 | Neutralization of wild type, B.1.1.7, B.1.351, and B.1.617.2 in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) convalescent and 2-dose BNT162b2 mRNA vaccinated kidney transplant recipients.** (**a**) SARS-CoV-2 IgG antibodies in 16 SARS-CoV-2 convalescent and 25 seroconverted BNT162b2 vaccinated kidney transplant recipients 2 to 3 months after infection or prime vaccination, respectively. The dashed black line represents the cutoff for detection. A semiquantitative index $\geq 1$ defined positivity. (**b**) SARS-CoV-2 neutralizing antibodies, as determined by a surrogate virus neutralization test in SARS-CoV-2 convalescent and BNT162b2 vaccinated transplant recipients. The dashed black line represents the cutoff for detection. Binding inhibition $\geq 30\%$ defined positivity. (**c**) Antibodies against the SARS-CoV-2 receptor-binding domain (RBD) protein, as determined by a bead-based assay, in SARS-CoV-2 convalescent and BNT162b2 vaccinated kidney transplant recipients. The mean fluorescence intensity (MFI) value of the reactivity is given on the y axis, with the dashed black line indicating the cutoff for detection. (**d**) Titers of neutralizing antibodies against wild type, B.1.1.7, B.1.351, and B.1.617.2 variants of concern (VoCs) in SARS-CoV-2 convalescent and BNT162b2 vaccinated kidney transplant recipients, as determined by serial 2-fold serum dilutions using VeroE6 target cells. The $ID_{50}$ equals the serum dilution that inhibits 50% of the infectivity. (**e**) Titers of neutralizing antibodies against wild type, B.1.1.7, B.1.351, and B.1.617.2 VoCs in 16 SARS-CoV-2 convalescent kidney transplant recipients 2 to 3 months after hospitalization compared with 12 and 13 2-dose BNT162b2 vaccinated kidney transplant recipients with and without mycophenolate mofetil (MMF) maintenance therapy, respectively. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. NS, nonsignificant.

target epitopes, convalescent KTRs showed a broader reactivity against various SARS-CoV-2 target epitopes with significantly higher anti-S2 and anti-nucleocapsid antibody levels compared with vaccinated KTRs (for both, $P < 0.001$; Supplementary Figure S1A). No significant differences in antibody levels to the S1 proteins of the 4 common cold coronaviruses were detected in convalescent compared with vaccinated KTRs (Supplementary Figure S1B).

In a full virus assay, convalescent KTRs had significantly higher activity of neutralizing antibodies against wild-type and all tested VoCs with a median (IQR) serum dilution that inhibits 50% of the infectivity ($ID_{50}$) of 60 (20–320) for neutralization against the wild-type, 640 (160–1280) for

B.1.1.7, 120 (40–160) for B.1.351, and 120 (80–320) for B.1.617.2 compared with 2-dose vaccinated KTRs with a median (IQR) $ID_{50}$ of 10 (0–60), 80 (80–240), 10 (0–30), and 20 (0–40), respectively ($P < 0.01$ for wild type and $P < 0.001$ for B.1.1.7, B.1.351, and B.1.617.2; Figure 1d). Higher neutralizing activity against the wild type and all tested VoCs in COVID-19 convalescent KTRs seemed to be independent of the initially causative SARS-CoV-2 strain (Supplementary Figure S2). In addition, KTRs infected with the wild-type strain (N = 8) were analyzed separately and showed significantly higher neutralizing activity against the wild type and all tested VoCs compared with 2-dose vaccinated KTRs (Supplementary Figure S3). Neutralization against B.1.1.7 was

higher compared with neutralization against B.1.351 or B.1.617.2 in both convalescent and vaccinated KTRs, which has also recently been demonstrated for BNT162b2 vaccinated and COVID-19 convalescent healthy cohorts.[6,7]

Kantauskaite *et al.* showed that seroconversion in SARS-CoV-2–vaccinated KTRs is impaired in patients on mycophenolate mofetil (MMF) maintenance therapy.[8] As no patient remained on MMF therapy during COVID-19 infection, it was not possible to differentiate the effect of infection from cessation of immunosuppression in mounting a broad humoral response in our cohort. However, cessation of MMF does not exclusively explain the higher neutralization titers in COVID-19–infected KTRs as seroconverted KTRs without antimetabolite therapy (12 of 25, 48%) still showed lower neutralization titers against B.1.351 and B.1.617.2 compared with COVID-19 convalescent KTRs (Figure 1e).

Despite detectable seroconversion in commercially available assays, 8 of 25 (32%), 12 of 25 (48%), and 8 of 25 (32%) 2-dose vaccinated KTRs did not show neutralization against wild type, B.1.351, or B.1.617.2, respectively. In contrast, only 2 of 16 (13%) and 1 of 16 (6%) COVID-19 convalescent KTRs did not show detectable neutralizing activity against wild type and B.1.617.2, respectively. Until now, it was not possible to define humoral or cellular cutoff values that confer protective immunity. To address this question, Khoury *et al.* modeled SARS-CoV-2 immune protection across different convalescent and vaccine studies. They estimated a "50% protective neutralization level" at an *in vitro* neutralization titer ($ID_{50}$) between 1:10 and 1:30, which best predicted protection against severe COVID-19.[9] However, these estimates are derived from the general population and may not be applicable to immunosuppressed patients.

Our data suggest that 60 to 80 days after SARS-CoV-2 infection, most convalescent KTRs showed strong neutralization against all tested VoCs. In contrast, at least one-third of vaccinated KTRs showed insufficient neutralization against the VoCs B.1.351 or B.1.617.2, even though antibodies were detectable in commercially available tests. Therefore, a third booster dose seems reasonable even in vaccinated KTRs with seroconversion to protect against breakthrough infections caused by VoCs.

### DISCLOSURE

All the authors declared no competing interests.

### DATA STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

### SUPPLEMENTARY MATERIAL

Supplementary File (Word)
**Supplementary Study Design.**
**Supplementary Methods.**
**Table S1.** Baseline characteristics of kidney transplant recipients at the time of COVID-19 hospitalization or prime vaccination.
**Table S2.** Clinical courses of COVID-19 in infected kidney transplant recipients.
**Table S3.** Management of immunosuppression in COVID-19–infected kidney transplant recipients.
**Figure S1.** IgG antibodies against different SARS-CoV-2 target epitopes and against the spike S1 protein of common cold coronaviruses in SARS-CoV-2 convalescent and 2-dose BNT162b2 vaccinated kidney transplant recipients.
**Figure S2.** Neutralization against wild type, B.1.1.7, B.1.351, and B.1.617.2 variants of concern in SARS-CoV-2 convalescent kidney transplant recipients stratified for causative SARS-CoV-2 strain and in 2-dose BNT162b2 vaccinated kidney transplant recipients.
**Figure S3.** Neutralization of wild type, B.1.1.7, B.1.351, and B.1.617.2 in wild-type SARS-CoV-2 convalescent and 2-dose BNT162b2 mRNA vaccinated kidney transplant recipients.
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# Observations on improving COVID-19 vaccination responses in kidney transplant recipients: heterologous vaccination and immunosuppression modulation

**To the editor:** Solid organ transplant recipients have a weaker humoral response to coronavirus disease 2019 (COVID-19) vaccination because of several factors, including lymphopenia associated with immunosuppressive therapies (particularly belatacept, antiproliferative drugs, and steroids).[1] Because of the high probability of severe COVID-19 symptoms in this at-risk population,[2] a third vaccine dose has been proposed for immunocompromised patients by the French National Authority for Health to improve humoral responses and vaccine efficiency.[3] Despite this improved vaccination schedule, >30% of kidney transplant recipients (KTRs) do not develop a humoral response and remain at risk of severe COVID-19 infection.

ChAdOx1-nCov vaccine (i.e., AstraZeneca) has been sparingly used by transplant centers, because of the low representation of patients with vulnerability in the initial trial[4] but also its rare but serious thrombotic complications.[5] Recently, emerging data reported that heterologous vaccination using an mRNA booster after ChAdOx1-nCov primed vaccination induced a good—and in some cases an even better—humoral response than exclusive mRNA vaccination.[6] There are currently no data that assess the benefit of heterologous vaccination in solid organ transplant recipients, or whether this can improve the humoral response. A total of 373 KTRs from our institution had a serologic assessment 1 month after the third vaccine injection (screening and binding antibody unit [BAU]/ml quantification of anti-spike IgG by ECLIA Roche, Architect Abbott, or Diasorin). Among them, 28 had a heterologous vaccination schedule (ChAdOx1-nCov priming, 1 or 2 injections, followed by 1 or 2 mRNA injections), and 345 received 3 mRNA injections. On the basis of established risk factors of nonhumoral response after mRNA vaccination, we identified a matched 2:1 control cohort having received 3 mRNA vaccines (mRNA exclusive) based on age ($\pm 5$ years), lymphopenia ($<1500/mm^3$), and use of antiproliferative drugs and steroids. Conditional logistic regression was used to compare heterologous and mRNA exclusive cohorts. The average age of both cohorts was 59 years, 71% received antiproliferative drugs, 39% received steroids, and the mean lymphocyte count was $1700/mm^3$. There was a trend of lower allograft function (assessed by the Modification of Diet in Renal Disease) in the heterologous cohort (44.6 vs. 51.5 ml/min; $P = 0.06$; Table 1). No difference in serious adverse events was observed among patients from the 2 groups. Median times of serologic screening in the heterologous group and the mRNA exclusive group were 33 and 34 days, respectively. Seroconversion (i.e., anti-spike IgG superior to laboratory threshold) was observed in 75% of patients with heterologous vaccination and 67.8% of patients with mRNA exclusive vaccination (odds ratio, 1.72; 95% confidence interval, 0.59–4.99; $P = 0.32$). Mean anti-spike IgG titers were 159 BAU/ml in the heterologous group and 125 BAU/ml in the mRNA exclusive group ($P = 0.36$; Figure 1). Recent data by Behrens et al. demonstrated a higher immune response induced by a heterologous schedule, including neutralization of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) delta variant.[7] To our knowledge, we report the first study assessing humoral responses to a heterologous vaccination schedule in immunocompromised KTRs. Seroconversion rates and antibody titers induced by heterologous vaccination were at least equal to mRNA-exclusive vaccination in immunocompromised transplant recipients; although they trended higher in the heterologous group, this did not reach statistical significance because of the small cohort size. Moreover, the lower allograft function in the heterologous cohort may have weakened the observed humoral response.[1] Overall, heterologous vaccination appears to induce a robust humoral response in KTRs and may be considered to improve vaccine response in this immunocompromised population.

Otherwise, there are important concerns for KTRs treated with belatacept, the only costimulation blocker that has received approval for clinical use.[8] Although poor humoral responses following 2 mRNA vaccine injections in KTRs treated with belatacept has been well demonstrated,[9–11] whether a third dose could overcome these issues, as in patients receiving conventional therapy, remains controversial. Indeed, published rates of seroconversion vary dramatically