KI REPORTS
KIReports.org

Humoral Response to Heterologous SARS-CoV-2 Vaccination in Kidney Transplant Patients Is Heterogeneous and Dose Dependent

Mariana Seija[1,2,11], Florencia Rammauro[3,4,11], Javier Noboa[1,4], José Santiago[1], Natalia Orihuela[5], Catherine Zulberti[5], Danilo Machado[6], Cecilia Recalde[6], Rossana Astesiano[1], Federico Yandián[1], Victoria Frantchez[7], Ana Guerisoli[1], Álvaro Morra[5], Daniela Cassinelli[8], Cecilia Coelho[8], Belén de Aramburu[8], Paulina González-Severgnini[8], Romina Moreno[8], Aldana Pippolo[8], Gabriela López[9], Mónica Lemos[9], Lorena Somariva[9], Eliana López[9], Soledad Fumero[9], Carla Orihuela[9], Ana Laura Suárez[2], Rosalía Rodríguez[6], Gonzalo Acuña[6], Victoria Rabaza[6], Nancy Perg[6], Rossana Cordero[6], Cristina Reisfeld[6], Paula Olivera[6], Paola Montero[6], Cecilia Nogueira[6], Catheryn Nalerio[5], Sergio Orihuela[5], Lilián Curi[5], Ema Bugstaller[6], Otto Pritsch[3,4], Marcelo Nin[1,5], Oscar Noboa[1] and Sergio Bianchi[2,10]

[1]Centro de Nefrología, Hospital de Clínicas, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay; [2]Departamento de Fisiopatología, Hospital de Clínicas, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay; [3]Laboratorio de Inmunovirología, Institut Pasteur de Montevideo, Montevideo, Uruguay; [4]Departamento de Inmunobiología, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay; [5]Centro de Trasplante INU, Hospital Italiano, Montevideo, Uruguay; [6]Centro de Trasplante, Hospital Evangélico, Montevideo, Uruguay; [7]Cátedra de Enfermedades Infecciosas, Hospital de Clínicas, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay; [8]Students of Scientific Methodology, Medical Doctor Degree, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay; [9]Departamento de Enfermería, Hospital de Clínicas, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay; and [10]Laboratorio de Genómica Funcional, Institut Pasteur de Montevideo, Montevideo, Uruguay

Correspondence: Sergio Bianchi, Departamento de Fisiopatología, Hospital de Clínicas, Facultad de Medicina, Universidad de la República, Av. Italia s/n, Montevideo 11600, Uruguay. E-mail: sbianchi@fmed.edu.uy

[11]MS and FR contributed equally to this work.

Received 6 April 2022; revised 29 April 2022; accepted 2 May 2022; published online 13 May 2022

Kidney Int Rep (2022) **7,** 1887–1892; https://doi.org/10.1016/j.ekir.2022.05.005
KEYWORDS: COVID-19; SARS-CoV-2 vaccine; transplantation

Vaccination is the cornerstone in the fight against the ongoing COVID-19 pandemic.[1–5] Kidney transplant recipients (KTRs) had a weak immunologic response after 2 doses of either vaccine scheme.[1–3,5,S1–S9] Severe COVID-19 breakthrough infection has been reported in KTR with 2 doses of mRNA vaccine.[3,S10,S11] On the basis of these data and evidence that boosters improve immunogenicity, international societies recommended a third dose in KTR and recently a fourth dose in a case-by-case basis.[4–8,S12–S14]

Different methods to assess immune response after vaccines, such as humoral and T cell function, have been used. However, many of these studies cannot be compared because of the lack of standardized assays.[9] The World Health Organization International recommended standardization of antibody measurement by using binding antibody units per milliliter (BAU/ml) for binding serologic tests.[S9] A cutoff value of 264 BAU/ml anti-receptor binding domain (RBD) IgG had been correlated with protection of symptomatic disease in the healthy population,[S15] whereas Barrière *et al*.[S16] proposed a 3-zone classification of anti–SARS-CoV-2 antibody levels for patients with cancer: a group of nonresponders with specific antibody levels <40 BAU/ml, a group that responds to vaccines with levels >260 BAU/ml, and a third area of uncertainty between these 2 values. Although they may be approximate values, the idea of this kind of classification can also be useful for its application in KTR patients.

We are carrying on a multicenter, prospective, observational study for monitoring anti–SARS-CoV-2 specific IgG in KTR.[1] Here, we present a second report

Table 1. Clinical characteristics of patients according to the number of doses to achieve seroconversion after 3D homologous and 4D heterologous SARS-CoV-2 vaccinations

	Seroconversion achieved after SARS-CoV-2 vaccination					
Variables	2D After 2 doses	3D After first booster	4D After second booster	No seroconversion	Total	*P* value
n (%)	29 (26.6)	35 (31.2)	15 (13.8)	31 (28.4)	109 (100)	
Vaccine scheme						
Heterologous, *n* (%)	23 (79)	28 (82.5)	15 (100)	26 (84)	92 (84)	0.326
Homologous, *n* (%)	6 (21)	6 (17.5)		5 (16)	17 (16)	
Age, yr, median (IQR)	55 (40–62)	54 (45–77)	59 (54–65)	54 (45–77)	58 (45–72)	0.245
Sex, men, *n* (%)	17 (57)	25 (73)	8 (28)	18 (62)	68 (64)	0.564
Diabetes mellitus, *n* (%)	3 (10)	8 (23)	6 (40)	7 (23)	24 (22)	0.160
Type of transplant, *n* (%)						
Kidney	28 (97)	33 (97)	15 (100)	30 (90)	106 (98)	0.687
Kidney-pancreas	1 (3)	1 (3)	—	—	2 (2)	
Time of transplant, mo, median (IQR)	113 (30–148)	84 (32–174)	68 (34–155)	45 (32–112)	68 (32–146)	0.415
Patients in the first year of transplant, *n* (%)	2 (7)	2 (6)	1 (7)	2 (7)	7 (7)	0.998
Triple immunosuppression, *n* (%)	17 (59)	22 (65)	11 (73)	26 (87)	76 (71)	0.097
Antimetabolite, *n* (%)						0.000
None	10 (35)	4 (12)	1 (7)	1 (3)	16 (15)	
Mycophenolate	13 (45)	28 (85)	13 (87)	29 (96.7)	83 (78)	
Azathioprine	6 (20)	1 (3)	1 (7)	0 (0)	8 (7)	
CNI, *n* (%)						0.312
None	3 (11)	5 (15)	1 (7)	1 (3.3)	10 (9)	
Tacrolimus	23 (79)	22 (65)	9 (60)	25 (83)	79 (73)	
Cyclosporine	3 (10)	7 (21)	5 (33)	4 (13)	19 (17)	
Prednisone, *n* (%)	28 (97)	32 (94)	13 (87)	28 (9%)	101 (94)	0.654
Everolimus, *n* (%)	10 (35)	10 (30)	1 (6.7)	1 (3)	22 (21)	0.006
Rituximab, *n* (%)	0	2 (6)	0	0	2 (2)	0.224
Thymoglobulin, *n* (%)	15 (47)	5 (15)	3 (20)	6 (21)	19 (18)	0.931
Rejection in last 3 mo, *n* (%)	0	0	1 (6.7)	0	8 (2.9)	0.103
Lymphocyte count, cells/μl, median (IQR)	2400 (1800–2552)	2010 (1560–2570)	2600 (1800–3440)	1900 (1288–2286)	2108 (1560–2600)	0.073
Lymphocyte count < 1400 cells/l, *n* (%)	2 (7)	6 (18)	3 (20)	10 (36)	21 (20)	0.058
Serum creatinine, μmol/l, median (IQR)	109 (90–134)	113 (96–141)	114 (87–122)	119 (87–138)	114 (92–134)	0.886
eGFR ml/min per 1.73 m^2, mean ± SD	62 ± 18	56 ± 21	59 ± 17	57 ± 20	58 ± 19	0.661
IgG anti-RBD SARS-CoV-2 (BAU/ml), median (IQR)						
After 2D	42 (23–173)	0	0	0	0 (0–8)	0.000
After 3D	2453 (1255–3505)	179 (41–412)	0	2.5	25 (0–1770)	0.000
After 4D	4659 (2778–13,394)	621 (254–1913)	74 (33–283)	0	254 (7–2775)	0.000

2D, 2 doses; 3D, 3 doses; 4D, 4 doses; CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate using CKD-EPI Formula; IQR, interquartile range; RBD, receptor binding domain; triple immunosuppression: antimetabolite + calcineurin inhibitor + prednisone.
Homologous vaccination: 3 doses of BNT162b2 mRNA vaccine 30 days apart; heterologous vaccination: 2 doses of inactivated SARS-CoV-2 vaccine (CoronaVac) and 2 BNT162b2 mRNA boosters 30 days apart. Lymphocyte counts were assessed previous to vaccination. The threshold to define seroconversion was 10 BAU/ml (black dotted line).

on antibody levels after 4 doses of heterologous vaccination and 3 doses of homologous scheme stratified by the number of doses required to achieve seroconversion compared with healthy control.

In Uruguay, 90% of KTRs received inactivated SARS-CoV-2 (CoronaVac, Sinovac Biotech Ltd., Beijing, People's Republic of China) and 10% BNT162b2 mRNA (Pfizer, Manhattan, NY/BioNTech, Mainz, Germany), the most used vaccines worldwide, according to definitions of the Ministry of Public Health (details in the Supplementary Material). After the demonstration that only 29% of KTR seroconverted,[1] the Ministry of Public Health approved heterologous and homologous booster/s in all KTRs, irrespectively of seroconversion status. The heterologous vaccination group received 2 doses of inactivated SARS-CoV-2 (CoronaVac) and 2 BNT162b2 mRNA boosters 30 days apart, and the homologous vaccination group received 3 doses of the BNT162b2 mRNA vaccine (Supplementary Figure S1).

Among all 1400 KTRs in follow-up in Uruguay (90% received 2 doses of CoronaVac and 10% 2 doses of mRNA-based vaccines), all were invited to participate in the study. Of these, 289 patients accepted to participate and were enrolled to study immunologic response to SARS-CoV-2 after 2 doses of vaccines.[1] From this initial cohort, we only included for the present analysis patients who had blood samples available after 30 days of 2 initial doses and boosters according to the vaccination scheme (heterologous $n = 92$; homologous $n = 17$). A total of 180 patients who did

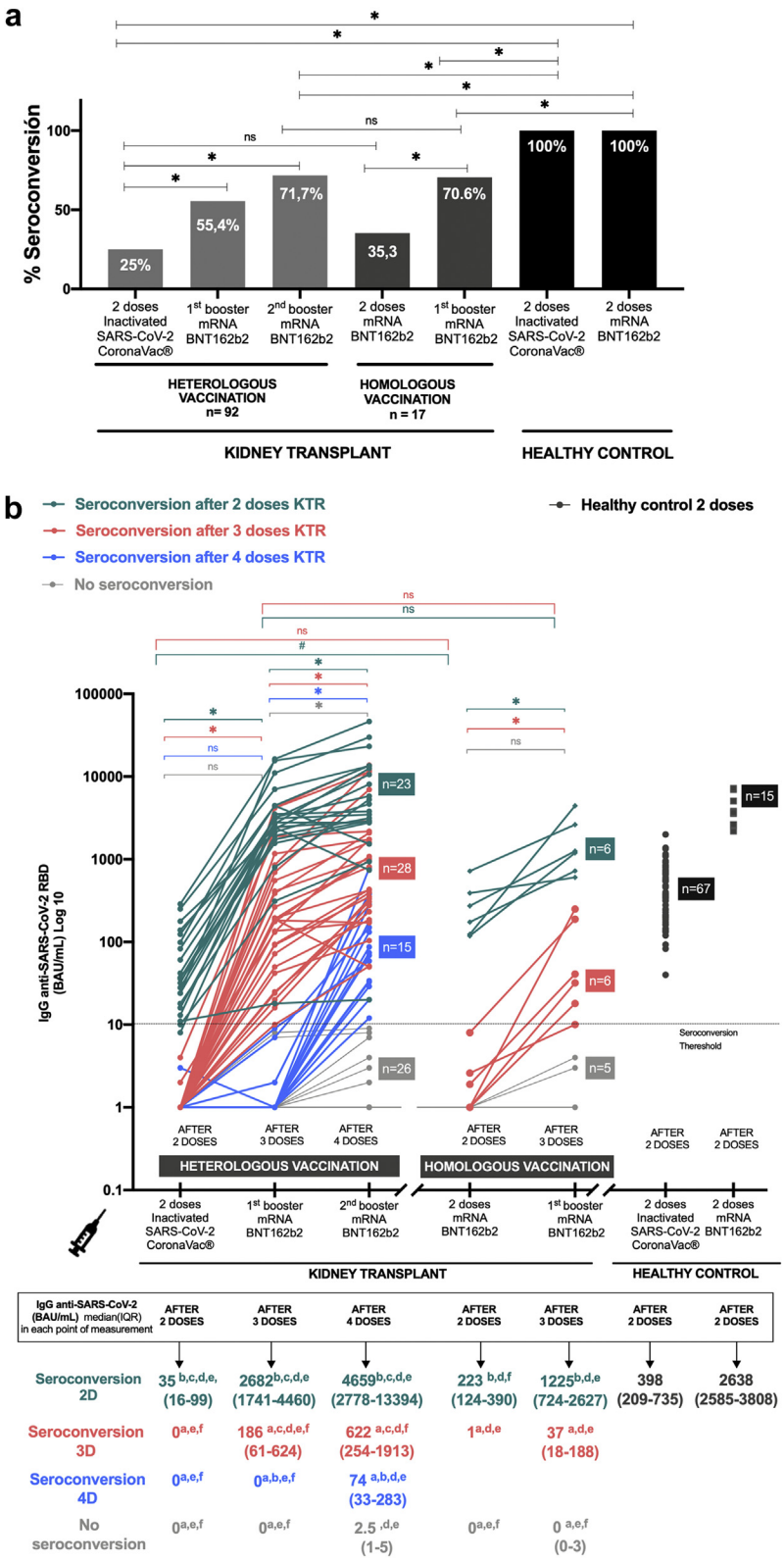

Figure 1. Serologic response after 3D-homologous and 4D-heterologous SARS-CoV-2 vaccination in KTRs. (a) Percentage of seroconversion. $^{*}P < 0.05$. (b) Anti-RBD SARS-CoV-2 IgG levels (BAU/ml) according to the number of doses to achieve seroconversion. Friedman test and Bonferroni correction were used for paired analysis within each seroconversion group (green: seroconversion-2D; pink: seroconversion-3D; blue: seroconversion-4D; gray: no seroconversion), $^{*}P < 0.05$. Comparison between homologous and heterologous vaccination was performed in seroconversion groups 2D and 3D in each point of measurement with Mann-Whitney, $^{\#}P < 0.05$. In each point of antibody measurement, Kruskal-Wallis test and Bonferroni *post hoc* test were used to compare between seroconversion and control groups. Pairwise analysis at each point of measurement (after 2 doses, 3 doses, and 4 doses of vaccine): $^{a}P < 0.05$ versus seroconversion 2D group; $^{b}P < 0.05$ versus seroconversion 3D group; $^{c}P < 0.05$ versus seroconversion 4D; $^{d}P < 0.05$ versus no seroconversion; $^{e}P < 0.05$ versus control (continued)

not have all the samples available were excluded. Patients included in the study had no prior confirmed COVID-19 diagnosis until homologous or heterologous vaccination scheme was completed.

All individuals declared not to have had any sign or symptom compatible with COVID-19 before vaccination. All patients underwent serologic testing for anti-RBD specific IgG 30 days after 2 initial doses and posteach booster. Patients were stratified into the following 4 groups according to the number of doses required to achieve seroconversion: seroconversion achieved after 2 doses (seroconversion-2D), 3 doses (seroconversion-3D), 4 doses (seroconversion-4D), and no seroconversion. In addition, a subanalysis between homologous or heterologous vaccine schemes was performed. Control group was constituted of 82 healthy individuals, fully vaccinated, with 2 doses of CoronaVac or BNT162b2 between April 1 and May 31, according to the recommendations of the Uruguayan National Health Authority considering age and comorbidities. No specific anti-RBD IgG antibodies were found before vaccination in the serum samples of the healthy control group (baseline seronegative). We do not had information on this regard in the KTR group. Seroconversion was defined as anti–RBD-SARS-CoV-2 specific IgG >10 BAU/ml.

Baseline patients' characteristics according to seroconversion groups are detailed in Table 1 and according to vaccine scheme in Supplementary Tables S1 and S2. Seroconversion after 2 doses was 26% globally, and it improved an additional 31.2% with the third dose (30.4% heterologous and 34.7% homologous vaccination, respectively) (Figure 1a). In the heterologous regimen, 21% of the patients seroconverted after the fourth dose of BNT162b2 mRNA (Figure 1a). At the end of heterologous and homologous vaccination, 71.7% and 70.6% of the patients seroconverted, respectively. In the seronegative group, a greater proportion of patients were treated with mycophenolate (96.7% vs. 45%, 85% and 87% in Seroconversion-2D, -3D, and -4D groups: respectively, $P = 0.000$) (Table 1); they also had less time since transplantation (median of 45 months vs. 113, 84, and 68 months in Seroconversion-2D, -3D, and -4D, respectively, $P = 0.415$) and lower lymphocyte count (median 1900/μl vs. 2400, 2010, and 2600/μl inSeroconversion-2D, -3D, and -4D, $P = 0.415$), statistically nonsignificant.

Serum levels of anti-RBD IgGs depend on the number of doses to achieve seroconversion (Figure 1b; green, pink, blue, and gray). Anti-RBD IgG serum levels were significantly higher in the Seroconversion-2D group (Figure 1b; green) in comparison with Seroconversion-3D and -4D (Figure 1b; pink and blue) at the 3-time point measurement (after 2, 3, and 4 doses) in both vaccine schedules.

In the heterologous regimen, anti-RBD IgG increased with each dose. For example, in the Seroconversion-2D-group, we observed a 14-fold after the third dose and 1.7 after the fourth dose (from a median of 186 to 2682 and 4659 BAU/ml respectively, $P = 0.000$). Meanwhile, with the homologous scheme, antibody titer increased only 5.5-fold after the third dose (from a median of 223 to 1225 BAU/ml, $P = 0.000$).

A comparison between the heterologous and homologous schemes at each point of measurement can be found in Figure 1b. Antibody levels were higher in patients after 2 doses of BNT162b2 mRNA than with 2 doses of CoronaVac (median 223 BAU/ml [interquartile range (IQR) 124–390] vs. 35 BAU/ml [IQR 16–99], $P = 0.003$). After the third dose of the heterologous scheme, the antibody response was enhanced compared with the homologous (seroconversion 2D, median 2682 [IQR 1741–4460] vs. 1225 BAU/ml [724–2627], $P = 0.158$ and in seroconversion 3D group median186 BAU/ml [IQR 61–624] and 37 BAU/ml [IQR 18–188], $P = 0.074$). Although not significant, these differences have a tendency to be considered when defining possible booster dose regimens.

Only patients in the seroconversion-2D group after the third and fourth doses in heterologous vaccination achieved antibody titers comparable with 2 doses of BNT162b2 mRNA in the healthy group. Meanwhile, only 26.7% of patients with seroconversion after the fourth dose have levels of antibody >264 BAU/ml (Supplementary Figure S2 and Supplementary Material).

In multivariate analysis, variables associated with low antibody level (<264 BAU/ml) after heterologous or homologous vaccination were mycophenolate and time post-KT (Supplementary Table S3). We used antibody level of 264 BAU/ml, suggested by other authors as a correlate of protection in healthy individual[S15] and KTR,[S17] to compare the number of patients in each group that reached this level, making it easier to visualize the response to vaccines. We are not

Figure 1. (continued) group vaccinated with inactivated SARS-CoV-2; $^{\dagger}P < 0.05$ versus control group vaccinated with BNT162b2 mRNA. Homologous vaccination: 3 doses of BNT162b2 mRNA vaccine 30 days apart; heterologous vaccination: 2 doses of inactivated SARS-CoV-2 vaccine (CoronaVac) and 2 BNT162b2 mRNA boosters 30 days apart. Healthy control received 2 doses of inactivated SARS-CoV-2 vaccine (CoronaVac) or 2 doses of BNT162b2 mRNA vaccine 28 days apart. Threshold to define seroconversion was 10 BAU/ml (black dotted line). 2D, 2 doses; 3D, 3 doses; 4D, 4 doses; BAU, binding antibody unit; IQR, interquartile range; KTR, Kidney transplant recipient; ns, not significant; RBD, receptor-binding domain.

suggesting a specific level of anti–SARS-CoV-2 antibodies that correlates with protection in KTR. During the 5-month follow-up, 10 KTRs had COVID-19 confirmed by polymerase chain reaction during the second wave of the pandemic in Uruguay (between December 2021 and February 2022). Two rejection episodes and 1 IgA nephropathy recurrence were reported. The 2 patients with rejection belonged to the group with higher antibody titers (seroconversion-2D, heterologous: 17,413 and 3470 BAU/ml) (details in the Supplementary Material and Supplementary Table S4). No deaths were reported.

We provided data that heterologous vaccination with 4 doses induced an enhanced humoral response in KTR. Although antibody titers are highly heterogeneous, it could be predicted by taking into consideration the number of doses to achieve seroconversion. We described the following 4 different groups: seroconversion-2D, -3D, -4D, and no response associated with the magnitude of humoral immune response after vaccination. These differences in humoral response were associated with the use of mycophenolate or everolimus and time since kidney transplant.

Patients with seroconversion after 2 doses of either vaccination scheme have higher antibody titers than those who seroconverted after the third or fourth dose and were similar to healthy control after 30 days of 2 doses of mRNA vaccines.

Although nonstatistically significant, heterologous vaccination achieved higher antibody titers than homologous. In the healthy population, heterologous vaccination had a better immunologic response than homologous,[6,S18] but in KTR, this effect cannot be found.[S16] This difference could be since prior studies included groups of patients with heterogeneity in immunologic response.[S19]

Potential biases for this study are the low number of patients in the homologous vaccination group; most patients were approximately 50 years old and lacked evaluation of cellular immunity.

Our results provide additional evidence of heterogeneous humoral response among KTRs after 4 doses of heterologous vaccination or 3 doses of mRNA homologous. Although there is information of response to the third and fourth dose vaccinations, as far as we know, it is the first work to study immune response after 4 doses of SARS-CoV-2 vaccines administered to every kidney transplant patient without considering seroconversion status. Patients who seroconverted after 2 doses achieved higher levels of SARS-CoV-2 antibody than those with seroconversion after the third or fourth dose and reached antibody titer similar to healthy control. Meanwhile, most patients with seroconversion after the fourth dose have low levels of antibodies. These differences in immune response depend on mycophenolate use and time since kidney transplant. This stratification strategy according to the number of doses to achieve seroconversion could be used to personalize boosters in KTR.

Heterologous vaccination seemed to induce an enhanced humoral response compared with homologous vaccination. However, future studies with larger size groups stratified by the magnitude of humoral response are needed to settle this debate.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

This work was supported by Fondo para la Convergencia Estructural del Mercosur (FOCEM, COF 03/11); Agencia Nacional de Investigación e Innovación (ANII), Uruguay; and Fondo de Investigación en Nefrología (FOINE), Hospital de Clínicas, Uruguay.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Figure S1. Types of vaccination schemes and IgG anti-RBD measurement.

Figure S2. Percentage of patients with IgG anti-SARS-CoV-2 RBD above 264 BAU/ml after 4-dose heterologous and 3-dose homologous vaccination scheme.

Table S1. Clinical characteristics of patients according to number of doses to achieve seroconversion after 4-dose heterologous SARS-CoV-2 vaccination.

Table S2. Clinical characteristics of patients according to number of doses to achieve seroconversion after 3-dose homologous SARS-CoV-2 vaccination.

Table S3. Multivariate analysis for IgG anti-RBD SARS-CoV-2 $<$ 264 BAU/ml as a surrogate of protection.

Table S4. Adverse events reported post-vaccination.

Supplementary References.

STROBE Statement.

REFERENCES

1. Seija M, Rammauro F, Santiago J, et al. Comparison of antibody response to SARS-CoV-2 after two doses of inactivated virus and BNT162b2 mRNA vaccines in kidney transplant. *Clin Kidney J*. 2021;15:527–533. https://doi.org/10.1093/ckj/sfab291
2. Benotmane I, Gautier-Vargas G, Cognard N, et al. Low immunization rates among kidney transplant recipients who received 2 doses of the mRNA-1273 SARS-CoV-2 vaccine. *Kidney Int*. 2021;99:1498–1500. https://doi.org/10.1016/j.kint.2021.04.005
3. Quiroga B, Soler MJ, Ortiz A, et al. Safety and immediate humoral response of COVID-19 vaccines in chronic kidney disease patients: the SENCOVAC study. *Nephrol Dial Transplant*. 2021;gfab313. https://doi.org/10.1093/ndt/gfab313

4. Benotmane I, Gautier G, Perrin P, et al. Antibody response after a third dose of the mRNA-1273 SARS-CoV-2 vaccine in kidney transplant recipients with minimal serologic response to 2 doses. *JAMA*. 2021;326:1063. https://doi.org/10.1001/jama.2021.12339

5. Westhoff TH, Seibert FS, Anft M, et al. A third vaccine dose substantially improves humoral and cellular SARS-CoV-2 immunity in renal transplant recipients with primary humoral nonresponse. *Kidney Int*. 2021;100:1135–1136. https://doi.org/10.1016/j.kint.2021.09.001

6. Reynolds CJ, Gibbons JM, Pade C, et al. Heterologous infection and vaccination shapes immunity against SARS-CoV-2 variants. *Science*. 2022;375:eabm0811. https://doi.org/10.1126/science.abm0811

7. Caillard S, Thaunat O, Benotmane I, et al. Antibody response to a fourth messenger RNA COVID-19 vaccine dose in kidney transplant recipients: a case series. *Ann Intern Med*. 2022;175:455–456. https://doi.org/10.7326/L21-0598

8. Prendecki M, Thomson T, Candice L, et al. Comparison of humoral and cellular responses in kidney transplant recipients receiving BNT162b2 and ChAdOx1 SARS-CoV-2 vaccines. medRxiv. Published July 14, 2021. https://doi.org/10.1101/2021.07.09.21260192

9. Ikizler TA, Coates PT, Rovin BH, Ronco P. Immune response to SARS-CoV-2 infection and vaccination in patients receiving kidney replacement therapy. *Kidney Int*. 2021;99:1275–1279. https://doi.org/10.1016/j.kint.2021.04.007