Predictive Factors for Humoral Response After 2-dose SARS-CoV-2 Vaccine in Solid Organ Transplant Patients

Olivier Marion, MD,1,2,3 Arnaud Del Bello, MD,1,3 Florence Abravanel, PharmD, PhD,2,3,4 Stanislas Faguer, MD, PhD,1,3 Laure Esposito, MD,1 Anne Laure Hebral, MD,1 Julie Bellière, MD, PhD,1,3 Jacques Izopet, PharmD, PhD2,3,4 and Nassim Kamar, MD, PhD1,2,3

Background. A weak immunogenicity has been reported in solid organ transplant (SOT) recipients after 2 doses of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine. The aim of this retrospective study was to identify the predictive factors for humoral response in SOT patients. Methods. Three hundred and ninety-three SOT patients from our center with at least 4 wk of follow-up after 2 doses of mRNA-based vaccine were included in this study. Anti-SARS-CoV-2 spike protein antibodies were assessed before and after vaccination. Results. Anti-SARS-CoV-2 antibodies were detected in 34% of the patients: 33.7% of kidney transplant patients, 47.7% of liver transplant patients, and 14.3% of thoracic transplant patients (P = 0.005). Independent predictive factors for humoral response after vaccination were male gender, a longer period between transplantation and vaccination, liver transplant recipients, a higher lymphocyte count at baseline, a higher estimated glomerular filtration rate and receiving the tacrolimus + everolimus ± steroids combination. Conversely, the nondevelopment of anti-SARS-CoV-2 antibodies after vaccination was associated with younger patients, thoracic organ recipients, induction therapy recipients, and tacrolimus + mycophenolic acid ± steroids recipients. Conclusions. The immunosuppressive regimen is a modifiable predictive factor for humoral response to SARS-CoV-2 vaccine.
response at 4 wk after the second vaccine dose was 34%.\textsuperscript{16} Our aim is to identify the risk factors for humoral response in our cohort of SOT patients.

MATERIALS AND METHODS
On April 26, 1024 out of 2666 SOT patients at our center had received at least 1 vaccine dose. Of these, 393 patients had at least 4 wk follow-up after the second dose (288 kidney transplant patients, 65 liver transplant patients, 35 thoracic transplant patients, and 5 isolated pancreas transplant patients). A comparison between patients with 4 wk of follow-up after the second dose and those with insufficient follow-up is presented in Table S1, SDC, http://links.lww.com/TXD/A382. All the patients received an mRNA-based vaccine (BNT162b2 vaccine, Pfizer-BioNTech, n = 391; mRNA-1273 vaccine, Moderna, n = 2). In accordance with the Francophone Transplantation Society’s recommendation, patients were asked to participate in biological monitoring, including the anti–SARS-CoV-2 spike protein antibodies before and after vaccination, to assess the safety and efficacy of the vaccine. We also retrospectively collected clinical data such as demographic data, the period between transplantation and vaccination, immunosuppressive regimens, and any history of acute rejection. According to French law (Loi Jardé), anonymous retrospective studies do not require Institutional Review Board approval.

Virological Analyses
Anti–SARS-CoV-2 spike protein antibody detection was performed using the Wantai total antibody (IgG/IgM/IgA) microplate assay ELISA test (Beijing Wantai Biological Pharmacy Enterprise, Ltd, China) in 80% of the patients.\textsuperscript{17} The remaining patients were tested with another anti-spike total or immunoglobulin G assay validated by the French National Reference Center.

Statistical Analyses
Continuous variables are presented as means (±SEM). The proportion of patients who developed antibodies is reported with exact binomial 95% confidence interval (CI). Proportions were compared by the $\chi^2$ test or Fisher exact test. Quantitative variables were compared by either the Student $t$ test or the Mann-Whitney test. Independent factors associated with nonresponse to vaccine were examined with a multivariate logistic regression model that used initial inclusion criteria with a significance of $P < 0.05$. A $P$ value of $<0.05$ was considered to be statistically significant. Data analysis was performed using GraphPad Prism version 9.0.2 (GraphPad Software, San Diego, CA) and R (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS
Humoral Response According to the Transplanted Organ
Four weeks after the second vaccine dose, anti–SARS-CoV-2 antibodies were detected in 97 out of 288 kidney transplant patients (33.7%; 95% CI, 28.2%-39.5%), 31 out of 65 liver transplant patients (47.7%; 95% CI, 35.2%-60.5%), 5 out of 35 thoracic transplant patients (14.3%; 95% CI, 4.8%-30.3%), and 1 of the 5 pancreas transplant patients (20.0%; 95% CI, 0.5%-71.6%) ($P = 0.005$). Liver transplant patients were more likely to develop anti–SARS-CoV-2 antibodies compared to other transplant patients (odds ratio [OR] = 2.0; 95% CI, 1.1-3.5). Conversely, thoracic transplant patients developed anti–SARS-CoV-2 antibodies less frequently compared to other transplant patients (OR = 0.3; 95% CI, 0.1-0.8).

Comparison Between Patients With a Humoral Response to the Vaccine and Those Without
Compared with nonresponders, patients who developed anti–SARS-CoV-2 antibodies after vaccination were mainly male and younger with a longer period between transplantation and vaccination (Table 1). With respect to immunosuppression, those who received an induction therapy at transplantation significantly less frequently developed antibodies compared with those who did not (OR = 0.6; 95% CI, 0.4-1.0). However, no difference was observed between polyclonal antibodies and anti–interleukin-2 receptor blockers. Transplant patients who received mycophenolic acid (MPA) (OR = 0.5; 95% CI, 0.3-0.7), steroids (OR = 0.6; 95% CI, 0.3-1.0), or belatacept (OR = 0.3; 95% CI, 0.1-0.7) developed anti–SARS-CoV-2 antibodies significantly less often. Conversely, those who were treated with mammalian target of rapamycin (mTOR) inhibitors were more likely to develop a humoral response (OR = 1.8; 95% CI, 1.1-3.0).

Interestingly, patients who received tacrolimus + MPA with or without steroids developed significantly less antibodies than those treated with tacrolimus + everolimus with or without steroids (27% versus 47%, $P = 0.0004$). The characteristics of patients according to their immunosuppressive regimen are detailed in Table S2, SDC, http://links.lww.com/TXD/A382. SOT recipients who developed anti–SARS-CoV-2 antibodies had a higher lymphocyte count before vaccination compared to nonrecipients. More precisely, when assessed, they had both a higher CD4+ and a higher CD19+ lymphocyte count. Conversely, CD8+ and natural killer cell counts were similar in both groups.

Finally, patients with anti–SARS-CoV-2 humoral response after vaccination had a higher estimated glomerular filtration rate (eGFR) compared with those who did not. This was observed in kidney transplant patients and in non-kidney transplant patients.

Predictive Factors for Humoral Response to SARS-CoV-2 Vaccines
The following variables were included in the multivariate analysis: gender (male versus female), age, the type of organ transplant (liver versus nonliver transplant and thoracic versus nonthoracic transplants), the period between transplantation and vaccination, induction therapy (induction versus no induction), the immunosuppressive regimen (use versus nonuse of MPA, steroids, mTOR inhibitors, or belatacept), the lymphocyte count, and the eGFR at baseline (Table 2).

Male gender, a longer period between transplantation and vaccination, and a higher eGFR level were independent predictive factors for humoral response after vaccination (Table 2). Conversely, younger patients, thoracic organ recipients, MPA, steroid, or belatacept recipients were associated with the nondevelopment of anti–SARS-CoV-2 antibodies after vaccination.

Since patients are treated with a combination of immunosuppressive drugs rather than a single immunosuppressant,
we performed a second multivariate analysis in which we included the most frequent combinations, that is, tacrolimus + MPA ± steroids and tacrolimus + mTOR inhibitors ± steroids, instead of considering each immunosuppressant separately (Table 3). Independent predictive factors for humoral response after vaccination were male gender, a longer period between transplantation and vaccination, liver transplant recipients, a higher eGFR, and receiving the combination of tacrolimus + everolimus ± steroids. Conversely, the non-development of anti–SARS-CoV-2 antibodies after vaccination was associated with younger patients, thoracic organ recipients, induction therapy recipients, and tacrolimus + MPA ± steroid recipients.

DISCUSSION

Several studies have reported weak immunogenicity in SOT patients who are at high risk for severe COVID-19 disease and the related mortality.12,14-16 In this retrospective study, we aimed to determine the predictive factors for humoral response to mRNA-based anti–SARS-CoV-2 vaccine in a large cohort of SOT patients. Our findings were 3-fold: (1) anti–SARS-CoV-2...
antibodies were detected in 34.0% of patients 4 wk after the second vaccine; (2) the humoral response differed significantly according to the grafted organ, that is, the best response was observed in liver transplant patients, and the weakest in thoracic organ transplant patients; and (3) patients receiving mTOR-based immunosuppression with calcineurin inhibitors were more likely to be responders than those on a mycophenolic based immunosuppressive regimen.

Few studies have assessed the humoral response to 2 doses of mRNA vaccine in SOT patients. Most studies have included patients who received 1 type of transplant organ. Anti–SARS-CoV-2 antibodies were detected in 22% to 58.8%. We recently reported a 34% humoral response in 367 patients followed at our center and patients from other centers who had sufficient follow-up. One hundred thirty-four out of 393 patients developed anti–SARS-CoV-2 antibodies (34.0%). We found that the use of mTOR inhibitors was associated with a decreased humoral response, the tacrolimus and mTOR inhibitor combination with or without steroids is associated with a better immunosuppressive regimen. These findings were confirmed by other groups. Conversely, we found that the use of mTOR inhibitors was associated with a decreased humoral response, the tacrolimus and mTOR inhibitor combination with or without steroids is associated with a better immunosuppressive regimen. These findings are of interest since the

TABLE 2.

Predictive factors for humoral response after 2 doses of mRNA-based vaccination (model 1)

|                              | Adjusted multivariable OR | 95% CI          | P        |
|------------------------------|---------------------------|-----------------|----------|
| Male gender                  | 1.964                     | [1.145-3.371]   | 0.012    |
| Age                          | 0.963                     | [0.944-0.982]   | <0.001   |
| Liver transplant (vs nonliver transplant) | 1.469                   | [0.726-2.973]   | 0.275    |
| Thoracic transplant (vs nonthoracic transplant) | 2.024                   | [0.860-4.692]   | 0.009    |
| Time between vaccination and transplantation | 1.004                   | [1.001-1.007]   | 0.005    |
| Induction therapy (vs no induction) | 0.597                   | [0.351-1.015]   | 0.052    |
| Immunosuppressive regimen including MPA | 0.231                   | [0.113-0.473]   | <0.001   |
| Immunosuppressive regimen including steroids | 0.463                   | [0.231-0.929]   | 0.027    |
| Immunosuppressive regimen including mTOR inhibitors | 1.072                   | [0.529-2.173]   | 0.845    |
| Immunosuppressive regimen including belatacept | 0.267                   | [0.092-0.775]   | 0.013    |
| Baseline lymphocyte count     | 1.000                     | [1.000-1.000]   | 0.227    |
| Baseline eGFR                | 1.024                     | [1.011-1.037]   | <0.001   |

Bold P values are significant.
CI, confidence interval; eGFR, estimated glomerular filtration rate; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; OR, odds ratio.

TABLE 3.

Predictive factors for humoral response after 2-dose mRNA-based vaccination (model 2)

|                              | Adjusted multivariable OR | 95% CI          | P        |
|------------------------------|---------------------------|-----------------|----------|
| Male gender                  | 1.691                     | [1.002-2.854]   | 0.045    |
| Age                          | 0.960                     | [0.941-0.980]   | <0.001   |
| Liver transplant (vs nonliver transplant) | 2.291                   | [1.174-4.471]   | 0.013    |
| Thoracic transplant (vs nonthoracic transplant) | 0.196                   | [0.057-0.676]   | 0.009    |
| Time between transplantation and vaccination | 1.005                   | [1.002-1.008]   | <0.001   |
| Induction therapy (vs no induction therapy) | 0.581                   | [0.345-0.977]   | 0.037    |
| Tacrolimus + MPA ± steroids  | 0.462                     | [0.255-0.837]   | 0.009    |
| Tacrolimus + mTOR ± steroids | 2.463                     | [1.139-5.328]   | 0.019    |
| Baseline lymphocytes count   | 1.000                     | [1.000-1.000]   | 0.107    |
| Baseline eGFR                | 1.020                     | [1.008-1.031]   | <0.001   |

Bold P values are significant.
CI, confidence interval; eGFR, estimated glomerular filtration rate; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin inhibitor; OR, odds ratio.
latter regimen has been shown to be efficient and safe after kidney, liver, and heart transplantation.\textsuperscript{24-26} Moreover, we observed that patients who developed anti-SARS-CoV-2 antibodies have both a higher CD4+ and CD19+ lymphocyte count. The presence of CD19+ peripheral B cells has been linked to anti-SARS-CoV-2 humoral immune response in nontransplanted patients.\textsuperscript{27} Lymphopenia is a common side effect of MPA, and its use has been associated with the inhibition of the immune response after vaccination, in contrast to mTOR inhibitors.\textsuperscript{28}

Finally, as previously reported, we found that patients who were younger, were male, had a longer period between transplantation and vaccination, and had a higher eGFR were more likely to develop antibodies.

Because of the weak immunogenicity of the vaccine, COVID-19 cases have been reported among vaccinated transplant patients.\textsuperscript{29,30} SOT recipients who received 2 doses of the vaccine remain at higher risk of developing COVID-19 with a higher risk of hospitalization and death compared with fully vaccinated immunocompetent patients.\textsuperscript{31} Therefore, different strategies have been or are considered to enhance the immunological response and consequently the protection rate. One such strategy is a vaccine with a higher dose. Boyarsky et al\textsuperscript{11} who vaccinated their kidney transplant patients with the mRNA-1273 vaccine, which has a higher dose than the BNT162b2 vaccine, were more likely to develop an antibody response. Benotmane et al,\textsuperscript{11} who vaccinated their kidney transplant patients with the mRNA-1273 vaccine, noted a higher humoral response (48%) compared with our kidney transplant patients who received the BNT162b2 vaccine (33.7%). However, no comparison between both mRNA-based vaccines was performed. Recently, monocentric reports and a randomized controlled trial have shown that a boost with a third dose can significantly increase the humoral response in up to 70% of patients.\textsuperscript{32-36} The third dose is now approved in several countries. However, this should be done under biological monitoring since acute rejection episodes have been reported after anti–SARS-CoV-2 vaccination.\textsuperscript{37}

Finally, based on our results, it can be hypothesized that modifying immunosuppression and using the combination of low-dose tacrolimus and mTOR inhibitors during the vaccination period improves the immunogenicity of the vaccine.

In conclusion, our study, which included a relatively large number of patients, confirmed the weak humoral response to 2 doses of mRNA vaccines in transplant patients and identified predictive factors for humoral response. Among these are immunosuppressive regimens that can be modified to improve the humoral response, especially when access to a third dose is not possible.

**ACKNOWLEDGMENTS**

We thank Mrs Célia Benzema and Marie Mattera for collecting the data.

**REFERENCES**

1. Akalin E, Azzi Y, Bartash R, et al. Covid-19 and kidney transplantation. N Engl J Med. 2020;382:2475–2477.
2. Caillaud S, Anglicheau D, Maitreton M, et al; French SOT COVID Registry. An initial report from the French SOT COVID Registry suggests high mortality due to COVID-19 in recipients of kidney transplants. Kidney Int. 2020;98:1549–1558.
3. Selfeela J-M, Friman G, von Zur-Mühlen B, et al. COVID-19 in solid organ transplant recipients: a national cohort study from Sweden. Am J Transplant. 2021;21:2762–2773.
4. Villanueva F, Mazuecos A, Pérez-Flores IM, et al; Spanish Society of Nephrology COVID-19 Group. Predictors of severe COVID-19 in kidney transplant recipients in the different epidemic waves: analysis of the Spanish Registry. Am J Transplant. 2021;21:2573–2582.
5. Chavarot N, Lurez-Ville M, Scemla A, et al. Decline and loss of anti-SARS-CoV-2 antibodies in kidney transplant recipients in the 6 months following SARS-CoV-2 infection. Kidney Int. 2021;99:486–488.
6. Kumar D, Blumberg EA, Danziger-Isakov L, et al; AST Infectious Diseases Community of Practice. Influenza vaccination in the organ transplant recipient: review and summary recommendations. Am J Transplant. 2011;11:2020–2030.
7. Boyarsky BJ, Werbel WA, Avery RK, et al. Immunogenicity of a single dose of SARS-CoV-2 messenger RNA vaccine in solid organ transplant recipients. JAMA. 2021;325:1784–1786.
8. Benotmane I, Gautier-Vargas G, Cognard N, et al. Weak anti-SARS-CoV-2 antibody response after the first injection of an mRNA COVID-19 vaccine in kidney transplant recipients. Kidney Int. 2021;99:1487–1489.
9. Grupper A, Rabinowich L, Schwartz D, et al. Reduced humoral response to mRNA SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients without prior exposure to the virus. Am J Transplant. 2021;21:2719–2727.
10. Marinaki S, Adamopoulos S, DeGiannis D, et al. Immunogenicity of SARS-CoV-2 BNT162b2 vaccine in solid organ transplant recipients. Am J Transplant. 2021;21:2913–2915.
11. 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.
12. Svobodch L, Grupper A, Baruch R, et al. Low immunogenicity to SARS-CoV-2 vaccine among liver transplant recipients. J Hepatol. 2021;75:435–438.
13. Boyarsky BJ, Werbel WA, Avery RK, et al. Antibody response to 2-dose SARS-CoV-2 mRNA vaccine series in solid organ transplant recipients. JAMA. 2021;325:2204–2206.
14. Korth J, Jahn M, Dorsch O, et al. Impaired humoral response in renal transplant recipients to SARS-CoV-2 mRNA-1273 vaccine with BNT162b2 (Pfizer-BioNTech). Viruses. 2021;13:756.
15. Ou MT, Boyarsky BJ, Motter JD, et al. Safety and reactogenicity of 2 doses of SARS-CoV-2 vaccine in solid organ transplant recipients. Transplantation. 2021;105:2170–2174.
16. Marion O, Del Bello A, Abravanel F, et al. Safety and immunogenicity of Anti-SARS-CoV-2 messenger RNA vaccines in recipients of solid organ transplants. Ann Intern Med. 2021;174:1336–1338.
17. Abravanel F, Meldouge M, Chapuy-Regaud S, et al. Clinical performance of a rapid test compared to a microplate test to detect total anti SARS-CoV-2 antibodies directed to the spike protein. J Clin Virol. 2020;130:104528.
18. Haflin J, Svorcova M, Dvorcekova E, et al. Immunogenicity of BNT162b2 mRNA COVID-19 vaccine and SARS-CoV-2 infection in lung transplant recipients. J Heart Lung Transplant. 2021;40:754–758.
19. Prangega L, Meller CH, Johnston F, et al. Immunosuppressive T-cell antibody induction for heart transplant recipients. Cochrane Database Syst Rev. 2013:CD008842.
20. Bitttermann T, Hubbard RA, Lewis JD, et al. The use of induction therapy in liver transplantation is highly variable and is associated with posttransplant outcomes. Am J Transplant. 2019;19:3319–3327.
21. Chavarot N, Ouedraogo A, Marion O, et al. Poor Anti-SARS-CoV-2 humoral and T-cell responses after 2 injections of mRNA vaccine in kidney transplant recipients treated with belatacept. Transplantation. 2021;105:e69–e95.
22. Bertrand D, Hamzaoui M, Lernvé E, et al. Antibody and T cell response to SARS-CoV-2 messenger RNA BNT162b2 vaccine in kidney transplant recipients and hemodialysis patients. J Am Soc Nephrol. 2021;32:2147–2152.
23. Noble J, Langello A, Bouchut W, et al. Immune response post-SARS-CoV-2 mRNA vaccination in kidney-transplant recipients receiving belatacept. Transplantation. 2021;105:e595–e602.
24. Pascual J, Berger SP, Witzke O, et al; TRANSFORM Investigators. Everolimus with reduced calcineurin inhibitor exposure in renal transplantation. J Am Soc Nephrol. 2018;29:1979–1991.
25. De Simone P, Nevens F, De Carli L, et al; H2304 Study Group. Everolimus with reduced tacrolimus improves renal function in de novo liver transplant recipients: a randomized controlled trial. Am J Transplant. 2012;12:3578–3589.
26. Bartten MJ, Hirt SW, Garbade J, et al. Comparing everolimus-based immunosuppression with reduction or withdrawal of calcineurin inhibitor reduction from six months after heart transplantation: the randomized MANDELA study. Am J Transplant 2019;19:3006–3017.
27. Mrak D, Tobudic S, Koblišček M, et al. SARS-CoV-2 vaccination in rituximab-treated patients: B cells promote humoral immune
responses in the presence of T-cell-mediated immunity. Ann Rheum Dis. 2021;80:1345–1350.

28. Struijk GH, Minnee RC, Koch SD, et al. Maintenance immunosuppressive therapy with everolimus preserves humoral immune responses. Kidney Int. 2010;78:934–940.

29. Wadei HM, Gorwa TA, Leoni JC, et al. COVID-19 infection in solid organ transplant recipients after SARS-CoV-2 vaccination. Am J Transplant. 2021;21:3496–3499.

30. Ali NM, Alnazari N, Mehta SA, et al. Development of COVID-19 infection in transplant recipients after SARS-CoV-2 vaccination. Transplantation. 2021;105:e104–e106.

31. Qin CX, Moore LW, Anjan S, et al. Risk of breakthrough SARS-CoV-2 infections in adult transplant recipients. Transplantation. 2021;105:e265–e266.

32. Kamar N, Abravanel F, Marion O, et al. Three doses of an mRNA COVID-19 vaccine in solid-organ transplant recipients. N Engl J Med. 2021;385:661–662.

33. Del Bello A, Abravanel F, Marion O, et al. Efficiency of a boost with a third dose of anti-SARS-CoV-2 messenger RNA-based vaccines in solid organ transplant recipients. Am J Transplant. [Epub ahead of print. July 31, 2021]. doi:10.1111/ajt.16775

34. Werbel WA, Boyarsky BJ, Ou MT, et al. Safety and immunogenicity of a third dose of SARS-CoV-2 vaccine in solid organ transplant recipients: a case series. Ann Intern Med. 2021;174:1330–1332.

35. Hall VG, Ferreira VH, Ku T, et al. Randomized trial of a third dose of mRNA-1273 vaccine in transplant recipients. N Engl J Med. 2021;385:1244–1246.

36. 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;326:1063–1065.

37. Del Bello A, Marion O, Delas A, et al. Acute rejection after anti-SARS-CoV-2 mRNA vaccination in a patient who underwent a kidney transplant. Kidney Int. 2021;100:238–239.