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Discordance Between SARS-CoV-2–specific Cell-mediated and Antibody Responses Elicited by mRNA-1273 Vaccine in Kidney and Liver Transplant Recipients

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Background. Severe acute respiratory syndrome coronavirus 2–specific cell-mediated immunity (SARS-CoV-2-CMI) elicited by mRNA-based vaccines in solid organ transplant (SOT) recipients and its correlation with antibody responses remain poorly characterized. **Methods.** We included 44 (28 kidney, 14 liver, and 2 double organ) recipients who received the full series of the mRNA-1273 vaccine. SARS-CoV-2-CMI was evaluated at baseline, before the second dose, and at 2 wk after completion of vaccination by an ELISpot-based interferon-γ FluoroSpot assay using overlapping peptides covering the S1 domain. SARS-CoV-2 immunoglobulin G seroconversion and serum neutralizing activity against the spike protein were assessed at the same points by commercial ELISA and an angiotensin-converting enzyme-2/spike antibody inhibition method, respectively. Postvaccination SARS-CoV-2-CMI was compared with 28 healthcare workers who received the BNT162b2 vaccine. **Results.** Positive SARS-CoV-2-CMI increased from 6.8% at baseline to 23.3% after the first mRNA-1273 dose and 59.5% after the completion of vaccination ($P<0.0001$). Lower rates were observed for immunoglobulin G seroconversion (2.3%, 18.6%, and 57.1%, respectively) and neutralizing activity (2.3%, 11.6%, and 31.0%). There was a modest correlation between neutralizing titers and the magnitude of SARS-CoV-2-CMI (Spearman's rho: 0.375; $P=0.015$). Fifteen recipients (35.7%) mounted SARS-CoV-2-CMI without detectable neutralizing activity, whereas 3 (7.1%) did the opposite, yielding poor categorical agreement (Kappa statistic: 0.201). Rates of positive SARS-CoV-2-CMI among SOT recipients were significantly decreased compared with nontransplant controls (82.1% and 100.0% after the first dose and completion of vaccination, respectively; $P<0.0001$). Kidney transplantation, the use of tacrolimus and prednisone, and the number of immunosuppressive agents were associated with lower cell-mediated responses. Results remained unchanged when 3 recipients with prevaccination SARS-CoV-2-CMI were excluded. **Conclusions.** Two-thirds of SOT recipients mounted SARS-CoV-2-CMI following vaccination with mRNA-1273. Notable discordance was observed between vaccine-induced cell-mediated and neutralizing humoral immunities. Future studies should determine whether these patients with incomplete responses are effectively protected.

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INTRODUCTION

Vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been revealed as the most effective measure to control the coronavirus disease 2019 (COVID-19) pandemic. Randomized trials[1,2] and observational studies[3,4] have shown excellent rates of seroconversion and clinical effectiveness for mRNA-based vaccines in the general population. Increasing evidence, however, confirms that immunogenicity after solid organ transplantation (SOT) is severely compromised.[5-18] As a consequence, SOT recipients may still develop COVID-19 requiring hospital admission despite the completion of vaccination series.[19,20] With few exceptions,[8,10,16-18] most previous studies have only assessed the induction of humoral immunity, either as total anti–SARS-CoV-2 immunoglobulin (Ig)G titers or neutralizing antibodies targeting the spike (S) glycoprotein. Vaccine-mediated immunity relies not only on long-term antibody responses but also on memory T cells.[21] Therefore, immunogenicity may have been underestimated in studies not evaluating the contribution of the cellular arm to vaccine-mediated protection. In addition, the concordance between cell-mediated cellular and humoral responses elicited by vaccination in the setting of long-term immunosuppression remains largely unknown.

On the other hand, the study population in reported experiences was mostly composed of kidney transplant (KT) recipients.[6-12,17] It is to be expected that vaccine-induced responses would vary across transplant types because liver transplant (LT) recipients require less intense immunosuppression than other SOT groups. The assessment of the relative impact of different agents on the ability to mount effective responses (such as antimetabolites[5,15]) is hampered by the fact that the majority of patients in previous cohorts were on triple therapy containing corticosteroids and a calcineurin inhibitor[8,12,13,16] or belatacept.[9,10]

With these knowledge gaps in mind, we have comprehensively assessed the response to the mRNA-1273 vaccine in a cohort of KT and LT recipients. We analyzed the development of SARS-CoV-2–specific cell-mediated immunity with a fluorescent ELISpot for cytokine secretion (interferon [IFN]-γ FluoroSpot), the seroconversion to SARS-CoV-2 IgG antibodies by means of a commercial ELISA, and the neutralizing activity of postvaccination sera against the S protein assessed by a human angiotensin-converting enzyme (hACE)-2/spike antibody inhibition ELISA-based method. Particular focus was put on quantifying the discordance between cell-mediated and humoral responses.

MATERIALS AND METHODS

Study Design and Setting

This prospective study was performed at the University Hospital "12 de Octubre" (Madrid, Spain). All KT and LT recipients with a functioning graft and regular follow-up at our institution who received the first dose of mRNA-1273 (Moderna Biotech)—a lipid nanoparticle-encapsulated mRNA vaccine expressing the prefusion-stabilized SARS-CoV-2 S protein[1]—between April 15 and April 27, 2021, were potentially eligible. Recipients with a prior history of suspected or documented COVID-19 were also included, provided that they were asymptomatic for >72 h and transmission-based precautions had been discontinued. Forty-five randomly selected patients who fulfilled these criteria were offered to participate.

All participants were evaluated for SARS-CoV-2–specific immunity at 3 time points: baseline (ie, immediately before the first dose of mRNA-1273), at a 4-wk interval (ie, immediately before the second dose), and 2 wk after the completion of the full vaccine series. Peripheral blood lymphocyte subpopulations ($CD3^+$, $CD4^+$, and $CD8^+$ T cells, B cells, and $CD56^+$ $CD16^+$ natural killer cells) and serum immunoglobulin levels were also measured at baseline. Demographics, comorbidities, immunosuppressive regimen, and trough serum levels at the time of vaccination, laboratory values, and vaccine-related adverse events (AEs) were prospectively recorded using a standardized case report form. Patients were followed up on until June 25, 2021.

The study was performed in accordance with the ethical standards as laid down in the Declarations of Helsinki and Istanbul. The study protocol was approved by the institutional research ethics committee, and all participants provided written informed consent.

SARS-CoV-2–specific Cell-mediated Immunity

Details of the IFN-γ FluoroSpot assay have been described elsewhere.[22] In brief, whole blood specimens were processed within 24 h from sampling. Peripheral blood mononuclear cells (PBMCs) were freshly isolated by density-gradient centrifugation using Ficoll-Paque and seeded at 300 000 cells/well onto IFN-γ FluoroSpot plates (MabTech, Nacka Strand, Sweden) with cell culture medium containing Roswell Park Memorial Institute medium, 1% L-glutamine, 1% penicillin/streptomycin, 10% fetal bovine serum, and anti-CD28 monoclonal antibody (1 μg/mL). Test wells were performed in duplicate and supplemented with 15-mer overlapping peptides covering the S1 domain of the S protein (166 individual peptides) (SARS-CoV-2 S1 scanning pool, MabTech) at a final concentration of 1 μg/mL. To assess the presence of SARS-CoV-2–specific responses indicative of natural immunity before vaccination, baseline samples were also stimulated with the viral nucleoprotein (N protein)—102 peptides—(EMPS SARS-CoV-2 NCAP-1, JPT Peptide Technologies GmbH, Berlin, Germany) and the membrane (M) protein—53 peptides (EMPS SARS-CoV-2 VME1, JPT). Negative control wells lacked peptides, and positive control wells included anti-CD3 antibodies (MabTech). Assays were incubated for 16–18 h at 37 °C. Spots were counted using an automated IRIS FluoroSpot reader system (MabTech). To quantify specific cell-mediated responses, spots of the negative control wells were subtracted from the mean spots test wells, and the results were expressed as IFN-γ–producing spot-forming units (SFUs)/10^6 PBMCs. Results were excluded if negative control wells had >80 SFUs/10^6 PBMCs or positive control wells had <400 SFUs/10^6 PBMCs.

Responses were considered to be positive if the results were at least 3 times higher than the mean of the negative control wells and above the following antigen-specific cutoff values: >25 SFUs/10^6 PBMCs for the S protein, >14 SFUs/10^6 PBMCs for the N protein, and >21 SFUs/10^6 PBMCs for the SARS-CoV-2 M protein. These thresholds were established on the basis of a control group of 30 unvaccinated healthcare workers (HCWs) with no clinical or microbiological diagnosis of COVID-19, a negative SARS-CoV-2 IgG serology, and no prior known contact with COVID-19 cases recruited by July 2020. We also used a cohort of 234 patients recovered from COVID-19 who had been hospitalized during the acute phase because of moderate or severe infection

(World Health Organization ordinal scale 3–7). Samples in this latter group were obtained at a mean of 6 mo (range, 3–7) from symptom onset. The area under receiver operating characteristics curve analysis allowed us to determine optimal cutoff values to discriminate between uninfected HCWs and patients recovered from COVID-19, revealing excellent diagnostic accuracy (SDC methods, SDC, http://links.lww.com/TXD/A377).

We also assessed polyfunctional Th1-biased cell-mediated responses expressing both IFN-γ and interleukin-2. Because of the exploratory nature of this analysis, no cutoff values for assay positivity were established, and data were treated in a continuous manner only.

SARS-CoV-2 Serology

Serum IgG antibodies targeting the S1 protein were detected with the EUROIMMUN Anti-SARS-CoV-2 ELISA (Euroimmun AG, Lübeck, Germany) according to the manufacturer's instructions. Optical density (OD) values were measured at 450 nm using the PR 3100 microplate reader (Bio-Rad Life Science, Marnes-La-Coquette, France). Results were semiquantitatively evaluated by calculating the ratio of the OD value of the sample over the OD value of the calibrator (relative OD) with the following cutoff values: <0.8, negative; ≥0.8 to <1.1, borderline; and ≥1.1, positive.

Serum Neutralizing Activity Against the S Protein

The neutralizing activity elicited by the mRNA-1273 vaccine was analyzed with an in-house hACE-2/spike antibody inhibition ELISA-based method. Microtiter 96-well plates were coated overnight with a chimeric version of a monoclonal anti-Foldon antibody[23] at 8 ng/μL in PBS. Plates were next blocked with PBS supplemented with BSA fraction V (Sigma-Aldrich, Munich, Germany) at 1% (PBS-BSA 1%). Then, purified HexaPro-derived construct[24] containing the D614G substitution was captured by incubation at 1 ng/μL in PBS-BSA 1% at room temperature for 45 min. Following protein incubation, plates were washed with PBS, and successive incubations of sera dilutions and hACE-2 monomeric-StreTag receptor (20 ng/μL) complexed with StrepTactin-horseradish peroxidase (Bio-Rad, Hercules, CA) at 1:5000 were performed. Sera incubation was prolonged for 45 min, and after a 15-min incubation of receptor–StrepTactin-horseradish peroxidase complexes, receptor binding to captured S protein was revealed with the O-phenylenediamine dihydrochloride substrate (Sigma-Aldrich) and measured in a spectrophotometer at 493–620 nm. The assay background was determined in parallel plates with the S protein locked in the close conformation, which is unable to bind the hACE-2 receptor. For this purpose, a purified HexaPro-derived construct including a double cysteine substitution (S383C, D985C) was used. A pool of sera from healthy individuals with negative SARS-CoV-2 IgG antibodies and no prior known contact with COVID-19 cases and hACE-2 monomeric untagged receptor were used as negative and positive controls, respectively. After background subtraction, the percentage of neutralization was calculated as $(1-[OD_{495-620}$ test serum/$OD_{495-620}$ negative control$]\times 100)$. The incubation of untagged hACE-2 receptor at 200 ng/μL achieved a neutralization rate >85% in all the experiments. The neutralizing antibody titer for a given patient was established as the last dilution leading to 50% inhibition of the OD value of the negative control. Because 1/10 was the lowest dilution for which this criterion was met, we used this threshold as pragmatic cutoff value.

Nontransplant Control Group

To better characterize the impact of posttransplant immunosuppression on the ability of vaccination to elicit SARS-CoV-2–specific cell-mediated immunity, we used a control group of 28 nonimmunocompromised HCWs who had received the full series of the BNT162b2 mRNA vaccine (Comirnaty, Pfizer-BioNTech) at our institution between January 15 and February 15, 2021. The IFN-γ FluoroSpot assay was performed following a schedule that mirrored that of SOT recipients: at baseline, at a 3-wk interval (ie, immediately before the second dose of BNT162b2), and at 2 wk after the completion of vaccination.

Statistical Analysis

Quantitative data were reported as the mean ± SD or the median with interquartile range (IQR). Qualitative variables were expressed as absolute and relative frequencies. Categorical variables were compared using the χ^2 test. The Student *t* test or the Mann-Whitney U test was used for continuous variables. Repeated measures were compared with the Wilcoxon signed-rank test or the McNemar test, as appropriate. Correlations between continuous variables were evaluated with Pearson's correlation coefficient or Spearman's rho. The categorical agreement between the results of the IFN-γ FluoroSpot assay and the SARS-CoV-2 IgG serology and serum neutralizing activity was evaluated by the Kappa statistic. Statistical analysis was performed using SPSS version 20.0 (IBM Corp, Armonk, NY).

RESULTS

Study Population

One out of 45 approached SOT recipients refused to participate. Therefore, 44 patients (28 KT recipients, 14 LT recipients, and 2 double organ [kidney-pancreas and liver-kidney] recipients) were included (Table 1). One patient (2.3%) had a history of prior COVID-19 diagnosed 6 mo ago. All but 2 participants (95.5%) received the full mRNA-1273 vaccine series. One patient refused to receive the second dose. One further patient—a 39-y-old LT recipient that was receiving tacrolimus, mofetil mycophenolate (MMF), and prednisone—was hospitalized because of SARS-CoV-2 infection without pneumonia or oxygen therapy requirement 3 wk after the receipt of the first vaccine dose, and the second dose had to be postponed until the resolution of symptoms.

Induction of SARS-CoV-2–specific Cell-mediated Immunity Following Vaccination

Three patients (6.8%) exhibited positive IFN-γ–producing cell-mediated responses against the 3 SARS-CoV-2 structural proteins tested (S, N, and M) at baseline, which demonstrated the acquisition of natural immunity before vaccination. None of these patients had a previous history suggestive of COVID-19.

The proportion of positive S protein–specific responses (>25 SFUs/10^6 PBMCs) by the IFN-γ FluoroSpot assay increased to 23.3% (10 of 43) after the first dose ($P = 0.039$) and 59.5% (25 of 42) at 2 wk after the completion of the vaccine series ($P = 0.0003$) (Figure 1A).

TABLE 1.

Demographics and clinical characteristics of the study population (n = 44)

Variable	
Age, y, mean ± SD	52.4 ± 11.5
Male gender, N (%)	27 (61.4)
Smoking habit, N (%)	7 (15.9)
Comorbidities, N (%)	
Hypertension	32 (72.7)
Dyslipidemia	12 (27.3)
Diabetes mellitus	11 (25.0)
Cardiovascular disease	14 (31.8)
Obesity	6 (13.6)
Chronic pulmonary disease	4 (9.1)
Venous thromboembolic disease	7 (15.9)
Ethnicity, N (%)	
Caucasian	38 (86.4)
Latino	3 (6.8)
Other	3 (6.8)
Type of transplantation, N (%)	
Kidney	28 (63.6)
Liver	14 (31.8)
Simultaneous kidney-pancreas	1 (2.3)
Simultaneous liver-kidney	1 (2.3)
Previous solid organ transplantation, N (%)	5 (11.4)
Time interval since transplantation, y, median (IQR)	2.3 (1.3–4.8)
Type of immunosuppression regimen, N (%)[a]	
Tacrolimus, MMF/MPS and prednisone	23 (52.3)
Tacrolimus and MMF/MPS	5 (11.4)
Tacrolimus, MMF/MPS and mTOR inhibitor	4 (9.1)
Tacrolimus monotherapy	4 (9.1)
MMF/MPS and mTOR inhibitor	2 (4.5)
Other[b]	6 (13.6)
Laboratory and immunological parameters, mean ± SD[a]	
eGFR, mL/min/1.73 m^2	57.5 ± 21.8
CD3$^+$ T-cell count, cells/μL	926 ± 481
CD4$^+$ T-cell count, cells/μL	444 ± 273
CD8$^+$ T-cell count, cells/μL	446 ± 313
B-cell count, cells/μL	112 ± 85
NK cell count, cells/μL	189 ± 154
Serum IgG levels, mg/dL	1140 ± 476
Serum IgA levels, mg/dL	276 ± 140
Serum IgM levels, mg/dL	121 ± 86

[a]At the time of the administration of the first dose of the mRNA-1273 vaccine.
[b]Cyclosporine, MMF and prednisone (n = 1); MPA, everolimus, and prednisone (n = 1); tacrolimus, azathioprine, and prednisone (n = 1); tacrolimus and prednisone (n = 1); everolimus and prednisone (n = 1); MMF monotherapy (n = 1).
eGFR, estimated glomerular filtration rate (estimated by the Modification of Diet in Renal Disease-4 equation); IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IQR, interquartile range; MMF/MPS, mycophenolate mofetil or mycophenolate sodium; mTOR, mammalian target of rapamycin; NK, natural killer; SD, standard deviation.

The magnitude of the cell-mediated response, as measured by the median number of S protein–specific IFN-γ–producing SFUs per 10^6 PBMCs, increased from baseline (0.0 [IQR, 0–3.3]) to the assessment following the first vaccine dose (8.3 [IQR, 3.3–20.0]) and after 2 wk from the completion of vaccination (35.8 [IQR, 8.3–185.0]) ($P < 0.0001$) (Figure 1B). Polyfunctional IFN-γ/interleukin-2–producing responses exhibited a similar trend, although absolute magnitudes were lower (Figure S1, SDC, http://links.lww.com/TXD/A377).

SARS-CoV-2 IgG Antibody Response

At the baseline (prevaccination) evaluation, only 1 patient (2.3%) tested positive for SARS-CoV-2 IgG antibodies. The rate of IgG seropositivity increased to 18.6% (8 of 43) after the first dose ($P = 0.016$) and 57.1% (24 of 42) at 2 wk after the completion of vaccination ($P < 0.0001$) (Figure 2A).

Out of a total of 129 monitoring points, 104 (80.6%) yielded concordant results between the S protein–specific cell-mediated immunity and the SARS-CoV-2 IgG serology, with both assays testing positive in 23 points (17.8%) and negative in 81 (62.8%). In 15 points (11.6%), the IFN-γ FluoroSpot assay was positive with negative ELISA, whereas the opposite results (lack of cell-mediated immunity with positive SARS-CoV-2 IgG) were observed in 10 points (7.8%). When focused on the evaluation at 2 wk after the completion of vaccination, 8 patients (19.0%) who remained seronegative exhibited detectable cell-mediated immunity, whereas 7 patients (16.7%) with positive IgG antibodies tested below the cutoff value for the IFN-γ FluoroSpot assay (Kappa statistic, 0.266).

When the results of both assays were analyzed as continuous variables, we observed a significant—albeit weak—positive correlation between the semiquantitative results of the IgG ELISA (expressed as relative OD) and the number of S protein–specific IFN-γ–producing SFUs per 10^6 PBMCs following the first vaccine dose (Spearman's rho, 0.354, $P = 0.019$) and after the completion of the series (Spearman's rho, 0.472, $P = 0.002$) (Figure S2, SDC, http://links.lww.com/TXD/A377).

Serum Neutralizing Activity Against SARS-CoV-2 S Protein

One patient (2.3%) exhibited neutralizing activity by the hACE-2/spike antibody inhibition method at baseline. The proportion increased to 11.6% (5 of 43) after the first vaccine dose ($P = 0.046$) and 31.0% (13 of 42) at 2 wk after the completion of vaccination ($P = 0.005$) (Figure 2B).

We obtained concordant results between the S protein–specific cell-mediated immunity and serum neutralizing activity in 104 out of 129 monitoring points (80.6%), with both assays concurrently testing positive and negative in 16 (12.4%) and 88 (68.2%) points, respectively. The presence of SARS-CoV-2–specific cell-mediated immunity was not accompanied by detectable neutralizing activity in 17 points (13.2%). Conversely, neutralizing activity was not associated with a positive IFN-γ FluoroSpot assay in 8 points (6.2%). By 2 wk from the administration of the second vaccine dose, 15 patients (35.7%) mounted cell-mediated responses without detectable neutralizing activity, whereas 3 of them (7.1%) did the opposite (Kappa statistic, 0.201) (Figure 3).

There was a significant correlation between neutralizing titers against the S protein and the number of S protein–specific IFN-γ–producing SFUs per 10^6 PBMCs after the first vaccine dose (Spearman's rho, 0.513; $P = 0.0004$) and at 2 wk from the second dose (Spearman's rho, 0.375; $P = 0.015$) (Figure 4).

Comparison With Nontransplant Controls

We compared SARS-CoV-2–specific cell-mediated immunity elicited by mRNA-based vaccines between SOT recipients and a nontransplant control group of 28 HCWs (21 females, mean age, 43.1 ± 15.1 y [range, 22.9–64.1]) who received the BNT162b2 vaccine. None of these HCWs had any relevant

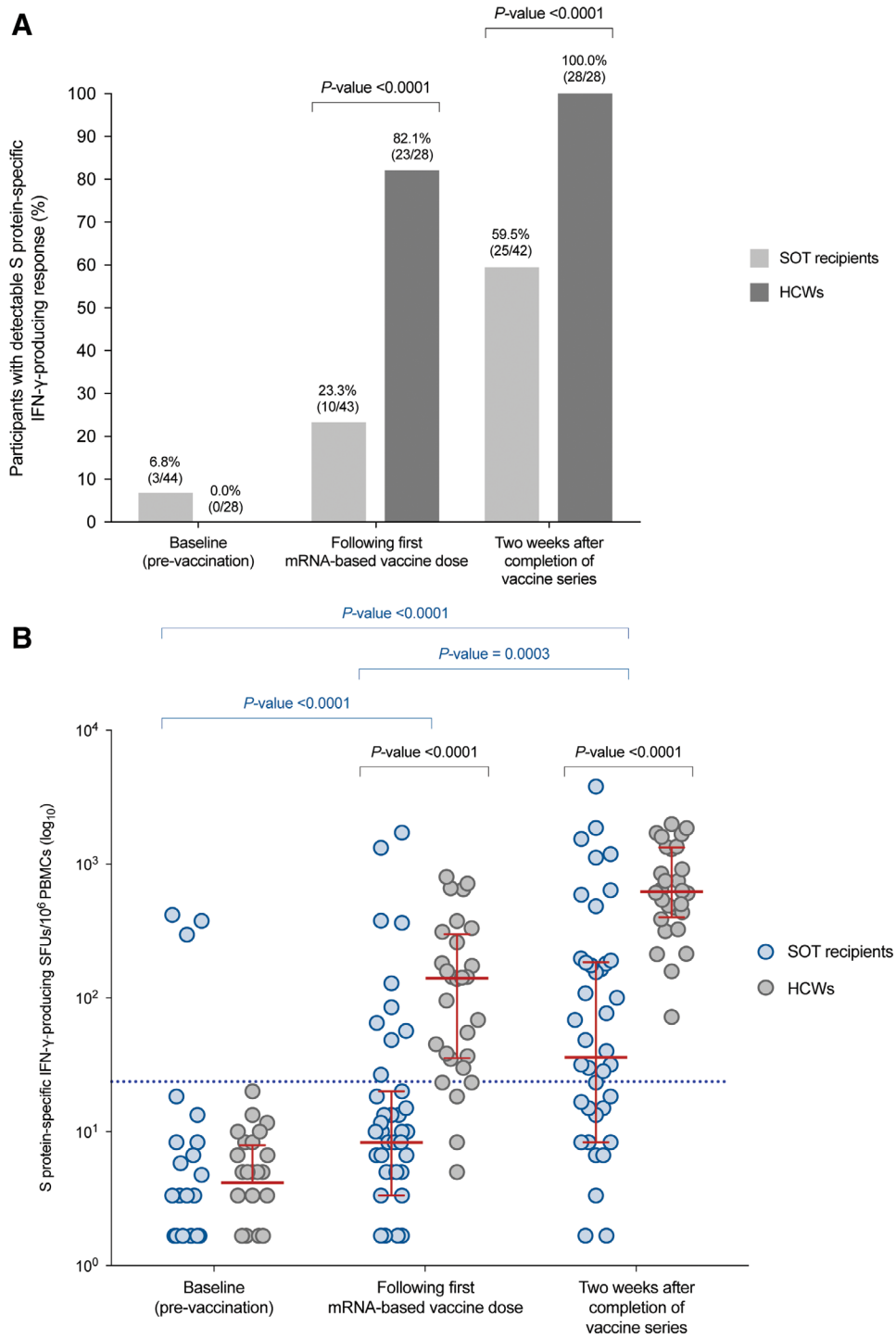

FIGURE 1. SARS-CoV-2–specific cell-mediated immunity in SOT recipients and HCWs who received the mRNA-1273 and BNT162bs vaccines, respectively: (A) proportion with detectable S protein–specific IFN-γ–producing response at baseline, after the first vaccine dose vaccine (ie, 4 and 3 wk apart for mRNA-1273 and BNT162b2), and at 2 wk after the completion of the vaccine series; (B) kinetics of the number of S protein–specific IFN-γ–producing SFUs at the same time points. Red bars and whiskers represent median values and interquartile ranges, respectively. The cutoff value for positivity in the IFN-γ FluoroSpot assay (>25 SFUs/10^6 PBMCs) is denoted by the blue dotted line. Comparisons between repeated measures were performed with the McNemar test or the Wilcoxon signed-rank test, as appropriate. HCW, healthcare worker; IFN-γ, interferon-γ; PBMC, peripheral blood mononuclear cell; S, SARS-CoV-2 spike glycoprotein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFU, spot-forming unit; SOT, solid organ transplantation.

comorbidity potentially impacting vaccine immunogenicity or showed immunological or serological evidence of natural immunity against SARS-CoV-2 at baseline. The rate of positive S protein–specific responses was significantly higher in HCWs than in SOT recipients either after the first vaccine dose (82.1% [23 of 28] versus 23.3% [10 of 43]; $P < 0.0001$) or at 2 wk after the administration of the full series (100.0% [28 of 28] versus 59.5% [25 of 42]; $P < 0.0001$) (Figure 1A). The median number of S protein–specific SFUs per 10^6 PBMCs was also significantly higher among

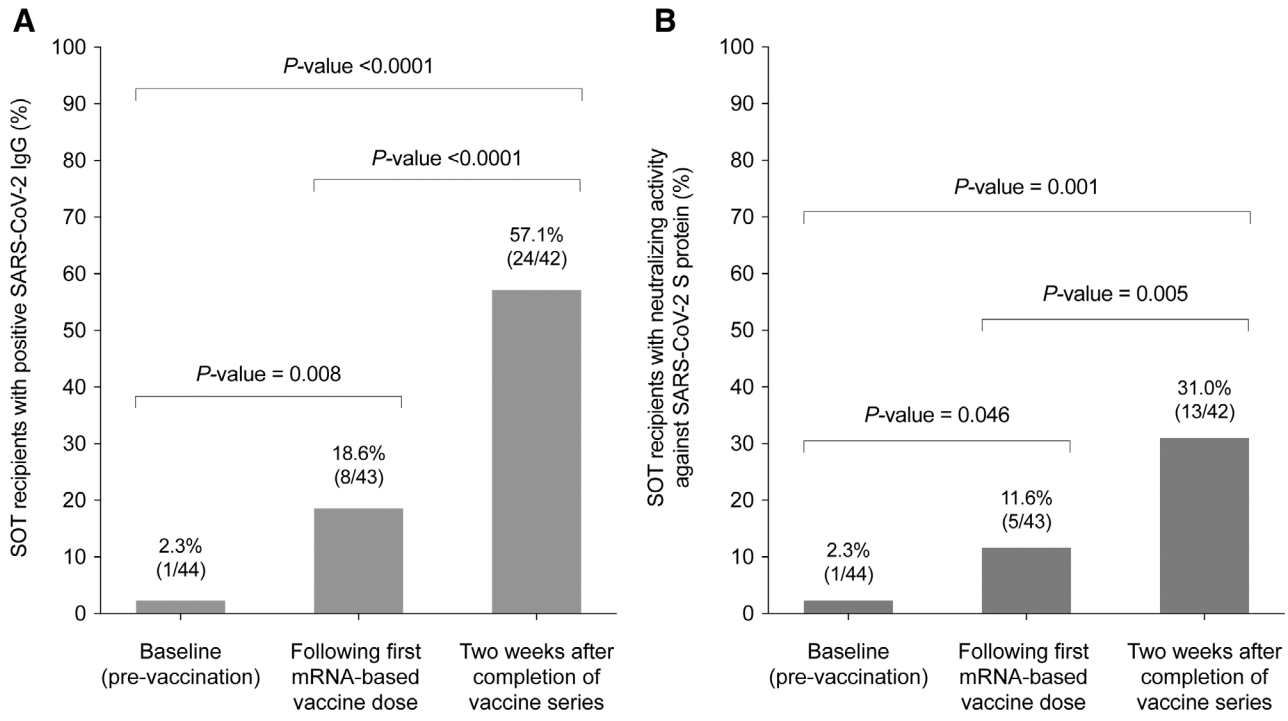

FIGURE 2. SARS-CoV-2–specific humoral immunity elicited by the mRNA-1273 vaccine in SOT recipients: (A) proportion of patients with IgG antibodies targeting the SARS-CoV-2 S protein assessed by commercial ELISA at baseline, after the first vaccine dose vaccine, and at 2 wk after the completion of the vaccine series; (B) proportion with serum neutralizing activity against the S protein analyzed with an *in-house* hACE-2/spike antibody inhibition ELISA-based method at the same time points. Comparisons between repeated measures were performed with the Wilcoxon signed-rank test. hACE, human angiotensin-converting enzyme; IgG, immunoglobulin; S, SARS-CoV-2 spike glycoprotein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SOT, solid organ transplantation.

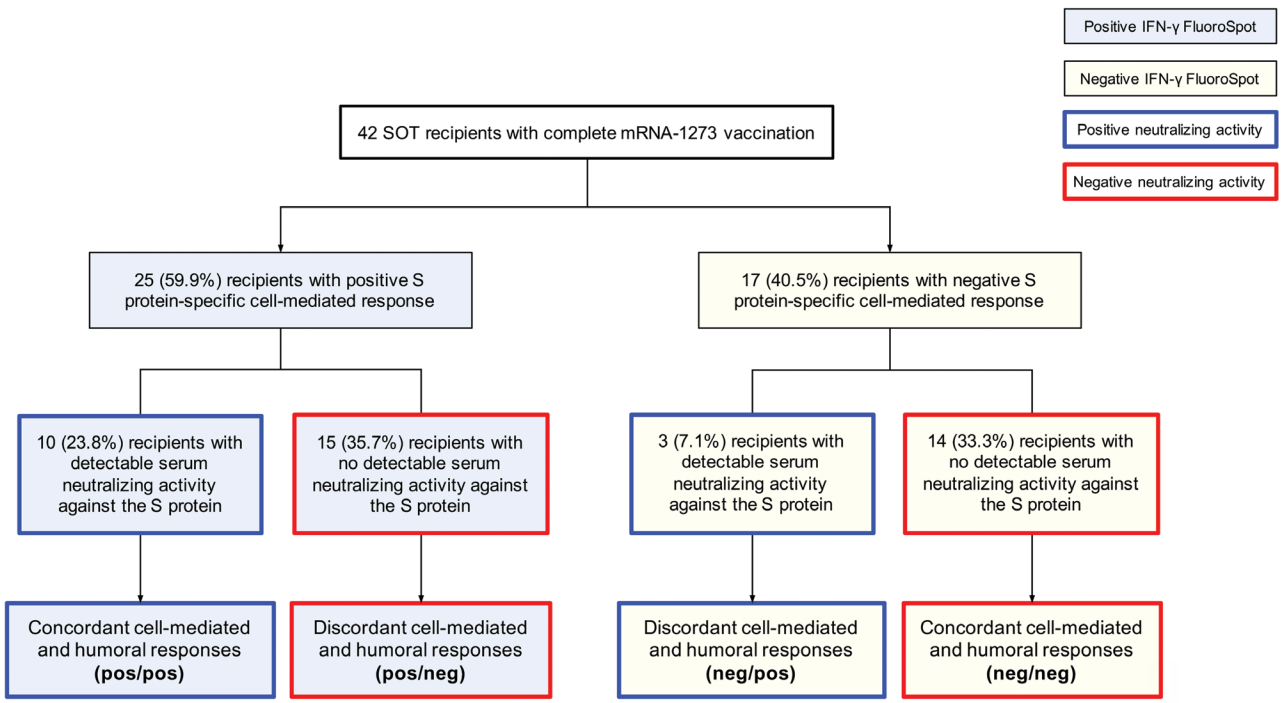

FIGURE 3. Overall disposition of SOT recipients according to the results of the IFN-γ FluoroSpot assay and the hACE-2/spike antibody inhibition ELISA-based method at 2 wk after the completion of the full mRNA-1273 vaccine series. hACE, human angiotensin-converting enzyme; IFN-γ, interferon-γ; S, SARS-CoV-2 spike glycoprotein; SOT, solid organ transplantation

HCWs at both points (140.0 [IQR, 35.4–298.6] versus 8.3 [IQR, 3.3–20.0]; 621.0 [IQR, 399.5–1328.0] versus 35.8 [IQR, 8.3–185.0], respectively; $P < 0.0001$ for both comparisons) (Figure 1B).

Factors Associated With the Mounting of SARS-CoV-2–specific Immunity After Vaccination

Recipients with positive S protein–specific cell-mediated responses at 2 wk after the completion of vaccination had

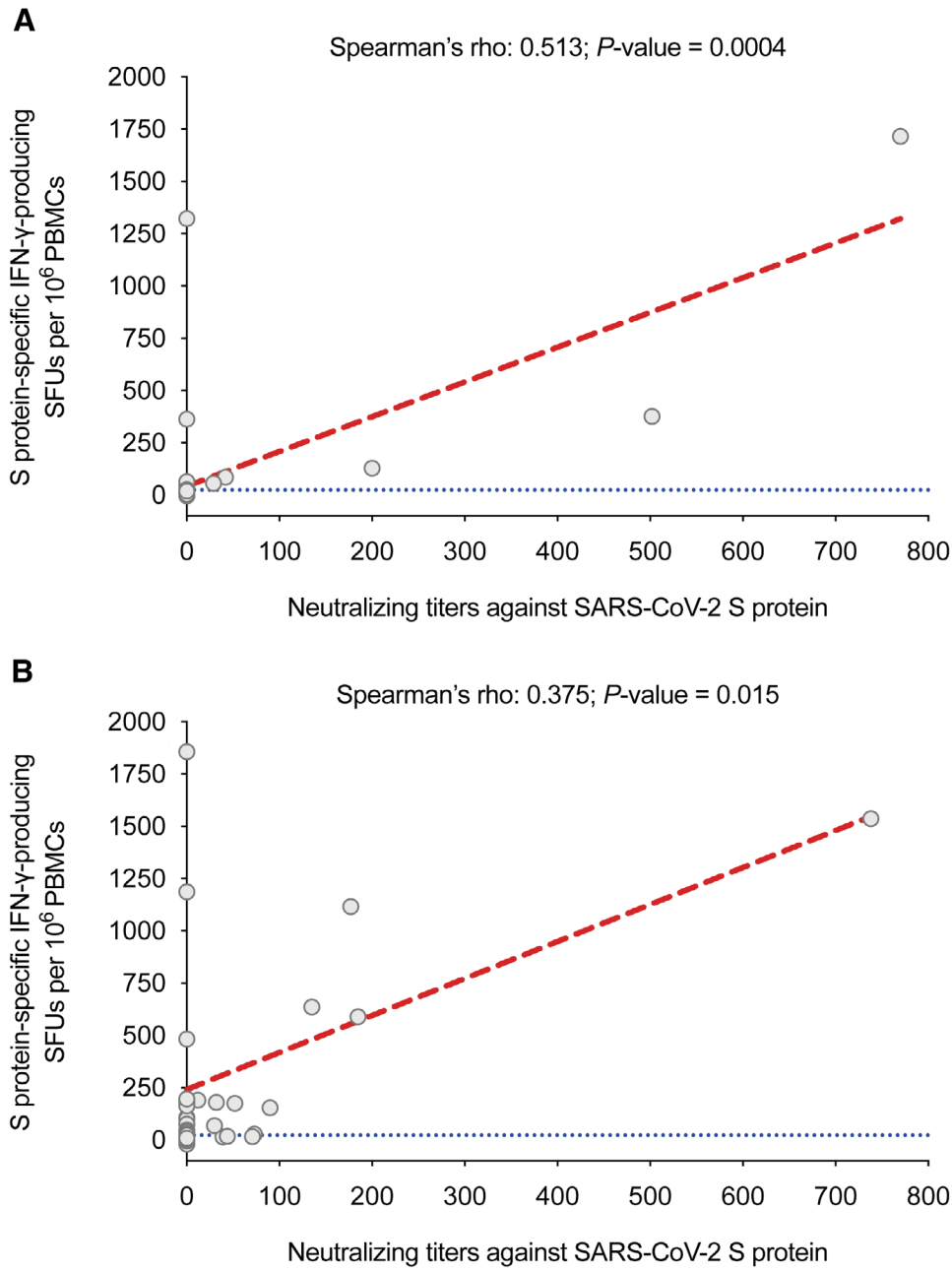

FIGURE 4. Correlation between the number of S protein–specific IFN-γ–producing SFUs per 10^6 PBMCs assessed by the IFN-γ FluoroSpot assay and the serum neutralizing activity against the S protein determined with an hACE-2/spike antibody inhibition ELISA-based method: (A) after the first dose of the mRNA-1273 vaccine; (B) at 2 wk after the completion of the full vaccine series. The cutoff value for positivity in the IFN-γ FluoroSpot assay (>25 SFUs/10^6 PBMCs) is denoted by the blue dotted line. hACE, human angiotensin-converting enzyme; IFN-γ, interferon-γ; PMBC, peripheral blood mononuclear cell; S, SARS-CoV-2 spike glycoprotein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFU, spot-forming unit.

significantly higher $CD4^+$ T-cell counts and lower IgM levels at baseline than those without them. In addition, the time interval since transplantation was longer in this group. On the other hand, the presence of serum neutralizing activity was associated with a higher estimated glomerular filtration rate and $CD8^+$ T-cell counts. Although not achieving statistical significance, LT recipients were more likely than KT recipients to exhibit neutralizing activity elicited by vaccination (Table 2).

We also analyzed the magnitude of SARS-CoV-2–specific cell-mediated immunity and serum neutralizing activity as continuous variables. The number of S protein–specific IFN-γ–producing SFUs were significantly lower among KT recipients (compared with LT recipients), as well as in patients receiving tacrolimus, prednisone, and a triple immunosuppression regimen (as compared with 1- or 2-drug regimens) (Figure S3, SDC, http://links.lww.com/TXD/A377). In addition, there was a significant positive correlation with baseline $CD3^+$ and $CD4^+$ T-cell counts (Table S1, SDC, http://links.lww.com/TP/C311). In contrast, neutralizing titers against the S protein were also significantly lower in KT recipients and those receiving prednisone (Figure S4, SDC, http://links.lww.com/TXD/A377), whereas titers positively correlated with the $CD8^+$ T-cell count and estimated glomerular filtration rate at baseline (Table S1, SDC, http://links.lww.com/TP/C311).

TABLE 2.

Comparison of clinical characteristics between SOT recipients who did or did not develop SARS-CoV-2–specific cell-mediated immunity (>25 S protein–specific IFN-γ–producing SFUs/10^6 PBMCs) or detectable serum neutralizing activity against the S protein (hACE-2/spike antibody inhibition ELISA-based method) at 2 wk after the completion of the full mRNA-1273 vaccine series

	SARS-CoV-2–specific cell-mediated immunity			Serum neutralizing activity against SARS-CoV-2 S protein		
Variable	Vaccine response (n = 25)	No vaccine response (n = 17)	*P*	Vaccine response (n = 13)	No vaccine response (n = 29)	*P*
Age of recipient, y, mean ± SD	53.0 ± 12.4	52.0 ± 10.6	0.789	52.0 ± 12.2	52.8 ± 11.5	0.836
Gender of recipient (male), N (%)	17 (68.0)	10 (58.8)	0.744	8 (61.5)	19 (65.5)	1.000
Smoking habit, N (%)	5 (20.0)	2 (11.8)	0.681	2 (15.4)	5 (17.2)	1.000
Comorbidities, N (%)						
Hypertension	17 (68.0)	14 (82.4)	0.477	8 (61.5)	23 (79.3)	0.270
Dyslipidemia	6 (24.0)	5 (29.4)	0.733	4 (30.8)	7 (24.1)	0.713
Diabetes mellitus	5 (20.0)	6 (35.3)	0.305	4 (30.8)	7 (24.1)	0.713
Cardiovascular disease	10 (40.0)	4 (23.5)	0.266	6 (46.2)	8 (27.6)	0.298
Obesity	3 (12.0)	3 (17.6)	0.672	2 (15.4)	4 (13.8)	1.000
Chronic pulmonary disease	4 (16.0)	0 (0.0)	0.134	3 (23.1)	1 (3.4)	0.080
Venous thromboembolic disease	4 (16.0)	3 (17.6)	1.000	1 (7.7)	6 (20.7)	0.405
Ethnicity other than Caucasian, N (%)	3 (12.0)	3 (17.6)	0.672	13 (100.0)	23 (79.3)	0.153
Type of transplantation, N (%)			0.179			0.068
Kidney (including double organ)	15 (60.0)	14 (82.4)		6 (46.2)	23 (79.3)	
Liver	10 (40.0)	3 (17.6)		7 (53.8)	6 (20.7)	
Previous solid organ transplantation, N (%)	2 (8.0)	3 (17.6)	0.379	3 (23.1)	2 (6.9)	0.162
Time interval since transplantation, y, median (IQR)	2.9 (1.5–5.1)	1.9 (0.8–3.2)	0.053	2.9 (1.9–4.1)	1.9 (0.8–5.1)	0.318
Immunosuppression regimen containing, N (%)[a]						
Tacrolimus	20 (80.0)	16 (94.1)	0.374	10 (76.9)	26 (89.7)	0.353
Trough serum levels, ng/mL, mean ± SD	8.0 ± 3.9	9.1 ± 2.7	0.377	8.2 ± 4.5	8.6 ± 2.9	0.749
MMF/MPS	20 (80.0)	11 (64.7)	0.305	8 (61.5)	23 (79.3)	0.270
Trough serum levels, ng/mL, mean ± SD	3.8 ± 2.2	3.9 ± 1.9	0.938	2.9 ± 1.8	4.2 ± 2.1	0.392
mTOR inhibitor	5 (20.0)	3 (17.6)	1.000	4 (30.8)	4 (13.8)	0.226
Trough serum levels, ng/mL, mean ± SD	5.4 ± 2.8	6.6 ± 4.4	0.656	5.5 ± 3.1	6.1 ± 3.7	0.807
Prednisone	15 (60.0)	15 (88.2)	0.081	6 (46.2)	24 (82.8)	0.026
Laboratory and immunological parameters, mean ± SD[a]						
eGFR, mL/min/1.73 m^2	59.5 ± 20.7	54.6 ± 20.7	0.457	69.4 ± 21.7	52.2 ± 18.0	0.011
$CD3^+$ T-cell count, cells/μL	1010 ± 483	738 ± 382	0.074	1067 ± 490	836 ± 440	0.165
$CD4^+$ T-cell count, cells/μL	520 ± 300	334 ± 208	0.044	471 ± 278	436 ± 285	0.733
$CD8^+$ T-cell count, cells/μL	456 ± 283	368 ± 233	0.327	560 ± 320	365 ± 221	0.038
B-cell count, cells/μL	118 ± 85	108 ± 90	0.733	135 ± 113	105 ± 74	0.350
NK cell count, cells/μL	165 ± 133	224 ± 187	0.258	188 ± 110	188 ± 174	0.991
Serum IgG levels, mg/dL	1032 ± 259	1109 ± 303	0.393	1064 ± 217	1062 ± 302	0.990
Serum IgA levels, mg/dL	238 ± 123	302 ± 140	0.139	245 ± 129	272 ± 135	0.552
Serum IgM levels, mg/dL	92 ± 67	146 ± 87	0.036	89 ± 41	124 ± 90	0.213

[a]At the time of the administration of the first dose of the mRNA-1273 vaccine.

eGFR, estimated glomerular filtration rate (Modification of Diet in Renal Disease-4 equation); hACE, human angiotensin-converting enzyme; IFN-γ, interferon-γ; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IQR, interquartile range; MMF/MPS, mycophenolate mofetil or mycophenolate sodium; mTOR, mammalian target of rapamycin; NK, natural killer; PBMC, peripheral blood mononuclear cell; S, SARS-CoV-2 spike glycoprotein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; SFU, spot-forming unit; SOT, solid organ transplantation.

Sensitivity Analysis Excluding Patients With Natural Immunity

In view of the well-known impact of preexisting natural immunity on vaccine immunogenicity in SOT recipients,[8] we performed a sensitivity analysis in which the 3 patients with positive IFN-γ–producing cell-mediated responses against S, N, and M proteins or serum neutralizing activity at baseline were excluded. In comparison with the overall cohort, no meaningful differences in the kinetics of SARS-CoV-2–specific cell-mediated (Figure S5, SDC, http://links.lww.com/TXD/A377) or humoral immunity after vaccination (Figure S6, SDC, http://links.lww.com/TXD/A377) were observed. Likewise, clinical factors found to be associated with the development and magnitude of vaccine-induced immune responses remain unchanged (Tables S2 and S3, SDC, http://links.lww.com/TXD/A377).

AEs

There were no serious vaccine-related AEs. At least 1 solicited or unsolicited event of local or systemic reactogenicity was reported by 12 recipients (27.3%): pain at the injection site (n = 6), headache (n = 3), fatigue (n = 2), fever (n = 1), tachycardia (n = 1), and nausea (n = 1). None of the patients experienced graft rejection during vaccination or in the subsequent follow-up.

There were no differences in the occurrence of AEs according to the presence of SARS-CoV-2–specific cell-mediated immunity at baseline (0.0% [0 of 3] versus 29.3% [12 of 41] for those with or without prevaccine response, respectively; $P = 0.551$).

DISCUSSION

In contrast to most previous studies,[5-7,9,11-15] we have performed a comprehensive evaluation of vaccine immunogenicity that comprised different methodological approaches to both SARS-CoV-2–specific cell-mediated and humoral immunities. Less than two-thirds of the SOT recipients that received the 2-dose regimen of the mRNA-1273 vaccine mounted positive cellular responses. This response rate was significantly lower than that obtained in the nontransplant control group and appeared to be influenced by the type of transplantation and the amount of immunosuppression. A considerable proportion of recipients exhibiting S protein–specific IFN-γ–producing SFUs above the threshold established for positivity in the IFN-γ FluoroSpot assay had no detectable vaccine-induced humoral immunity analyzed either by SARS-CoV-2 IgG serology or serum neutralizing activity (19.0% and 35.7%, respectively). Moreover, discordance was also observed in the opposite direction, resulting in a less than moderate categorical agreement between cell-mediated and antibody responses.

A few recent studies have investigated SARS-CoV-2–specific cell-mediated responses among vaccinated SOT recipients.[8,10,16,17] Cucchiari et al found that 54.7% of KT recipients had a positive S protein–reactive ELISpot assay after the second dose of mRNA-1273. Although this figure was close to that of our experience, it should be noted that one-quarter of the patients in that study were also reactive to the N protein, raising the possibility of acquired natural—rather than vaccine-induced—immunity or cross-reactivity with seasonal coronaviruses.[8] In a cohort of 101 KT recipients treated with belatacept, the rates of S1-specific cell-mediated immunity by ELISpot after the first and second dose of the BNT162b2 vaccine were 5.0% and 30.4%, respectively.[10] The deleterious impact of the costimulation blocker belatacept on mRNA vaccine immunogenicity has also been reported for IgG antibody responses.[9] In a mixed population mostly consisting of KT recipients, Schmidt et al reported that heterologous boosting with the mRNA-based vaccine after priming with the adenovirus-vector vaccine ChAdOx1 nCoV-19 led to the most pronounced induction of SARS-CoV-2–specific $CD4^+$ T cells.[17]

To our knowledge, only 1 previous study has assessed cell-mediated immunity elicited by SARS-CoV-2 vaccination in the LT population.[17] Although not achieving statistical significance, LT recipients were more likely than KT recipients to mount S protein–specific responses (76.9% versus 51.7%, respectively). In addition, the number of IFN-γ–producing SFUs per 10^6 PBMCs was significantly higher among LT recipients. This difference across transplant types was concordant with the results we have observed for neutralizing activity and with other studies assessing IgG seroconversion.[5] Herrera et al[17] also found more robust cell-mediated responses for LT recipients compared with heart transplant recipients. Ours, however, is the first study to date to compare immunogenicity across different groups of abdominal SOT recipients.

One of the most relevant findings was the notable discordance observed between SARS-CoV-2–specific cell-mediated and humoral responses, including neutralizing activity against the S protein. Although pointed out in some previous studies,[8,16,18] this issue had not been assessed in detail. Cucchiari et al[8] analyzed by Luminex the presence of IgG antibodies against the receptor-binding domain and found that half of the KT recipients who remained seronegative after the completion of vaccination exhibited a positive S-reactive ELISpot. In the same line, 46.2% of SOT recipients with negative IgG antibody in a recent study still had a positive $CD4^+$ T-cell response.[16] In contrast, only 1.9% of 205 hemodialysis patients who received either the BNT162b2 or mRNA-1273 vaccine mounted a cell-mediated response without IgG antibodies detectable by the chemiluminescent immunoassay.[25] A single dose of the mRNA-1273 vaccine elicited robust neutralizing activity and Th1-biased responses in a murine model.[26] Moreover, all the participants in the phase-1 trial who received two 100-μg mRNA-1273 doses generated consistent antibody and $CD4^+$ T-cell responses.[27] Therefore, it may be hypothesized that long-term immunosuppressive therapy contributes to discordant cellular and humoral vaccine-induced responses after SOT. A recent study in nontransplant patients treated with rituximab reported that B-cell depletion affects seroconversion but does not necessarily abrogate T-cell–mediated responses following vaccination.[28] Because the generation of long-lived antibody-secreting plasma cells requires the collaboration between $CD4^+$ subsets (such as follicular helper T cells) and activated B cells, it remains to be assessed whether IgG seronegative recipients with a positive IFN-γ FluoroSpot assay or those who seroconverted but remained below the threshold established for IFN-γ–producing SFUs—which accounted for 19.0% and 16.7% of our cohort, respectively—are effectively protected against SARS-CoV-2 infection.[29]In addition to the type of SOT, we found that a lower prevaccination $CD4^+$ T-cell count, a shorter interval since transplantation, a maintenance immunosuppression regimen including tacrolimus or prednisone, and the number of immunosuppressive drugs received at the time of vaccination (triple therapy versus 1- or 2-drug regimens) predicted the development and magnitude of SARS-CoV-2–specific cell-mediated immunity. These associations remain essentially unchanged when the 3 patients exhibiting natural immunity at baseline were excluded. Taken together, these findings point to the overall amount of immunosuppression as a major determinant of vaccine immunogenicity, as supported by the differences observed with HCWs in the control group. A previous study also identified lymphopenia as a risk factor for defective cellular response.[8] Our study further shows that this association is mainly mediated by the $CD4^+$ T-cell subset. We have previously reported the deleterious impact of tacrolimus on the amount of cell-mediated immunity elicited by natural SARS-CoV-2 infection in SOT recipients recovered from COVID-19.[19,30] In the same line, calcineurin inhibitors have been shown to decrease polyfunctional T-cell responses upon in vitro stimulation with cytomegalovirus antigens.[31] In contrast, no apparent effect on vaccine responsiveness was observed for the use of antimetabolites, which is in contrast with other studies focused on antibody responses.[5,15] Interestingly, a high MMF dose—but not tacrolimus levels—is associated with a lower likelihood of seroconversion after influenza vaccination in KT recipients.[32] These findings should be borne in mind if a strategy based on MMF dose reduction—with a compensatory increase in tacrolimus exposure—is attempted to improve vaccine immunogenicity because calcineurin inhibitors, rather

than antimetabolites, seem to act as major determinants of the capacity to mount protective SARS-CoV-2–specific cell-mediated immunity.

A number of limitations should be acknowledged. First, because of the limited sample size, no multivariate analysis could be performed, and the associations found between clinical characteristics and vaccine-induced immunity remain exploratory. Second, the cutoff value applied to define positive responses by the IFN-γ FluoroSpot assay has not been clinically validated, and different protective thresholds may be applicable to the transplant setting. Nevertheless, these thresholds were established on the basis of a reference group of patients who recovered from severe COVID-19 with naturally acquired immunity. This approach mirrors what followed to determine positive—and presumably protective—cell-mediated responses against other viruses (ie, cytomegalovirus) in commercial ELISA- and ELISpot-based assays, which also used the area under receiver operating characteristics curve analysis to discriminate between IgG seropositive and seronegative individuals.[33-35] Third, because of logistical reasons, the HCWs within the control group received the BNT162b2 vaccine rather than the mRNA-1273 administered to SOT recipients. Increasing evidence reveals that mRNA-1273 is able to elicit higher antibody titers than BNT162b2,[5,36] which is likely due to the higher mRNA content and the longer interval between priming and boosting. Data for cell-mediated immunity are much more limited, although no significant differences between both vaccine products in the magnitude of responses quantified by ELISpot have been reported for pregnant and lactating women,[37] rituximab-treated patients,[28] or KT recipients.[38] Nevertheless, because SOT recipients in our cohort received the mRNA-1273 vaccine (which is presumably associated with higher immunogenicity), the difference observed in cell-mediated responses with regard to the nontransplant control group may have been actually underestimated. Fourth, the dispersion of vaccine-induced responses among HCWs was relatively large, which was likely due to their wide age range. Finally, because we assessed SARS-CoV-2–specific responses at 1 single point after the completion of the vaccination course, the medium- and long-term decay of postvaccination immunity were not investigated.

In conclusion, 59.5% of SOT recipients mounted S protein–specific cell-mediated immunity by 2 wk after the second dose of the mRNA-1273 SARS-CoV-2 vaccine. This response rate was higher than that observed for serum neutralizing activity against the S protein (31.0%) but markedly decreased as compared with nontransplant healthy controls. The agreement between cellular and humoral immunities—particularly neutralizing antibodies—was poor. Our research adds to the emerging, albeit limited, evidence suggesting that KT and LT recipients may benefit from SARS-CoV-2 vaccination even in the absence of seroconversion to IgG antibodies because detectable cell-mediated responses can still be mounted. Future studies, however, must determine whether discordant responses effectively confer protection against COVID-19, as well as the impact on SARS-CoV-2–specific cell-mediated immunity of novel strategies such as heterologous vaccination[17] or the administration of a third vaccine dose.[39,40]

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