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Humoral and Cellular Immune Responses After a 3-dose Course of mRNA-1273 COVID-19 Vaccine in Kidney Transplant Recipients: A Prospective Cohort Study

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Background. In kidney transplant recipients, there is discordance between the development of cellular and humoral response after vaccination against SARS-CoV-2. We sought to determine the interplay between the 2 arms of adaptive immunity in a 3-dose course of mRNA-1273 100 µg vaccine. **Methods.** Humoral (IgG/IgM) and cellular (N- and S-ELISpot) responses were studied in 117 kidney and 12 kidney-pancreas transplant recipients at the following time points: before the first dose, 14 d after the second dose, and before and after the third dose, with a median of 203 and 232 d after the start of the vaccination cycle, respectively. **Results.** After the second dose, 26.7% of naive cases experienced seroconversion. Before the third dose and in the absence of COVID-19, this percentage increased to 61.9%. After the third dose, seroconversion occurred in 80.0% of patients. Naive patients who had at any time point a detectable positivity for S-ELISpot were 75.2% of the population, whereas patients who maintained S-ELISpot positivity throughout the study were 34.3%. S-ELISpot positivity at 42 d was associated with final seroconversion (odds ratio, 3.14; 95% confidence interval, 1.10-8.96; $P=0.032$). Final IgG titer was significantly higher in patients with constant S-ELISpot positivity ($P<0.001$). **Conclusions.** A substantial proportion of kidney transplant recipients developed late seroconversion after 2 doses. Cellular immunity was associated with the development of a stronger humoral response.

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INTRODUCTION

Kidney transplant recipients (KTRs) with coronavirus disease 2019 (COVID-19) suffer from high morbidity and mortality due to their immunosuppressive status and the presence of comorbidities.[1] To protect this delicate population, the generation of a strong adaptive immunity through vaccination is vital to prevent infection or mitigate its severity. However, it is known that the rate of seroconversion after a 2-dose course of mRNA vaccination (either BNT162b2 from Pfizer or mRNA-1273 from Moderna) is as low as 29.9% to 56.9% in different case series.[2-5] As IgG titer is correlated with neutralizing antibodies capacity,[6] which is a strong predictor of clinical protection,[7] it is likely that absence of seroconversion after a 2-dose regimen will leave KTRs unprotected from COVID-19. As a matter of fact, different cases of severe breakthrough infections have been described in KTRs.[8]

Based on all these findings, several healthcare authorities approved the use of a third booster dose of mRNA vaccine for solid organ transplant (SOT) recipients, such as France in April,[9] the United States in August,[10] and Spain on September 7, 2021[11]. Preliminary data indicate that the administration of a third dose in SOT recipients is effective in increasing the percentage of seroconverted patients, even though a still high proportion of them remained unprotected.[9,12]

We have previously demonstrated that almost half of patients without detectable IgG against the S protein after the second dose developed a specific T-cell response assessed

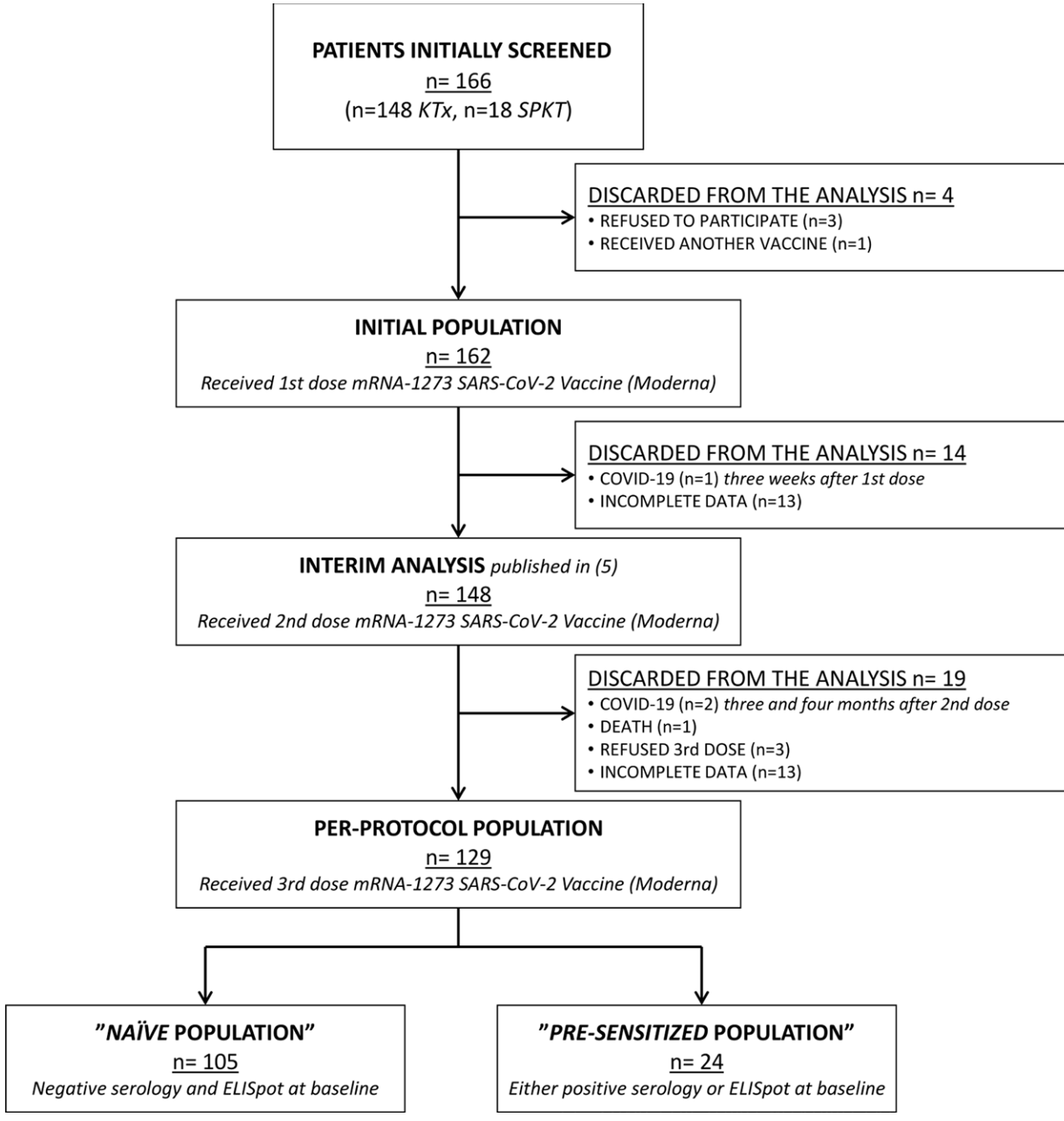

FIGURE 1. Study flowchart.

by IFN-γ–based ELISpot assay.[5] Whether the development of a detectable cellular response is associated with later development of a humoral response or with the effectiveness of a booster dose in KTRs is currently unknown.

To address this point and following up our previous experience,[5] we present herein a complete analysis of both humoral and cellular response in an 8-mo period, from February to October 2021, in which 3 doses of the mRNA-1273 COVID-19 vaccine have been administered to a cohort of KTRs.

MATERIALS AND METHODS

Setting

This is a single-center prospective cohort study of KTRs actively followed up at the Hospital Clínic of Barcelona, Catalunya, Spain who submitted to a 3-dose course of mRNA-1273 vaccine (Moderna, Cambridge, MA).

Vaccination Schedule

The mRNA-1273 vaccine (100 μg) has been administered in the deltoid region, the first 2 doses 4 wks apart and the third dose at the time it was indicated by the health authority (6.7 mo after the first dose).

Inclusion and Exclusion Criteria

The screened population included 166 KTRs. Exclusion criteria included age <18 y, transplantation within the last 3 mo before the first dose, having received antithymocyte globulins (ATG) or rituximab in the last 3 mo for rejection before the first dose, and an active SARS-CoV-2 infection. History of previous COVID-19 was not an exclusion criterion, and patients were considered for vaccination 3 mo after the infection episode. The interim analysis[5] after 2 doses included 148 patients, as 3 patients refused to participate to the study, 1 patient received another vaccine, 1 patient developed COVID-19 between the first and the second dose, and in 13 cases, data were incomplete. From the interim analysis,[5] 19 more cases were excluded because of COVID-19 (2 cases, 3 and 4 mo after the second dose), third dose refusal (n = 3), death for another cause (n = 1), and incomplete data about serological and/or cellular response.[13] The final population herein presented received all 3 doses of the mRNA-1273 vaccine and included 129 patients. Study flowchart is depicted in Figure 1.

Design

After signing informed consent, patients' data and immunologic response (cellular and humoral immunity) were studied at baseline, 2 wks after the second dose, just before receiving the third dose, and 1 mo after (Figure 2). At all the time points, the antibody response against the S protein (IgM/IgG) and the cellular response to the nucleocapside (N) and spike (S) proteins of SARS-CoV-2 virus by means of the ELISpot assay were studied. Patients were categorized as either SARS-CoV-2-naive or SARS-CoV-2-presensitized depending on the baseline status before receiving the first dose of the vaccine.

Outcomes

Because neutralizing antibodies are highly predictive of protection from symptomatic infection[7] and they are correlated with S-specific IgG titer in the general population[6] and in KTRs,[4] we chose IgG seroconversion after the third dose as the main outcome of the study. The secondary objectives included the analysis of late seroconversion after the second dose, a complete descriptive analysis of the changes of cellular and humoral immunity during the course of the study, and the analysis of the baseline factors associated with vaccine responsiveness.

Ethics

The institutional ethics committee approved the initial protocol of the study that was amended upon notification of the third dose by the Spanish Ministry of Health in September 2021 (code HCB/2021/0222).

Quantification of Antibodies to SARS-CoV-2 by Luminex

To quantify immunoglobulins titer, we used a serological assay based on the Luminex assay that has the benefit of a higher dynamic range than other assays.[14] We measured antibodies that were directed against the receptor-binding domain of the S protein by Luminex. Crude median fluorescent intensities (MFIs) were exported using the xPONENT software. Assay cutoff was calculated as 10 to the mean plus 3 standard deviations of log10-transformed MFIs of 47 negative controls. The data used for the calculations were the ratio of the raw MFI of the particular individual with the raw MFI obtained from the donor pool, and a value ≥1 was considered

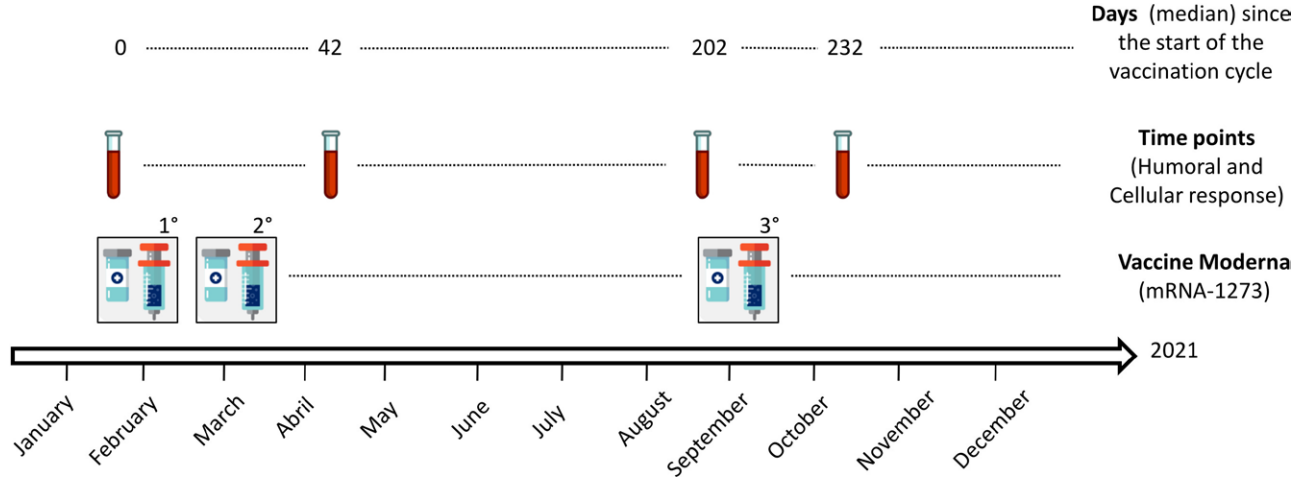

FIGURE 2. Study design.

to be positive. Sensitivity of the assay using samples from participants previously diagnosed with COVID-19 and with >10 d since the onset of symptoms was 97% for IgG and 75% for IgM, with specificities of 100% for IgG and IgM.

IFN-γ ELISpot Assay

Peripheral blood mononuclear cells (PBMCs) at a concentration of 2×10^5 PBMCs were processed immediately and stimulated in X-VIVO 15 medium (Lonza) supplemented with 10% heat inactivated AB serum and PepTivator SARS-CoV-2 Prot_S (1 μg/mL, Miltenyi Biotec) that cover the immunodominant sequence domains of the spike ("S") glycoprotein of SARS-CoV-2 (*GenBank MN908947.3*, *Protein QHD43423.2)* and N peptide pools. The latter peptide pool N-ELISpot represents a control for exposure to the SARS-CoV-2 nucleocapsid phosphoprotein because the mRNA-1273 vaccine is based on the S protein. Negative control wells lacked peptides, whereas positive control ones included anti-CD3-2 mAb. Cells were incubated overnight at 37 °C 5% CO_2 in precoated anti-IFN-γ MSIP white plates (mAb 1-D1K, Mabtech). After incubation, plates were washed 5 times with PBS (Sigma-Aldrich) and incubated 2 h at room temperature with horseradish peroxidase–conjugated anti-IFN-γ detection antibody (1 μg/mL; clone mAb-7B6-1; Mabtech). After 5 further washes with PBS, tetramethylbenzidine substrate was added, and spots were counted using an automated ELISpot Reader System (Autoimmun Diagnostika GmbH). To quantify positive peptide-specific responses, spots of the unstimulated wells were subtracted from the peptide-stimulated wells, and the results expressed as spot forming units SFU/2×10^5 PBMCs. We determined SARS-CoV-2–specific spots by spot increment, defined as stimulated spot numbers >6 SFU/2×10^5 PBMCs against the N and the S protein. This cutoff was defined calculating the mean ± 2 standard deviations in a group of healthy donors obtained before the start of the pandemic of SARS-CoV-2. Spot counting was done automatically and reevaluated manually in all cases.

Statistical Analysis

Baseline characteristics were analyzed as a whole and separately for naive and presensitized patients. Continuous variables have been described as mean with SD or median and interquartile range [25th; 75th percentiles] and analyzed by Student's *t*-test or Mann-Whitney *U* test, respectively, according to data distribution. Categorical variables are described as absolute frequencies and percentages and analyzed by Fisher's exact test. Estimation of vaccine responsiveness (IgG after the third dose) was assessed by odds ratio (OR) and their 95% confidence intervals (95% CI) by means of univariate logistic regression models. To establish independent factors predicting vaccine responsiveness, variables associated with the outcome at the univariate analysis with a $P \leq 0.05$ were finally entered into a multivariable logistic model. Changes in the ELISpot and antibodies titers through time points were assessed by Wilcoxon signed ranks test for related samples. Differences in the ELISpot and antibodies titers between groups were analyzed by Mann-Whitney *U* test for independent samples. Correlation between IgG titer and S-ELISpot was analyzed by Spearman's test. In all statistical analyses, we applied a 2-sided type I error of 5%. To perform all the analysis, the software SPSS, version 25 (Armonk, NY, IBM Corp), has been used. Figures were designed with GraphPad, version 5 (GraphPad Software, La Jolla, CA).

RESULTS

A total of 129 patients were included in the final analysis (Figure 1), of which 105 were naive to SARS-CoV-2 and 24 were presensitized. Baseline characteristics of the whole population and the 2 groups are listed in Table 1. Of the 24 presensitized patients, only in 9 cases was there a history of PCR-proven COVID-19 at least 3 mo before the first dose. Four patients had both positive IgG and IgM, 5 patients had only positive IgG, 5 had only positive IgM, and 10 tested positive for either N- or S-ELISpot in the absence of positive serology.

After receiving the first dose, the time points of the study were at 2 wks after the second dose (42 d after the first dose

TABLE 1.

Baseline demographic and clinical characteristics of the final population included at the time of receiving the third dose of the mRNA-1273 vaccine

	Total (n = 129)	Naive (n = 105)	Presensitized (n = 24)	*P*
Age (y)	59.1 ± 12.4	60.4 ± 11.6	53.2 ± 14.5	**0.010**
Sex (% female)	27.9	28.6	25.0	0.806
Diabetes (% yes)	21.7	23.8	12.5	0.282
BMI	25.6 ± 4.6	25.7 ± 4.7	25.2 ± 4.6	0.619
Ethnicity (%)				0.378
Caucasic	95.3	96.2	91.7	
Hispanic	3.9	2.9	8.3	
African	0.8	1.0	-	
Blood type (%)				0.301
A	51.9	48.6	66.7	
B	3.9	3.8	4.2	
0	42.7	45.7	29.2	
AB	1.6	1.9	-	
Type of donor (%)				0.833
Living	34.4	34.3	34.8	
DBD	40.0	38.2	47.8	
DCD II	6.4	6.9	4.3	
DCD III	19.2	20.6	13.0	
Type of transplantation				**0.010**
Kidney	90.7%	94.3%	75.0%	
Kidney-pancreas	9.3%	5.7%	25.0%	
Time from transplant (y)	1.6 [0.7-5.0]	1.6 [0.7-4.6]	1.6 [1.0-8.9]	0.677
Transplant < 1 y (%)	28.7	30.5	20.8	0.456
Dialysis vintage (mo)	18 [4.7-38]	19 [6-37.5]	9.5 [0-48.75]	0.391
Previously transplanted (yes)	26.4%	25.7%	29.2%	0.798
Any rejection (% yes)	19.4	19.0	20.8	0.782
Baseline cPRAI+II (%)	0 [0-24]	0 [0-31.5]	0 [0-19.75]	0.867
eGFR CKD-EPI (mL/min)	48.1 ± 19.3	46.0 ± 18.2	57.2 ± 21.8	**0.010**
Leukocytes (/mm^3)	6165 ± 2029	6088 ± 2048	6503 ± 1950	0.368
Hb (g/dL)	13.3 ± 1.7	13.3 ± 1.7	13.6 ± 1.5	0.306
Lymphocytes (/mm^3)	1369 ± 778	1343 ± 759	1479 ± 861	0.444
Lymphopenia (<1000/mm^3) (% yes)	32.6	31.4	37.5	0.631
Baseline immunosuppression				0.434
Tacrolimus + mycophenolate	51.9%	48.6%	66.7%	
Tacrolimus + mTOR inhibitors	27.1%	29.5%	16.7%	
Belatacept-based	7.8%	7.6%	8.3%	
Other	13.2%	14.3%	8.3%	
Treated during the last year with (% yes)				
ATG	11.6	13.3	4.2	0.301
Rituximab	1.6	1.9	-	1

ATG, antithymocyte globulins; BMI, body mass index; CKD, Chronic Kidney Disease; cPRA, calculated PRA; DBD, Donor after Brain Death; DCD, Donor after Circulatory Death; eGFR, estimated Glomerular Filtration Rate; mTOR: n/a.

and 14 d after the second dose in all patients), just before receiving the third dose (median 203 [201–204] d after the first dose) and 1 mo after the third dose (median time 232 [231–233] d after the first dose and 29 [28–32]) (Figure 2).

Results in the Naive Population

Evolution of Humoral Response Before and After the 3 Doses of the mRNA-1273 Vaccine

In naive patients, seroconversion occurred in 28 of 105 patients (26.6%) 2 wks after the second dose, with 5 patients having both IgG and IgM, 2 patients only IgM, and 21 only IgG. Before receiving the third dose, at a median of 6.7 mo after the first dose and in absence of clinically evident COVID-19, 65 out of 105 patients (61.9%) displayed a humoral response. Of these, 15 had both IgG and IgM, 8 only IgM, and 42 only IgG. After the third dose, the number of patients with positive serology had increased to 84 (80.0%), of which 23 had both IgG and IgM, 5 only IgM, and 56 only IgG (Figure 3A,B) (Table 2).

Evolution of Cellular Response Before and After the 3 Doses of the mRNA-1273 Vaccine

After receiving the second dose, 55 naive patients (52.3%) developed positivity at the ELISpot, of which 13 were for

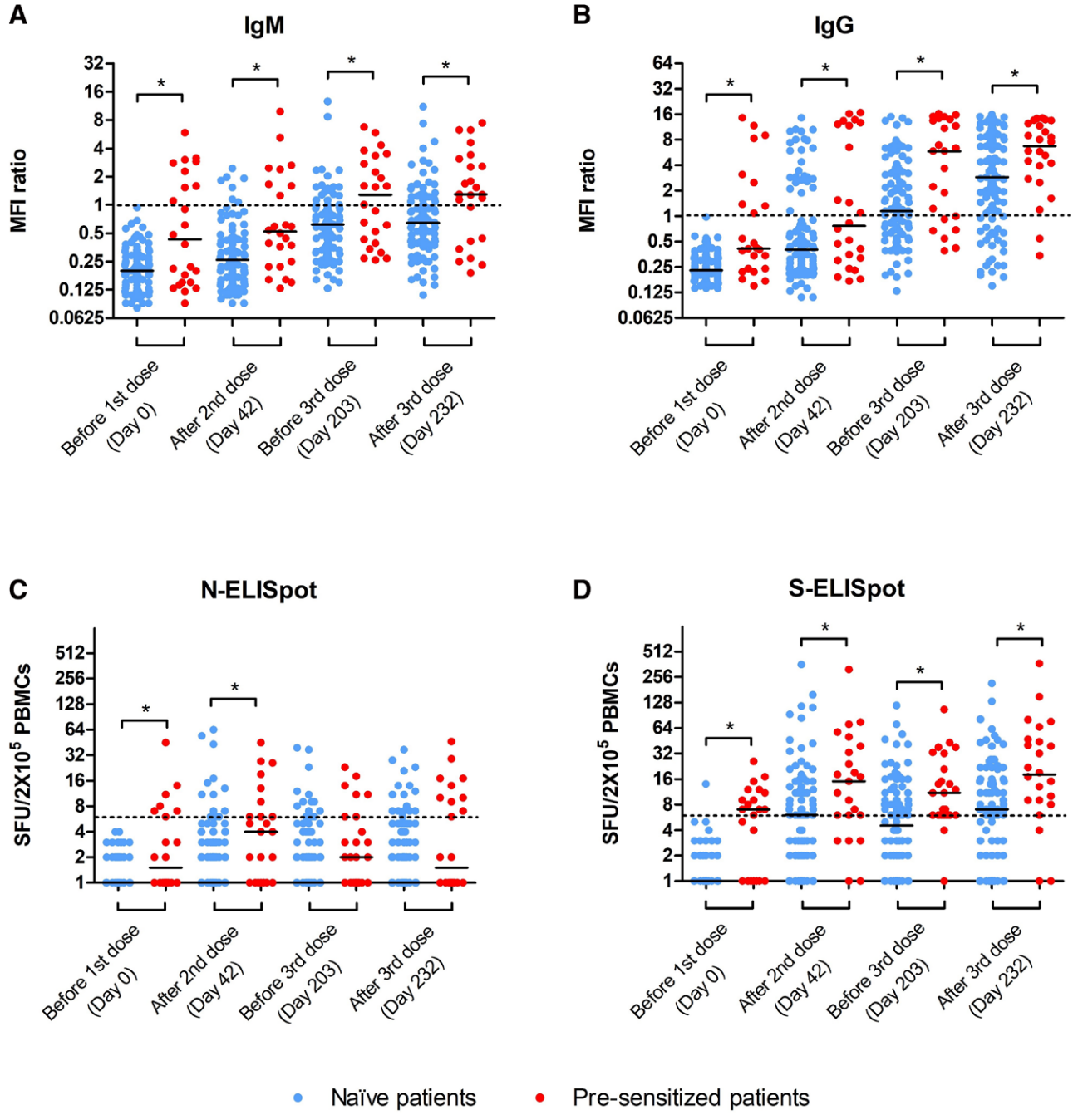

FIGURE 3. Changes in IgM (A) and IgG concentration (B), N-ELISpot (C), and S-ELISpot (D) spots before and after the 3 doses of the mRNA-1273 SARS-CoV-2 vaccine. Dashed lines indicate the normal range, whereas the median for every group is highlighted as a black line. Presensitized patients are marked in red, whereas naive patients are marked in blue. C and D, patients with 0 spots are not included in the graphs. *Statistical significance with a $P < 0.05$.

TABLE 2.

Seroconversion rates of the whole population and cellular immunity at all the time points studied of the whole population and according if patients were SARS-CoV-2-naive or presensitized

	Seroconversion (either IgG or IgM)	IgG	IgM	ELISpot (either S- or N-)	S-ELISpot	N-ELISpot
Total population (n = 129)						
Before first dose	10.8%	6.9%	6.9%	11.6%	10.0%	5.4%
After second dose	32.5%	28.6%	11.6%	57.3%	55.8%	17.8%
Before third dose	65.1%	58.1%	27.9%	58.1%	56.5%	16.2%
After third dose	82.9%	78.2%	34.1%	66.6%	65.1%	19.3%
Naive population (n = 105)						
Before first dose	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
After second dose	26.6%	24.7%	6.6%	52.3%	51.4%	13.3%
Before third dose	61.9%	54.2%	21.9%	49.5%	49.5%	13.3%
After third dose	80.0%	75.2%	26.6%	61.9%	60.9%	14.2%
Presensitized population (n = 24)						
Before first dose	58.3%	37.5%	37.5%	62.5%	54.1%	29.1%
After second dose	58.3%	45.8%	33.3%	79.1%	75.0%	37.5%
Before third dose	79.1%	75.0%	54.1%	91.6%	91.6%	29.1%
After third dose	95.8%	91.6%	66.6%	83.3%	83.3%	41.6%

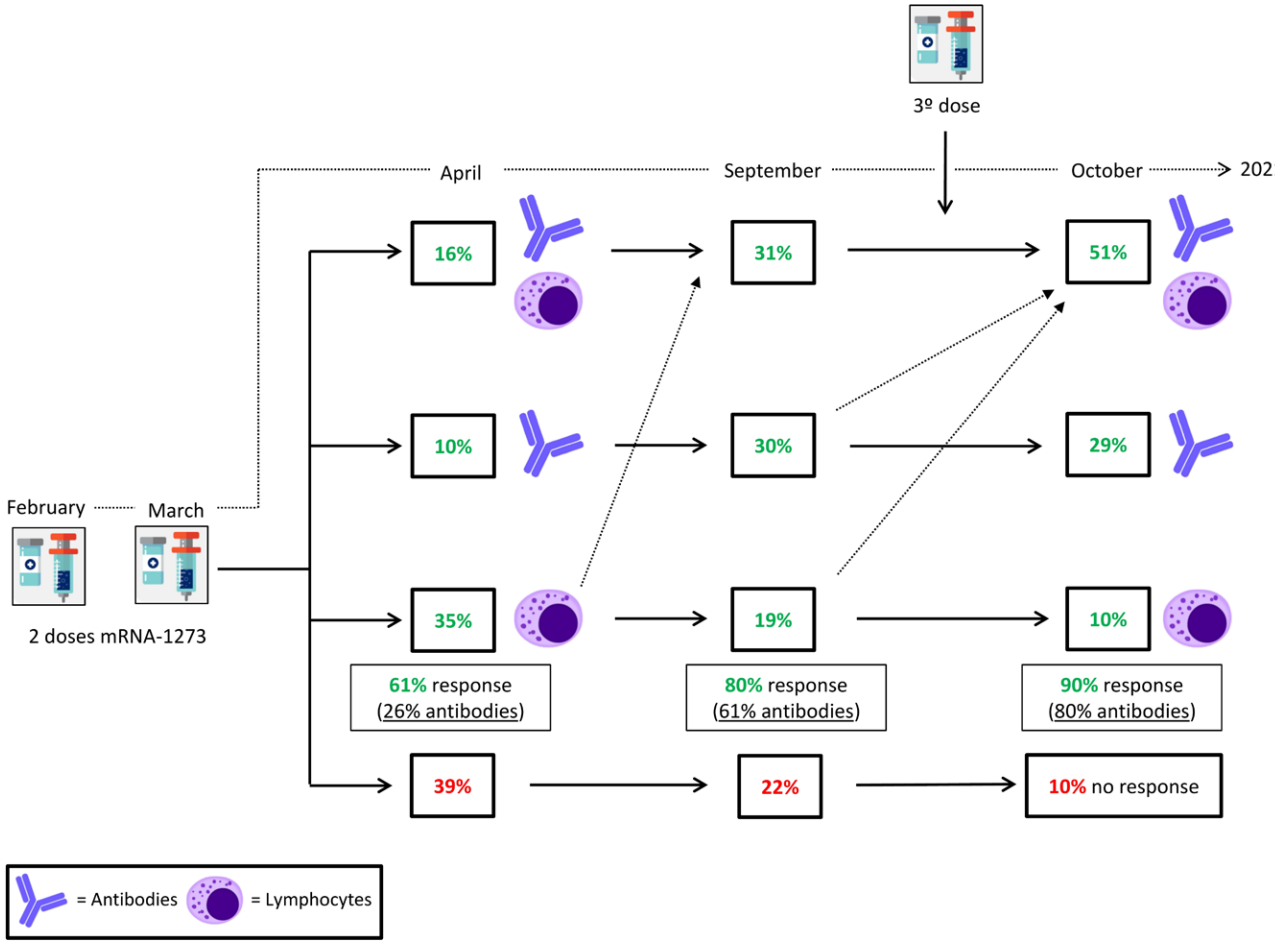

FIGURE 4. Evolution of both humoral and cellular immunity across all the time points of the study. Patients are divided among those who developed both humoral and cellular response (upper part), those who developed only humoral response or only cellular response (mid-part), or no-responders (lower part). Dashed lines indicate relative movements between time points of either cellular or humoral response.

both S- and N-ELISpot, 1 only for N-ELIspot, and 41 only for S-ELISpot. Before receiving the third dose, 53 patients were still positive at the ELISpot (49.5%), of which 12 were for both S- and N-ELIspot, 2 only for N-ELISpot, and 38 only for S-ELISpot. After receiving the third dose, the number of patients with ELISpot positivity increased to 65 (61.9%), of which 13 were positive for S- and N-ELISpot, 2 only for N-ELISpot, and 50 only for S-ELISpot (Figure 3C,D) (Table 2). Evolution of both humoral and cellular response throughout the study is summarized in Figure 4, whereas absolute values

and percentage differences for IgG and S-ELISpot across the time points are highlighted in Tables S1 and S2 (SDC, http://links.lww.com/TXD/A463), respectively.

A total of 79 patients (75.2%) displayed S-ELISpot positivity at some time point during the course of the study, and only 36 (34.3%) maintained S-ELISpot positivity during all the time points.

On the other side, 30 patients (28.6%) displayed N-ELISpot positivity sometimes during the course of the study, and only 3 (2.8%) maintained N-ELISpot positivity at all the time points after vaccination.

Late Seroconversion After the Second Dose

At the second time point of the study (median of 42 and 14 d after the first and the second doses, respectively), 77 patients had not developed either IgG or IgM. At the third time point (median of 203 d after the start of the vaccination cycle), 40 of these 77 patients (51.9%) had positive serology, of which 6 had both IgM and IgG, 7 only IgM, and 27 only IgG against the S protein. None were diagnosed with COVID-19 during this time frame, and only 5 (12.5%) of the seroconverted patients had positive N-ELIspot either after the second dose or before the third dose. Of the 40 patients who experienced late seroconversion, 21 (52.5%) had positive S-ELISpot after the second dose, whereas in the other 37 patients, S-ELISpot positivity at the same time point was observed in 16 cases (43.2%). Within the 77 seronegative patients after the second dose, we observed 11 cases of patients whose S-ELISpot was negative at this time point and turned positive before the third dose (14.2%). Neither N-ELISpot (OR, 0.57; 95% CI, 0.16-2.72; $P = 0.579$) nor S-ELISpot positivity (OR, 1.44; 95% CI, 0.59-3.51; $P = 0.418$) after the second dose were associated with late seroconversion. The only factor that was associated with the late development of positive IgG was having IgM above the median of 0.29 (MFI ratio) after the second dose (OR, 2.71; 95% CI, 1.00-2.71; $P = 0.048$). Conversely, IgG above the median of 0.40 (MFI ratio) after the second dose was not associated with late seroconversion (OR, 1.41; 95% CI, 0.56-3.56, $P = 0.460$). Final IgG titer was significantly higher in patients who were already positive after the second dose in comparison with late responders (9.37 [6.84-13.79] versus 1.89 [0.63-4.43] MFI ratio, $P < 0.001$).

Factors Associated With Final Seroconversion After the Third Dose of the mRNA-1273 Vaccine

The primary outcome of seroconversion after the 3-dose course of mRNA-1273 vaccine was achieved by 84 patients (80.0% of the naive population). The only factor that was positively correlated with the achievement of the outcome at univariate (OR, 3.13; 95% CI, 1.21-8.07; $P = 0.018$) and multivariate analysis (OR, 3.14; 95% CI, 1.10-8.96; $P = 0.032$) was S-ELISpot positivity after the second dose. More specifically, naive patients who had S-ELISpot positivity after the second dose (54 patients, 51.4%) were IgG-positive after the third dose in 85.2% of cases. Conversely, patients who were S-ELISpot negative after the second dose (51 cases, 48.6% of naive population) had a seroconversion rate after the third dose of 64.7% ($P = 0.023$, Fisher's exact test). Factors that were negatively associated with the outcome at univariate analysis included previous transplantation (OR, 0.34; 95% CI, 0.13-0.89; $P = 0.029$), having received ATG (OR, 0.26; 95% CI, 0.08-0.84; $P = 0.025$), and being transplanted within 1 y from the first dose (OR, 0.25; 95% CI, 0.09-0.64; $P = 0.004$). In multivariable analysis, the 2 factors that were still negatively associated with the outcome were previous transplantation (OR, 0.22; 95% CI, 0.06-0.78; $P = 0.020$) and time from transplant <1 y from the first dose (OR, 0.23; 95% CI, 0.07-0.80; $P = 0.021$). The positivity for S-ELISpot at any time during the course of the study was nonsignificantly associated with the outcome (OR, 2.46; 95% CI, 0.94-6.44; $P = 0.067$). Conversely, positivity for N-ELISpot at any time point was not associated with seroconversion at univariate analysis (OR, 0.72; 95% CI, 0.27-1.86; $P = 0.501$) (Table 3).

Efficacy of the Third Dose for Patients Still Seronegative After 2 Doses and as a Booster for Seropositive Patients

Considering seronegative patients at the time of receiving the third dose (40 cases, 38.1% of the naive population), a humoral response developed afterward in 23 cases (57.5%), of which 2 developed both IgG and IgM, 3 only IgM, and 18 only IgG. The IgG titer after the third dose was higher in patients who had positive serology before the booster in comparison with seronegative patients at the same time point

TABLE 3.

Univariable and multivariable analysis on factors associated with vaccine response, defined as IgG+ at 1 mo after third dose of mRNA-1273 SARS-CoV-2 vaccine, in naive patients at baseline

	Vaccine response (IgG+ 1 mo after third dose) in naive patients at baseline			
	Univariable	*P*	Multivariable	*P*
Age				
≤50 y	Ref	0.243		
51–60 y	0.71 [0.18-2.73]	0.624		
61–70 y	1.31 [0.30-5.55]	0.714		
>70 y	0.39 [0.11-1.33]	0.133		
Sex (female)	0.54 [0.21-1.38]	0.202		
Diabetes (yes)	0.48 [0.18-1.27]	0.140		
Previous Tx (yes)	0.34 [0.13-0.89]	**0.029**	0.22 [0.06-0.78]	**0.020**
Baseline immunosuppression				
TAC + MPA	Ref			
TAC + mTORi	1.57 [0.53-4.65]	0.410		
Belatacept	0.22 [0.04-1.07]	0.062		
Other	5.29 [0.63-44.12]	0.123		
ATG <1 y	0.26 [0.08-0.84]	**0.025**	2.22 [0.38-12.81]	0.371
Lymphopenia (yes)	1.04 [0.40-2.71]	0.933		
Time from Tx <1 y	0.25 [0.09-0.64]	**0.004**	0.23 [0.07-0.80]	**0.021**
eGFR (ml/min/1.73m2)				
>60	Ref	0.164		
45–60	1.52 [0.33-6.95]	0.582		
30–44	0.55 [0.15-2.03]	0.375		
<30	0.33 [0.07-1.44]	0.142		
S-ELISpot+ after second dose	3.13 [1.21-8.07]	**0.018**	3.14 [1.10-8.96]	**0.032**
S-ELISpot+ anytime	2.46 [0.94-6.44]	0.067		
N-ELISpot+ anytime	0.72 [0.27-1.86]	0.501		

Estimation of vaccine responsiveness was assessed by OR and their 95% CI by means of logistic regression models.

95% CI, 95% confidence interval; ATG, antithyocyte globulins; MPA, Mycophenolic Acid; mTORi, mTOR inhibitors; OR, odds ratio; TAC, Tacrolimus.

(6.72; 2.04-11.06; $P < 0.001$). Factors negatively associated with the efficacy of a third dose in seronegative patients were the use of ATG within the last year at baseline (OR, 0.10; 95% CI, 0.01-0.97; $P = 0.047$) and having been transplanted within the last year at baseline (OR, 0.19; 95% CI, 0.05-0.71; $P = 0.014$). Figure 5 displays an increase in the IgG titer before and after the third dose, according to whether or not patients were S-ELISpot positive. There was also an association, albeit not statistically significant, of S-ELISpot positivity at the time point after the second dose (OR, 3.50; 95% CI, 0.94-12.96; $P = 0.061$) or at any time point of the study (OR, 3.80; 95% CI,0.97-14.87; $P = 0.054$) with seroconversion after the third dose in seronegative patients. At multivariable analysis, however, none of these variables were finally associated with the outcome (Table 4). In seropositive patients at the time of receiving the third dose (61.9% of the naive population), the IgG titer increased substantially after the booster from 2.18 (1.22-5.34) to 6.72 (2.04-11.60) ($P < 0.001$).

Intensity of Cellular Immunity Is Associated With the Final IgG Titer

Naive patients who had at any time point after the first dose a detectable positivity for S-ELISpot (75.2% of the naive population) tended to have higher IgG titer at the end of the study (3.39 [1.17-9.85] versus 1.96 [0.506.90]; $P = 0.063$), whereas patients who maintained positivity for S-ELISpot after the first dose all throughout the study (34.3% of the naive population) had significantly higher IgG titer after the third dose (8.55 [1.74-11.62] versus 2.06 [0.70-6.18]; $P = 0.001$). The number of S-spots after second dose was not associated with IgG titer at the same time point ($r = 0.140$, $P = 0.155$) (Figure 6A), was weakly associated with IgG at 6 mo before the third dose ($r = 0.206$, $P = 0.036$) (Figure 6B), and was strongly associated with IgG titer after the third dose ($r = 0.344$, $P < 0.001$) (Figure 6C). This correlation with final IgG titer persisted also when taking into account the number of S-spots after the third dose ($r = 0.282$, $P = 0.004$) (Figure 6D).

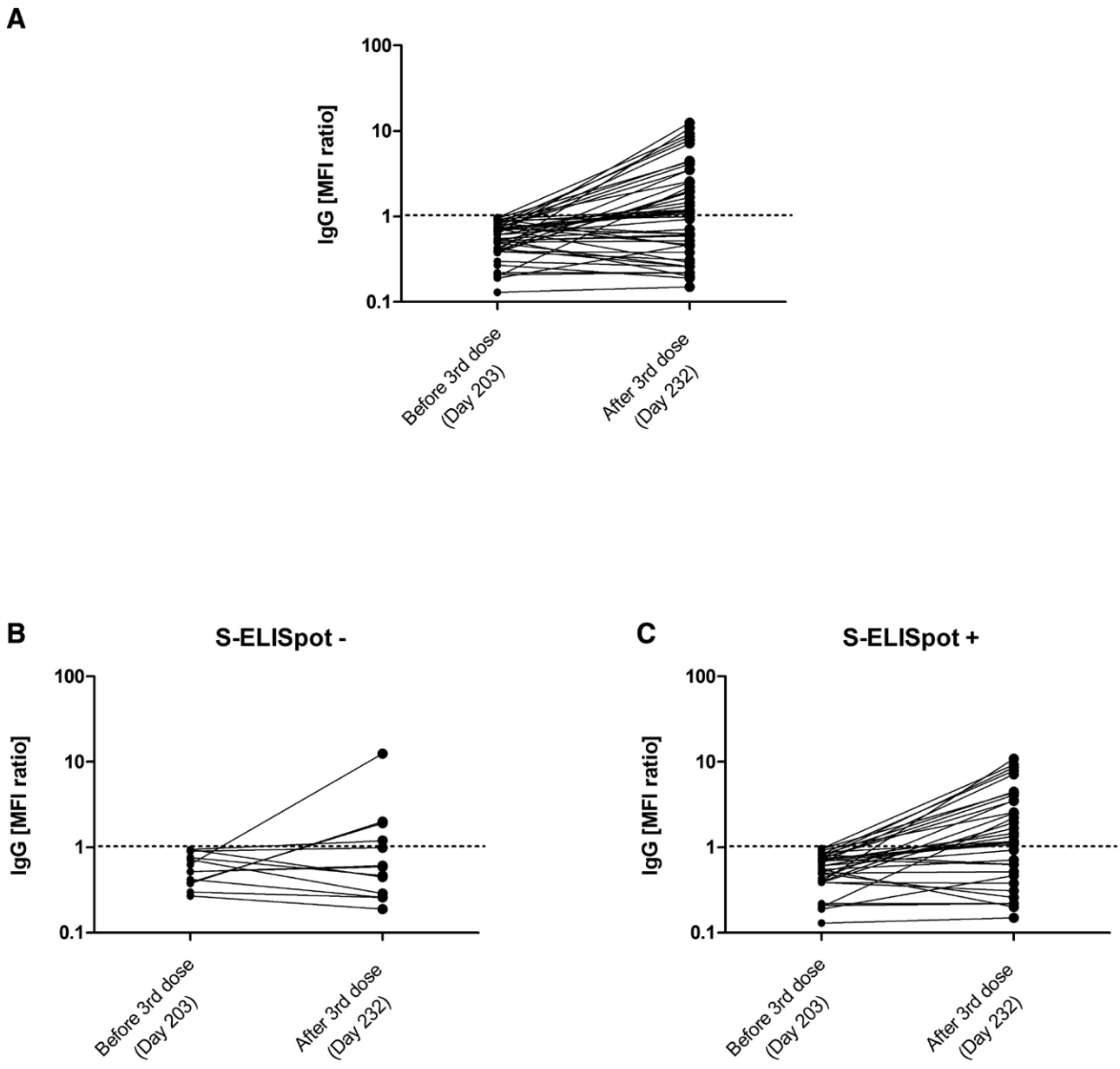

FIGURE 5. Efficacy of a third dose in 40 seronegative patients after 2 doses. Patients are displayed as a whole (A), if they had S-ELISpot negative all throughout the study (B), or if they had S-ELISpot positivity in any time point after the second dose (C). MFI, median fluorescent intensities.

TABLE 4.

Univariable analysis on factors associated with the administration of a third dose of mRNA-1273 vaccine, defined as IgG+ at 1 mo after, in seronegative patients after the first 2 doses

	Response to a third dose (IgG+ 1 mo after) in seronegative patients after 2 doses			
	Univariable	P	Multivariable	P
Age				
≤50 y	Ref	0.155		
51–60 y	1.00 [0.16-5.98]	1		
61–70 y	5.00 [0.64-39.05]	0.125		
>70 y	0.60 [0.10-3.33]	0.560		
Sex (female)	0.53 [0.15-1.79]	0.308		
Diabetes (yes)	0.77 [0.23-2.59]	0.682		
Previous Tx (yes)	0.34 [0.09-1.25]	0.106		
Baseline Immunosuppression				
Tacrolimus + mycophenolate	Ref	0.294		
Tacrolimus + mTOR inhibitors	1.62 [0.36-7.20]	0.523		
Belatacept-based	0.54 [0.08-3.51]	0.520		
Other	6.50 [0.68-62.1]	0.104		
ATG <1 yr at baseline	0.10 [0.01-0.97]	**0.047**	0.33 [0.02-4.18]	0.395
Lymphopenia (yes)	0.81 [0.20-3.22]	0.767		
Time from Tx <1 yr at baseline	0.19 [0.05-0.71]	**0.014**	0.25 [0.05-1.27]	0.095
eGFR <30 mL/min/1.73m2	0.52 [0.14-1.85]	0.316		
S-ELISpot+ after second dose	3.50 [0.94-12.96]	0.061		
S-ELISpot+ anytime	3.80 [0.97-14.87]	0.054		
N-ELISpot+ anytime	0.60 [0.15-2.41]	0.475		

ATG, antithyocyte globulins; eGFR, estimated Glomerular Filtration Rate; mTORi, n/a.

Results in the Presensitized Population

Presensitized patients displayed progressively higher rates of seroconversion (either IgG or IgM) from 58.3% before and after the first 2 doses to 95.8% after the third dose. In parallel, ELISpot positivity increased progressively, both for S-ELISpot (from 54.1% to 83.3%) and N-ELISpot (from 29.1% to 41.6%) before and after the 3-dose course, respectively (Table 2).

Presensitized Patients Maintained Higher Humoral and Cellular Immunity Throughout the Study, and Third Dose Did Not Increase Significantly IgG Titer

Presensitized patients (n = 24) displayed higher antibodies titers all the way throughout the study in comparison with naive patients (Figure 3A,B). Also S-ELISpot was higher at all the time points, whereas no difference was observed for N-ELISpot in the last 2 time points (Figure 3C,D). Although the first 2 doses were effective in increasing IgG titer in presensitized patients (0.41 [0.24-2.20] at baseline versus 0.77 [0.30-12.10] MFI ratio after second dose, $P = 0.002$), the third dose was not associated with a significant increase (before and after 5.88 [0.94-13.81] and 6.73 [3.05-12.33] MFI ratio, respectively, $P = 0.6001$) (Table S1, SDC, http://links.lww.com/TXD/A463), However, when taking into account patients without a history of clinically evident COVID-19 (15 of 24 cases), the increase in IgG after the third dose reached statistical significance (before and after 1.89 [0.67-5.90] and 4.47 [1.62-12.55] MFI ratio, $P = 0.025$). Conversely, patients with a history of PCR-proven COVID-19 expressed paradoxically a decrease in IgG titer (before and after third dose 13.95 [9.00-15.44] and 8.96 [5.85-12.66] MFI ratio, respectively, $P = 0.039$).

DISCUSSION

A substantial proportion of KTRs who fail to achieve seroconversion after 2 doses of mRNA-1273 vaccine develop cellular immunity against the S protein.[5] Whether the development of cellular immunity is associated with higher possibility to achieve a late seroconversion with or without a third dose is currently unknown. Preliminary studies in this setting were focused only on time points before and after the third dose and have not considered the whole evolution from the start of the vaccination cycle.[12,13]

By contrast, the present study comprises a longitudinal and prospective follow-up during an 8 mo–long vaccination cycle in 129 KTRs who have received 3 doses of the same product (mRNA-1273, 100 μg). Although combination of different vaccines has been proposed in the last months, based on the potential increase in effectiveness of the so-called "heterologus vaccination,"[15] it is especially relevant that this cohort received homogeneously only the Moderna vaccine. Differently from the other reports that are mainly focused on humoral response, we provide herein the complete evolution of cellular response also in all the time points across the 3-dose course of mRNA-1273 vaccine.

We demonstrated that the development of cellular immunity after the second dose was associated with final seroconversion (Table 3). The importance of developing a detectable cellular immunity is highlighted by the fact that patients who displayed positive ELISpot throughout the study (34.3%) had significantly higher IgG titer after the third dose and that the number of S-spots after the second dose were strongly correlated with final IgG as well (Figure 6).

The finding that the majority of patients with a cellular response did not display a constant S-ELISpot positivity during the study probably reflects that effector T cells are circulating only at a determinate time point before homing at secondary lymphoid organs. We know that, in a productive immune response, specific T-cell populations undergo numerical increases and differentiation to manifest appropriate effector functions to eliminate the pathogen. This phenomenon is usually followed by a substantial loss of effector cells though preserving an elevated number of enduring memory T cells.[16,17] Moreover, the discordance between humoral and cellular immunity has already been highlighted by different groups as a consequence of the immunosuppression received by KTRs.[5,18] In a group of 25 patients, Schrezenmeier et al did not observe a substantial increase in T-cell response after third dose.[13] However, this does not imply that, upon rechallenge with SARS-CoV-2, these effector T cells cannot proliferate and be detectable in peripheral blood.[19]

Regarding humoral immunity, it is known that the most important clinical correlate of efficacy is neutralizing antibodies[7,20] that, in turn, are associated with the IgG titer.[4,6] In this sense, achieving an 80.0% of seroconversion after the third dose is an encouraging finding for KTRs, considering the poor response rate after the first 2 doses.[2,5] Moreover, we observed that 57.5% of seronegative patients after 2 doses positivize IgG after a "rescue" third dose. Recent data from Reindl-Schwaighofer et al showed that the rate of seroconversion in seronegative patients after 2 doses was 35% and 42% for a mRNA (BNT162b2 or mRNA-1273) and a vector vaccine (Ad26COVS1), respectively.[15]

A striking finding is that many patients experienced late seroconversion because 51.9% of patients who were seronegative

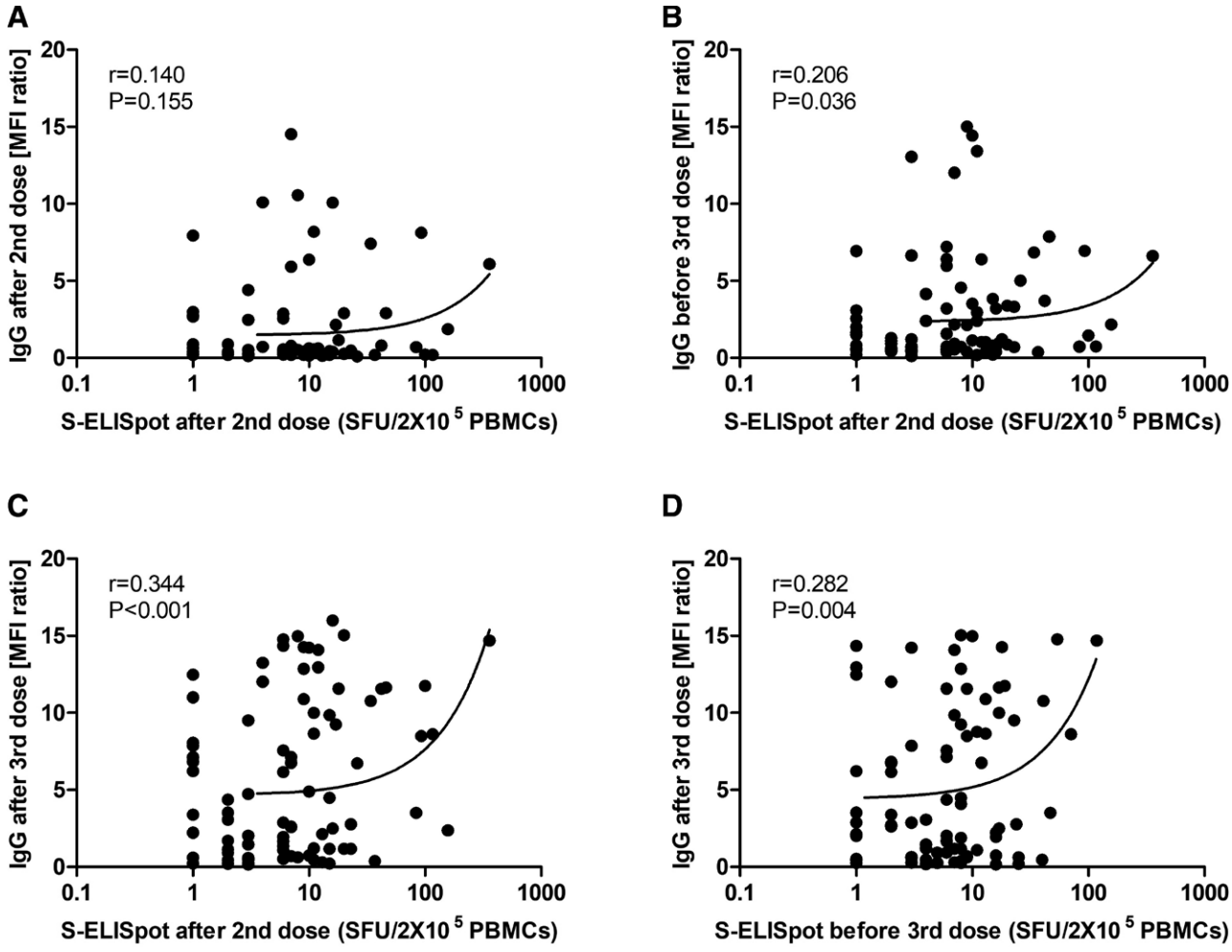

FIGURE 6. Correlation of the number of S-spots after second dose with IgG titer after second dose (A) and before (B) and after (C) the third dose. Correlation of the number of S-spots before the third dose with the final IgG titer after the third dose is displayed in (D). Correlation has been calculated by means of the Spearman test. MFI, median fluorescent intensities; PBMC, peripheral blood mononuclear cell.

after the second dose turned seropositive just before the third dose in the absence of clinically proven COVID-19. Only 5 of 77 patients with late seroconversion had N-specific ELISpot against SARS-CoV-2 in this time frame. This may suggest that, because of chronic immunosuppression, KTRs experience a delayed response to a 2-dose course of mRNA vaccine, and probably, seroconversion should be assessed later than 2 to 4 wks after the second dose, which is the classical time point studied in the general[21] and transplant populations.[2-5] A recent study also demonstrated that anti-S IgG production is delayed in KTRs after COVID-19 in comparison with general population.[22]

However, still, 20% of patients do not achieve seroconversion after this 3-dose course. This population is probably the most vulnerable to immunosuppression because factors negatively associated with seroconversion were kidney transplant within last year and having been transplanted before (Table 3). Also, having received ATG was associated with the inability of the booster to rescue seronegative patients after 2 doses (Table 4).

In presensitized patients, we did not observe a significant increase of IgG titer after the booster, especially in patients with a history of clinically evident COVID-19. This finding correlates with some studies published in previously infected individuals in whom no further increase in antibodies titer was observed upon administration of a second dose of vaccine.[23-25]

The main limitation of the study lies in the absence of another time point between the second and third dose that would have shed more light on the dynamic of late seroconversion after the first 2 doses. Our assumption that this late seroconversion developed in the absence of exposure to SARS-CoV-2 in the majority of patients may be debatable. However, patients with clinically proven COVID-19 (n = 3) were discarded from the final analysis (Figure 1). We also consider that, given the typical clinical course of COVID-19 in KTRs that is usually symptomatic,[1] it is unlikely that this high rate of seroconversion is only due to asymptomatic infections because only a minority of cases developed positive N-ELISpot during this time frame. Also, some evidence points out that N-specific ELISpot may also have cross-reactivity with other coronaviruses.[26]

The study was performed before the last Omicron wave. However, Massa et al demonstrated the very good cross-variant immunogenicity (including the Delta variant) of the 3-dose mRNA vaccination.[27] Finally, we do not provide safety data, although there are several publications showing that KTRs have an acceptable tolerability profile to the third vaccine booster[15,27].

In conclusion, the administration of a third dose in KTRs is a safe intervention that provides a valid option for seronegative patients and a booster for seropositive ones. The

development of cellular immunity is associated with final seroconversion and also with the IgG titer. Future studies will have to address the assessment of the antibody response months after the third dose and the response of a fourth dose either as a booster therapy for seropositive patients or a rescue therapy for seronegative ones.

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