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Trajectory of COVID-19 Vaccine Antibody Titers Over Time and Association of Mycophenolate Mofetil in Solid Organ Transplant Recipients

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ABSTRACT

The coronavirus disease 2019 vaccines will be safe and effective in solid organ transplant recipients (SOTs). However, the blunted antibody responses were also of concern. Few studies have reported prolonged serological follow-up after two doses of BNT162b2 vaccine in SOTs. We performed a single-center, prospective observational study of 78 SOTs who received two doses of BNT162b2 vaccine. We identified the trajectory of antibody titers after vaccination among SOTs with or without mycophenolate mofetil (MMF), or withdrawn from MMF.

We found low seroconversion rates (29/42: 69%) and low antibody titers in SOTs treated with MMF. An inverse linear relationship between neutralizing antibody titers and MMF concentration was confirmed in restricted cubic spline plots (*P* for effect <0.01, *P* for non-linearity = 0.08). For the trajectory of antibody responses, seroconversion and improved antibody titers were observed after withdrawal from MMF in SOTs who showed seronegative or low antibody titers at the first visit after two doses of vaccine (*P* for effect <0.01, *P* for non-linearity <0.05 and *P* for interaction <0.01). We identified increased B-cell counts after withdrawal from MMF (*P* <0.01). Recovery of antibody responses was seen in SOTs withdrawn from MMF. Trajectories of antibody responses were modified by MMF administration.

1 INTRODUCTION

Vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been found to be effective and safe in both clinical trials and real-world settings [1, 2]. Antibody responses after mRNA SARS-CoV-2 vaccination are well-established in the general population [3-7]. However, the previous pioneering works revealed blunted antibody responses in solid organ transplant recipients (SOTs) who need continuous immunosuppressive medications to prevent rejection of the transplanted organs [8-14]. There is a paucity of data in SOTs who use potent immunosuppressants because they have been specifically excluded from SARS-CoV-2 vaccine trials [1, 2].

Data from recent observational studies suggest that a substantial proportion of patients with solid organ transplant, particularly those undergoing immunosuppression with mycophenolate mofetil (MMF), show attenuated antibody response even after a second or third vaccination [8, 10, 12, 15, 16]. We have demonstrated marked attenuation of antibody titers among transplant recipients with MMF in a dose-dependent manner after second doses of a SARS-CoV-2 vaccine [10]. In addition, in a report of a case series, seroconversions were confirmed following withdrawal from MMF in vaccinated transplant recipients without a third vaccination [15]. Based on such results, identification

of the trajectories of antibody responses in SOTs in association with MMF administration is essential to elucidate optimal countermeasures in this vulnerable population.

Whether the mid-term immunogenicity of SARS-CoV-2 vaccine is maintained in SOTs remains unclear [17, 18]. Hence, to fill the gap in the evidence for this vulnerable population, we conducted the present study to identify the trajectory of antibody titers after SARS-CoV-2 vaccination among SOTs with or without MMF, or withdrawal from MMF. SOTs have a normal immune system but potently inhibited T- and B-cell responses to prevent transplant rejection. We thus also explored the associations between antibody titers and trough MMF concentrations, and B- and T-cell counts, together with CD4- and CD8-positive T-cell counts, in SOTs after the second dose of SARS-CoV-2 vaccine.

2 MATERIALS AND METHODS

2.1 Study design and population

A prospective, single-center observational cohort study including SOTs receiving immunosuppressive therapy from Matsunami General Hospital, Japan, was conducted between July 1, 2021 and April 30, 2022. We included all patients who

received second doses of the BNT162b2 mRNA SARS-CoV-2 vaccine (Pfizer/BioNTech). Patients with a history of polymerase chain reaction-confirmed diagnosis of coronavirus disease 2019 (COVID-19) or positive SARS-CoV-2 anti-nucleocapsid antibodies before the second vaccination were excluded.

SOT enrollment was stratified into three categories: with MMF; without MMF; and withdrawn from MMF. SOTs in the withdrawal category were tapered from MMF using a decremental schedule according to the discretion of the attending physician. Dose of other immunosuppressants could be changed as dictated by clinical necessity. Withdrawal from MMF was attempted in the period between the first and second measurements of antibody titer. With the exception of one patient, weaning from MMF was successful. One patient showed an elevated liver function test during the withdrawal, thus MMF was left at the maintenance dose due to concerns about rejection. No graft failure was observed in all patients.

The trajectory of antibody titer was evaluated using density plots, linear mixed-effects models of longitudinal analysis, and non-linear regression with a robust Huber-White sandwich estimator.

2.2 Sample collection and follow-up schedule

The first two doses were given at least 3 weeks apart, and blood samples for antibody measurements were taken at least 2 weeks after the second vaccination. Visit-to-visit blood sampling was scheduled over three regularly scheduled outpatient clinic visits.

2.3 Antibody quantification

Serum was obtained by centrifugation at 1,500×*g* for 10 min. S receptor-binding domain (RBD) immunoglobulin (Ig)G antibody titers were quantified using the SARS-CoV-2 S-IgG (IC) Assay Reagent assay kit (Fujirebio, Tokyo, Japan) to measure RBD-IgG antibody titers as described previously [10, 15]. All procedures were performed according to the instructions from the manufacturer. Briefly, 10 μL of serum sample, 250 μL of antigen-bound particles, and 80 μL of diluted SARS-CoV-2 S-IgG were mixed and incubated at 37°C for 10 min. After separating bound and free fractions, 150 μL of enzyme-labeled antibody was added and incubated at 37°C for 10 min. After separating bound and free fractions, 200 μL of substrate solution was added and incubated at 37°C for 5 min. Luminescence was then measured using a LUMIPULSE G1200 fully automated chemiluminescent enzyme immunoassay system (Fujirebio) and

the results were calculated. Titers greater than 1.0 arbitrary units (AU)/mL were considered to represent seropositivity.

To confirm whether previous infection had occurred, antibodies to the N antigen were also measured. A Cobas 8000 analyzer (Roche Japan, Tokyo) and Elecsys anti-SARS-CoV-2 reagent (Roche Japan) were used. All procedures were performed in accordance with the instructions from the manufacturer (measurements were contracted to SRL Corporation, Tokyo, Japan). Serum samples were incubated with biotinylated SARS-CoV-2 antigen and ruthenium-labeled SARS-CoV-2 antigen at 37°C for 9 min. After washing, streptavidin magnetic particles were added and incubated at 37°C for 9 min. The reaction mixture was aspirated into the measuring cell, and magnetic particles were attracted to the electrode by magnetic force for B/F separation. The target substance was then quantified by electrochemiluminescence. A result more than 1.00 units (cut-off index) was deemed positive.

2.4 Therapeutic drug monitoring of MMF

Drug monitoring of MMF was planned at the discretion of the attending physician at a regular outpatient clinic. The measurement of MMF was performed using serum samples, a BioMajesty 6070 G biochemistry analyzer (JEOL, Tokyo, Japan), and

the reagent Emit 2000 MPA assay (Siemens Healthcare Diagnostics Co., Tokyo, Japan). Assays were contracted to SRL Corporation. We examined the association between MMF concentration immediately before the second vaccination and RBD-IgG antibody titer at the first routine outpatient visit after completion of the second vaccination.

2.5 Lymphocyte subset counts before and after withdrawal from MMF

To investigate factors contributing to improved immunogenicity, we comprehensively analyzed counts of lymphocyte subsets including CD4+ and CD8+ T cells and B cells in patients who were successfully tapered and withdrawn from MMF, and compared the results before and after withdrawal from MMF. CD4- and CD8-positive T- and B-cell counts were measured using heparinized blood, a FACSCant II analyzer (BD Biosciences, NJ, United States), and monoclonal antibodies (Coulter T11-RD1/B4-FITC, T4-FITC and T8-RD1; Beckman Coulter Co., CA, United States). All measurements were contracted to SRL Corporation. Cell counts were calculated by multiplying the measured percentages of CD4- and CD8-positive T and B cells by the number of lymphocytes measured with a multiparameter automated hematology analyzer (XN-3100; Sysmex, Kobe, Japan).

2.6 Ethical considerations

This study conformed to the principles outlined in the Declaration of Helsinki and its later amendments. We obtained written informed consent from all study participants. The study protocol was approved by the Ethics Committee of Matsunami General Hospital (approval no. 498, 2021).

2.7 Statistical analysis

Continuous values are expressed as median and interquartile range (IQR). The Mann-Whitney U test or Kruskal-Wallis test was used to analyze differences between groups of continuous variables, as appropriate. Categorical data were compared using the chi-square test. We compared lymphocyte subset counts before and after withdrawal of the same individual from MMF using the Wilcoxon signed-rank test. The presence of a non-linear association between RBD-IgG antibody titer and MMF concentration was assessed using a restricted cubic spline regression model with 3 knots using the R rms package. To examine whether RBD-IgG antibody titers changed over time, a nonlinear regression with the Huber-White robust sandwich estimator of variance-covariance matrix was used. To examine the relationship between antibody titers and the number of days elapsed since the second vaccination among SOTs with or without MMF, or who

were successfully withdrawn from MMF, we used a non-linear regression with a robust Huber-White sandwich estimator. Statistical significance was set at $P<0.05$ (two-tailed), and P for interaction <0.15 was considered significant. Statistical analyses were performed using R version 4.1.3 (R Foundation for Statistical Computing, Vienna, Austria).

3 RESULTS

3.1 Baseline characteristics

We enrolled 78 patients (median age, 66.5 years; IQR, 56–73.8 years; 59 men [75.6%]; 19 women [24.4%]) receiving immunosuppressive regimen for SOT with no history of COVID-19 and a negative SARS-CoV-2 anti-nucleocapsid antibody test during the study period, indicating no previous exposure to COVID-19. Among these patients, 36 received an immunosuppressive regimen without MMF (46%), 19 received an immunosuppressive regimen with MMF continuously throughout the study period (24%), and 23 were successfully withdrawn from MMF after the second vaccination (29%) (Table 1). Median days of Visits 1, 2 and 3 after the second vaccination were 46 days (IQR, 22–66 days), 107 days (IQR, 94–136.2 days), and 188 days (IQR, 143–202 days), respectively.

3.2 Alluvial plot for temporal changes in seropositivity in SOTs with or without MMF, or withdrawn from MMF

Figure 1 illustrates the alluvial plot of how results of serological tests changed over time in three groups (SOTs without MMF, SOTs with MMF, and SOTs withdrawn from MMF). Sixty-four of the 78 SOTs (82%) were seropositive at Visit 1, comprising 35 non-MMF users (35/36, 97.2%), 15 MMF continuous users (15/19, 78.9%), and 14 users withdrawn from MMF (14/23, 60.8%) (Figure 1). Seropositivity in non-MMF users persisted and was maintained from Visit 1 to 3 (35/36, 97.2%; 23/24, 95.8%; and 20/21, 95.2%, respectively at Visits 1, 2 and 3). Interestingly, 5 patients in the MMF withdrawal group were seronegative at Visit 1, but seroconversion was obtained by Visit 2 after withdrawal from MMF without additional vaccination. In MMF continuous users who were seronegative at Visit 1 (n=5), we could not verify seroconversion at either Visit 2 or Visit 3 (Figure 1).

3.3 Relationship between RBD-IgG antibody titers and MMF concentration in SOTs

A restricted cubic spline plot (Figure 2) shows the relationship between antibody titers and MMF concentration in SOTs. An inverse linear relationship between antibody titers and MMF concentration was identified (P for effect < 0.01, P for non-linearity = 0.08).

3.4 Density plot for trajectories of RBD-IgG antibody titers from second vaccination

Figure 3 depicts the kernel density plot showing distributions of antibody titers for samples from SOTs without MMF, SOTs with MMF, and SOTs withdrawn from MMF at Visits 1-3. Comparison of RBD-IgG antibody titers against SARS-CoV-2 revealed higher antibody titers in SOTs without MMF compared to other recipient groups at Visit 1. However, as expected, the distribution of antibody titers shifted toward zero at Visits 2 and 3 (Figure 3).

3.5 Linear mixed-effects model for evaluating visit-to-visit RBD-IgG antibody titer variations

Mixed-effects models were used to compare serial changes to RBD-IgG antibody titers in two (with or without MMF) or three (with or without MMF, or

withdrawn from MMF) groups at Visits 1, 2 and 3. The linear mixed-effects model showed differences in antibody titers between groups at Visits 1, 2 and 3 (P=0.048 and P=0.11, respectively) (Figure 4).

3.6 Non-linear restricted cubic spline model between RBD-IgG antibody titers and days from second vaccination

We created a non-linear regression model analysis between time after the second vaccination and RBD-IgG antibody titers together with interaction analysis according to MMF administration. The Huber-White robust sandwich variance-covariance estimator was used to account for repeated observations. RBD-IgG antibody titers decreased over time (both P for effect < 0.01 and P for non-linearity < 0.05) (Figure 5). Regression models including an interaction term showed reverse U-shaped serial changes in SOTs withdrawn from MMF. Antibody titers were modified significantly depending on MMF administration (Figure 5) (P for interaction = 0.01 and P for interaction < 0.01, respectively).

3.7 Comparison of CD4- and CD8-positive T- and B-cell counts before and after withdrawal from MMF

For lymphocyte subset counts measured in 20 SOTs withdrawn from MMF, we compared CD4+ and CD8+ T- and B-cell counts before and after withdrawal from MMF. Figure 6 exhibits the result for B-cell counts before and after withdrawal from MMF within the same individual using the Wilcoxon signed-rank test. A significant difference in B-cell counts was seen between before and after withdrawal from MMF ($P < 0.01$). Conversely, no significant differences were detected in CD4+ or CD8+ T-cell counts between before and after withdrawal from MMF.

4 DISCUSSION

We reported in this study the trajectory of RBD-IgG antibody titers after two doses of BNT162b2 mRNA COVID-19 vaccine in SOTs. First, we found low antibody response rates and low antibody titers in SOTs treated with MMF. Second, an inverse linear relationship between RBD-IgG antibody titers and MMF concentration was detected. Third, seroconversion and improved antibody titers were observed after withdrawal from MMF in SOTs who showed seronegative or low antibody titers at Visit 1 after two doses of the vaccine. Last, we identified increased B-cell counts after withdrawal from MMF.

SOTs are at higher risk of COVID-19 due to the immunosuppressants needed to prevent graft rejection [9, 18-20]. This is because the immunosuppressive agents used may impair responses to COVID-19 vaccines. Patients treated with MMF before vaccination did not mount sufficient antibody response to BNT162b2 vaccination and might have been rendered unprotected from COVID-19, as suggested by breakthrough infections. We and others have reported poor antibody response after two doses of BNT162b2 mRNA vaccine in SOTs treated with MMF [8, 10, 12, 16]. We also confirmed previous findings that both MMF administration dose and concentration were inversely related to antibody titers in a linear, dose-response-dependent manner [8, 10]. Those receiving MMF as maintenance immunosuppression therapy were less likely to develop an antibody response and seroconversion. We found increased antibody titers and serological recovery after withdrawal from MMF. These findings were consistent with our report of seroconversion in SOTs who achieved withdrawal from MMF [15] and with the statement that temporarily withholding MMF could be considered when the disease condition is stable [21].

In SOTs requiring continuous immunosuppression, no specific strategy to reinforce vaccine immunogenicity has been proposed for these blunted antibody responses [20, 22, 23]. One potential strategy for enhancing antibody titers in SOTs could be

to minimize or potentially withhold MMF administration at the time of vaccination in patients who show failed seroconversion after completion of scheduled vaccinations [8, 15, 21]. The benefit-risk balance of this strategy, however, should be assessed individually and close monitoring is advised to avoid organ rejection and allograft complications. For example, long-standing SOTs with stable conditions, such as our study population, could be candidates for modulating immunosuppressive regimens at the time of booster vaccination. Also, the withdrawal of MMF to achieve a greater antibody titer is still a controversial issue, as SOTs are exposed to a risk of organ rejection while obtaining effective antibody titers. Continued research evaluating immunogenicity, clinical efficacy and safety, and development of strategies to improve antibody response in this vulnerable populations are thus warranted.

In SOTs, increased B-cell counts were observed after the withdrawal from MMF. Depletion of B-cell responses is a possible mechanism for the diminished antibody titers in SOTs receiving MMF [24-26]. Similar results have been reported in patients with hematological cancer who need anti-B-cell therapy [24-27], and in patients with chronic inflammatory diseases receiving B-cell-depleting therapies [27, 28]. However, a complex interplay between cellular and humoral immunity is required to achieve adequate antibody response after vaccination. Data on such immune responses after

vaccinations in SOTs on immunosuppressants are conflicting [29-31]. Whether impaired vaccination-induced humoral responses are associated with the level of circulating B cells and/or with CD4+ or CD8+ T-cell responses in SOTs remains unclear.

This study had several limitations. First, the investigation was limited by the single-center design and study participants had a relatively long period since transplantation. Therefore, further studies, registries, and/or clinical trials including participants with diverse backgrounds are warranted. Second, our study population comprised SOTs who had received two doses of BNT162b2 mRNA vaccine and none who had received a third (booster) vaccination. Long-term follow-up is thus needed to assess the durability of antibody responses and side effects, including organ rejection and allograft complications. Third, we assessed both CD4+ and CD8+ T- and B-cell counts in our study, but not viral-specific helper CD4+ T-cell responses or viral-specific CD8+ cellular assays [32], and their cytokines were not commercially available. Comprehensive analysis of immunogenicity in SOTs may thus reveal the mechanisms underlying diminished antibody titers in SOTs receiving immunosuppressants.

5 Conclusion

Antibody titers were diminished at the final visit in all three groups of SOTs: with or without MMF, and withdrawn from MMF. A third vaccination of SOTs has been

considered one alternative to achieve adequate antibody responses. Also, with careful consideration, withdrawal from MMF could provide a potential strategy for enhancing antibody titers in SOTs. In some SOTs who achieve only low antibody titers, clinicians and patients should continue non-pharmaceutical interventions, including mask wearing and social distancing.

List of abbreviations

COVID-19 = coronavirus disease 2019

MMF = mycophenolate mofetil

mTOR = mammalian target of rapamycin

RBD = S receptor-binding domain

SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

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DISCLOSURES

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets generated and/or analyzed during the present study are not publicly available due to ethical/privacy reasons, but are available from the corresponding author on reasonable request.

Authorship:

AS, TM, SL, KF, HT, HM: study idea, design, manuscript

AS, TM, KS, FH, TY, NS, HA, AT, MM, RY, AI, TS, YK, YM, MT, YY, HF, KS: study performance

AS, KS, TM: data analyses, manuscript

All authors: data ascertainment, manuscript discussion, manuscript revisions

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FIGURE LEGENDS

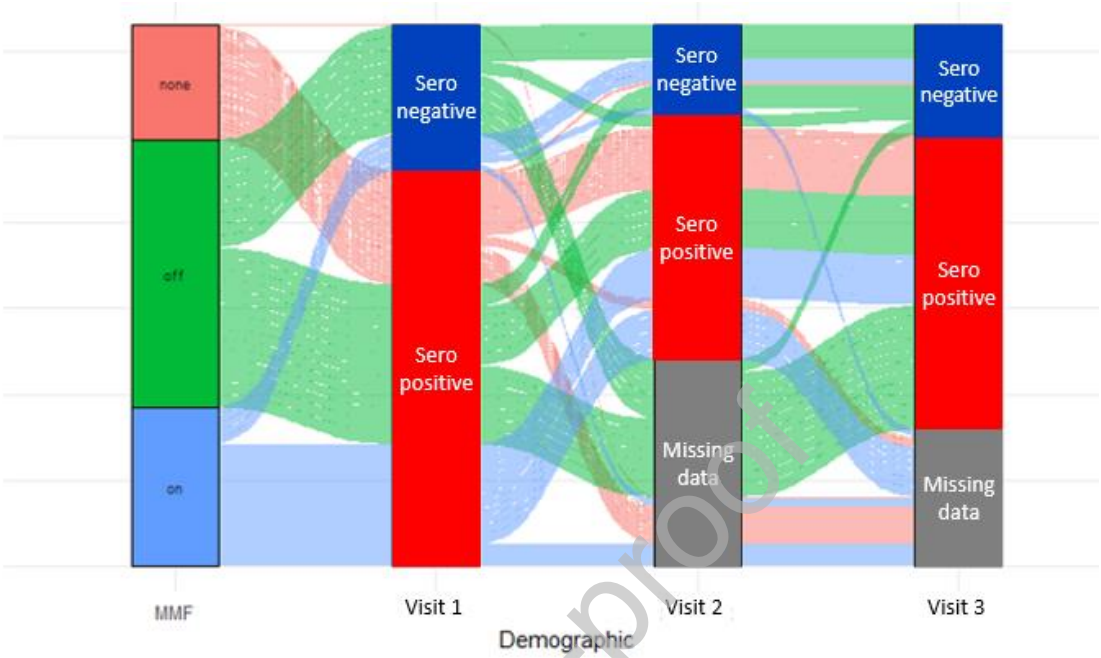

Figure 1. Alluvial plot for seroconversion in solid organ transplant recipients with or without MMF

MMF none: solid organ transplant recipients without MMF regimen.

MMF on: solid organ transplant recipients who continued MMF regimen.

MMF off: solid organ transplant recipients withdrawn from MMF regimen.

MMF, mycophenolate mofetil.

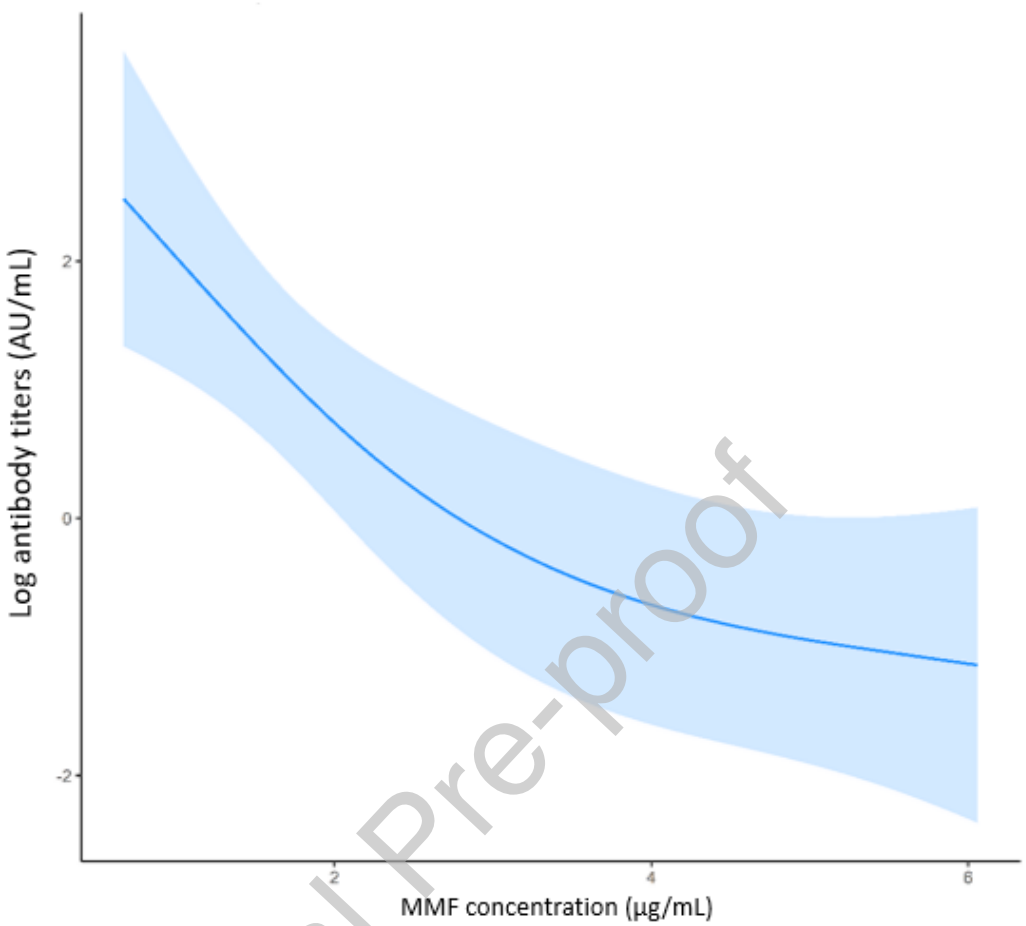

Figure 2. Association between antibody titers and MMF concentrations in solid organ transplant recipients by restricted cubic spline model with three knots

The shaded area represents the 95% confidence interval.

MMF, mycophenolate mofetil.

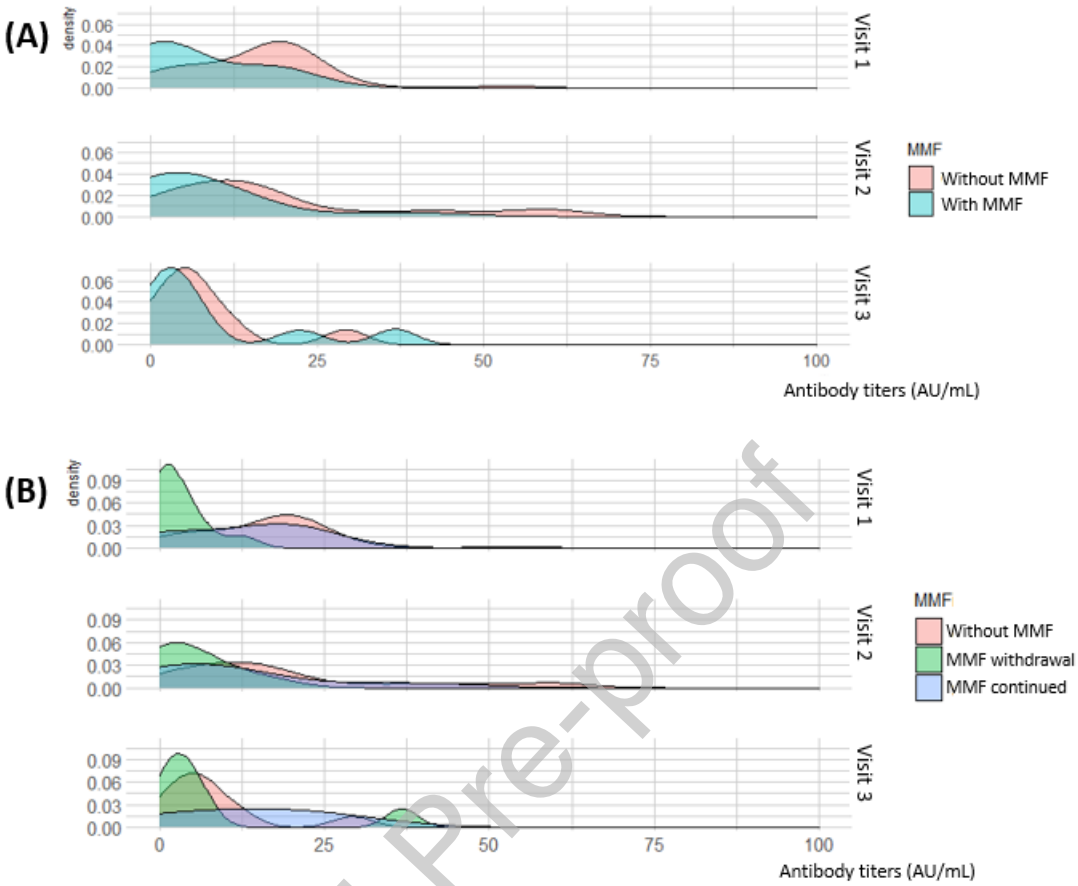

Figure 3. Density plot for trajectories of antibody titers from second vaccination

Kernel density plot showing distribution of antibody titers stratified by solid organ transplant recipients without MMF and those with MMF at baseline immunosuppressive regimen (A), or stratified by solid organ transplant recipients without MMF, solid organ transplant recipients with MMF, and solid organ transplant recipients with MMF withdrawing (B) at Visits 1–3.

MMF, mycophenolate mofetil.

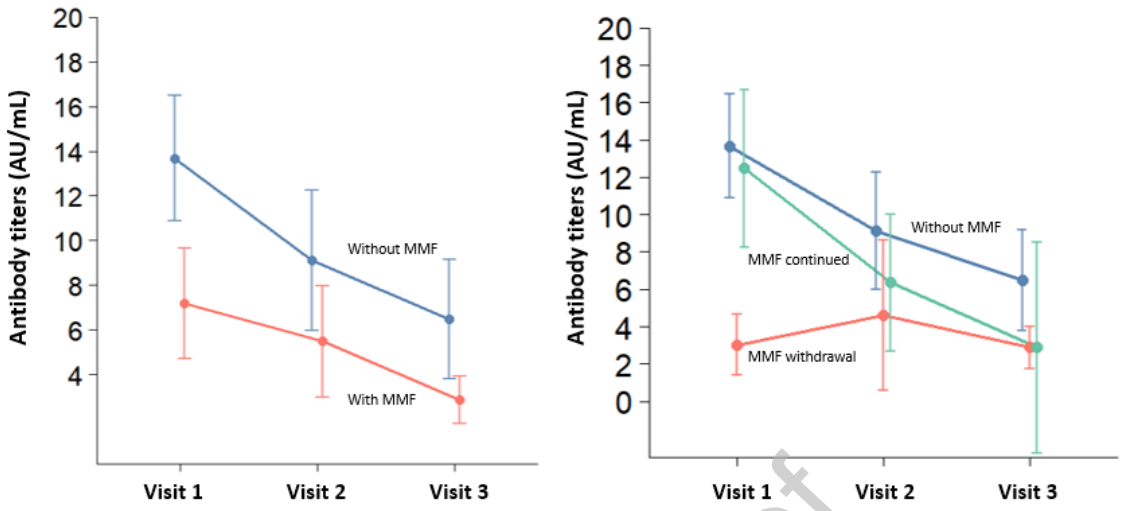

Figure 4. Linear mixed-effects model for evaluating visit-to-visit antibody titer variations

Linear mixed-effects models comparing serial changes of antibody titers in two (with or without MMF) (left panel) or three (with or without MMF, or withdrawn from MMF) (right panel) groups at Visits 1, 2 and 3. Open circles represent mean antibody titers, and bars represent 95% confidence intervals.

MMF, mycophenolate mofetil.

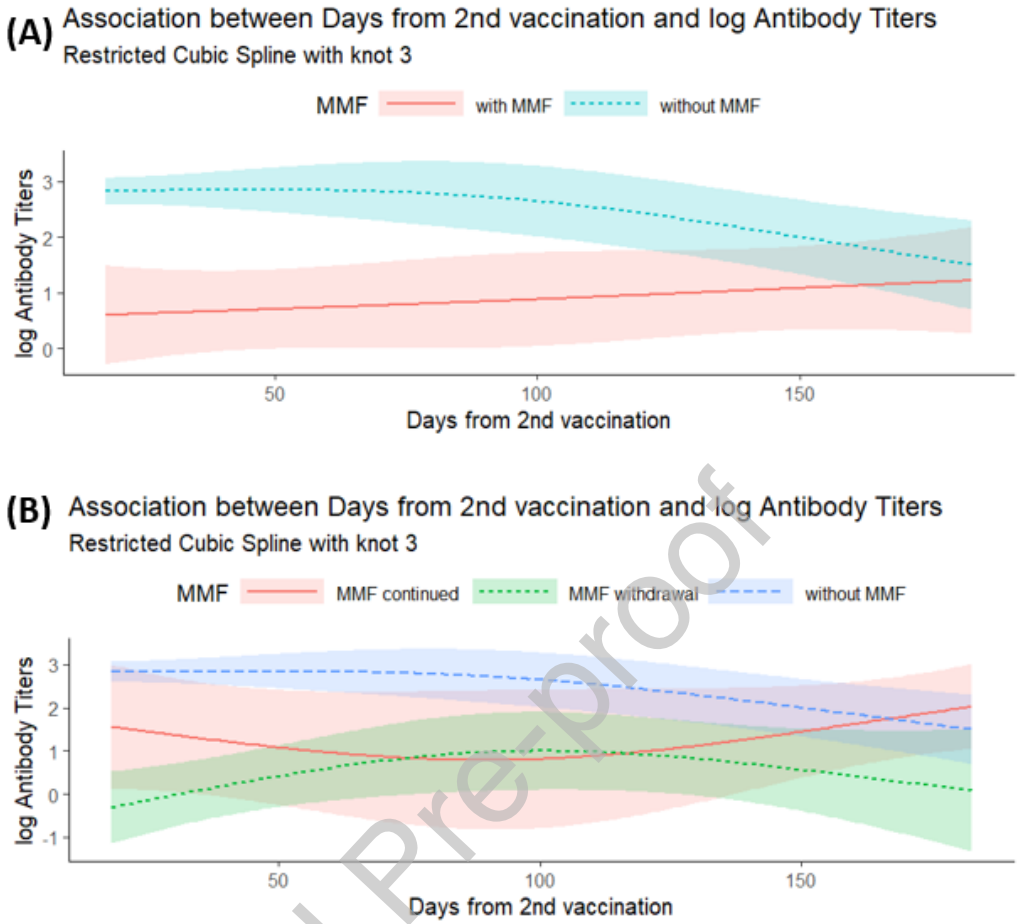

Figure 5. Association between days from second vaccination and log-transformed antibody titers (restricted cubic spline)

(A) Stratified by MMF administration at baseline regimen.

(B) Stratified by with or without MMF, or withdrawn from MMF

MMF, mycophenolate mofetil.

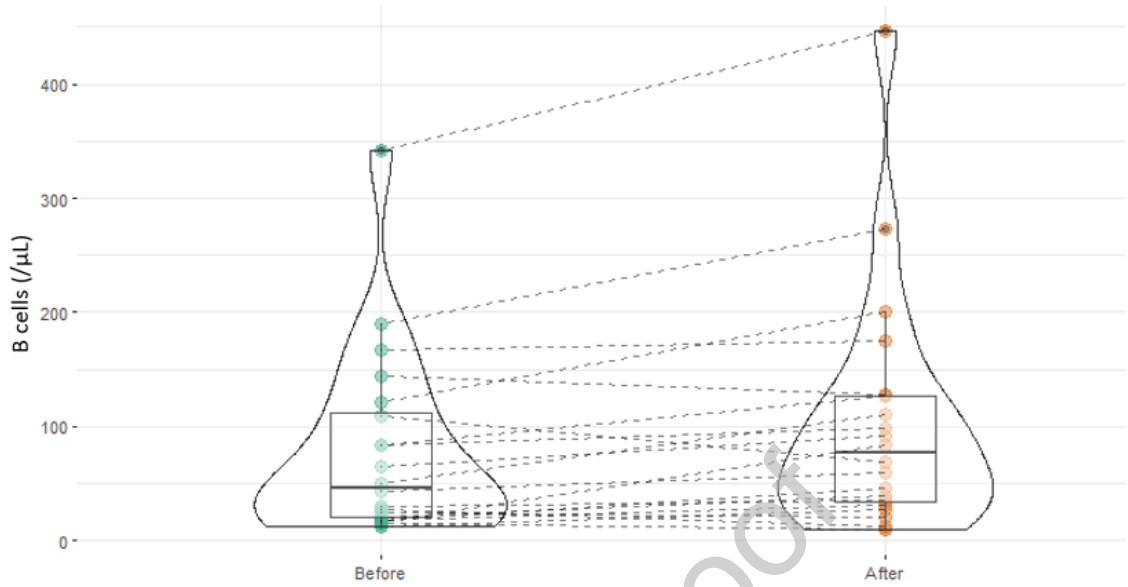

Figure 6. Violin charts wrapping a box plot for B-cell count in solid organ transplant recipients after MMF discontinuation

MMF, mycophenolate mofetil.

Table 1. Clinical characteristics of participants, stratified by MMF administration

	All subjects (N = 78)	Without MMF (n = 36)	With MMF (n = 19)	Withdrawal from MMF (n = 23)	*P*
Age, years*	66.5 [56.0, 73.8]	65.0 [50.8, 71.3]	64.0 [55.5, 73.5]	70.0 [58.5, 77.0]	0.20
Sex (male), n (%)	59 (75.6)	25 (69.4)	17 (89.5)	17 (73.9)	0.25
BMI, kg/m^2*	23.1 [21.1, 25.0]	22.8 [21.6, 25.0]	23.2 [20.8, 24.5]	23.9 [21.3, 25.3]	0.70
Organ					
Kidney/liver, n	9/70	0/36	4/15	5/19	<0.01
Donor type					
Living donor, n (%)	17/78 (21.8)	10 (27.8)	6 (31.6)	1 (4.3)	0.051
Deceased donor, n (%)	61/78 (78.2)	26 (72.2)	13 (68.4)	22 (95.7)	
Time from transplant, years	16.0 [15.0, 19.0]	18.0 [15.0, 21.0]	15.0 [15.0, 18.0]	15.0 [14.0, 16.5]	0.01
Immunosuppression maintenance therapy					
Calcineurin inhibitor, n (%)	73 (93.6)	34 (94.4)	18 (94.7)	22 (95.7)	0.98
Azathioprine, n (%)	4 (5.1)	4 (11.1)	0 (0.0)	0 (0.0)	0.09
Mycophenolate mofetil, n (%)	42 (53.8)	0 (0.0)	19 (100.0)	23 (100.0)	<0.01
Steroids, n (%)	1 (1.3)	1 (2.8)	0 (0.0)	0 (0.0)	0.55
mTOR inhibitor, n (%)	1 (1.3)	0 (0.0)	0 (0.0)	1 (4.3)	0.30

BMI, body mass index; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin.

*Values represent median [interquartile range].