Mycophenolic Acid Exposure Determines Antibody Formation Following SARS-CoV-2 Vaccination in Kidney Transplant Recipients: A Nested Cohort Study

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Despite (repeated) boosting, kidney transplant recipients (KTRs) may remain at increased risk of severe COVID-19 since a substantial number of individuals remain seronegative or with low antibody titers. In particular, mycophenolic acid use has been shown to affect antibody formation negatively and may be an important modifiable risk factor. We investigated the exposure–response relationship between mycophenolic acid 12-hour area under the curve (AUC_{0-12h}) exposure and seroconversion including antibody titers after vaccination using mRNA-1273 SARS-CoV-2 vaccine (Moderna) in 316 KTRs from our center that participated in the national Dutch renal patients COVID-19 vaccination – long term efficacy and safety of SARS-CoV-2 vaccination in kidney disease patients vaccination study. After two vaccination doses, 162 (51%) KTRs seroconverted. KTRs treated with mycophenolic acid showed less seroconversion and lower antibody titers compared with KTRs without mycophenolic acid (44% vs. 77%, and 36 binding antibody units (BAU)/mL vs. 340 BAU/mL; $P < 0.001$). The mean mycophenolic acid AUC_{0-12h} exposure was significantly lower in KTRs who seroconverted compared with KTRs who did not (39 vs. 29 mg·h/L; $P < 0.001$). High mycophenolic acid exposure (±90 mg·h/L) and no exposure to mycophenolic acid resulted in a seroconversion rate ranging from 10% to 80%. Every 10 mg·h/L increase in mycophenolic acid AUC_{0-12h} gave an adjusted odds ratio for seroconversion of 0.87 (95% confidence interval (CI), 0.79–0.97; $P = 0.010$) and 0.89 (95% CI, 0.85–0.93; $P < 0.001$) for KTRs on dual and triple maintenance immunosuppressive therapy, respectively. Higher mycophenolic acid AUC_{0-12h} correlated with lower antibody titers ($R = 0.44$, $P < 0.001$). This study demonstrates the exposure–response relationship between gold standard mycophenolic acid exposure and antibody formation to support interventional studies investigating mycophenolic acid adjustment to improve antibody formation after further boosting.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Despite (repeated) boosting, kidney transplant recipients (KTRs) may remain at increased risk of severe COVID-19 since a substantial number remains seronegative or with low antibody titers. Mycophenolic acid use is known to negatively impact antibody formation, but temporary cessation might result in an immune response towards the transplanted organ.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ The primary objective was to investigate the exposure–response relationship between gold standard mycophenolic acid 12-hour area under the curve (AUC_{0-12h}) and antibody formation in KTRs after vaccination.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Mycophenolic acid AUC_{0-12h} is significantly associated with seroconversion and antibody titers post vaccination in an exposure-dependent manner.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Therapeutic drug monitoring of mycophenolic acid may identify individuals with overexposure. Titration to the therapeutic range may possibly improve seroconversion. This study provides evidence for interventional trials that investigate the impact and timing of adjustment of mycophenolic acid on antibody formation in individuals that remain seronegative despite repeated boosting. This may perhaps not only benefit transplant recipients but also others on mycophenolic acid.

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Solid organ transplant recipients may remain at increased risk of severe COVID-19 from low seroconversion rates and antibody titers after repeated vaccination.[1–3] Despite beneficial effects of administration of a third, fourth, or even fifth dose, a substantial group (40–70%) remains seronegative or with low antibody titers, necessitating an additional intervention to improve the antibody yield.[4–8]

Mycophenolic acid use and dosage are significantly associated with lower rates of seroconversion and low antibody titers after vaccination.[3,9–12] The mycophenolate mofetil label describes a fixed dose of 2,000 mg/day, but it is known that clinically relevant interpatient variability exists in mycophenolic acid drug exposure, which can be assessed by therapeutic drug monitoring (TDM).[13] Temporary cessation of mycophenolic acid might facilitate antibody formation but may come at an increased risk of rejection or formation of donor-specific antibodies given its variability.

The aim of this observational study was to closely investigate the exposure–response relationship between 12-hour area under the curve (AUC_{0-12h}) mycophenolic acid exposure measurements, seroconversion rates, and antibody titers in kidney transplant recipients (KTRs) after vaccination.

METHODS

Study design and setting

For this observational cohort study, we included KTRs from our transplant center that participated in the Dutch national renal patients COVID-19 Vaccination – long term efficacy and safety of SARS-CoV-2 vaccination in kidney disease patients (RECOVAC LESS CoV-2) study (NCT04841785) from April 2021 until March 2022.[14] The RECOVAC LESS CoV-2 study is a prospective observational national multicenter cohort study designed to evaluate the efficacy and safety of SARS-CoV-2 vaccination in patients with chronic kidney disease stages G4-5, dialysis patients, and KTRs. The study included patients with and without a history of COVID-19. We excluded participants of the RECOVAC LESS CoV-2 study, if they had opted out for research consent in the Dutch National Transplantation Register (NOTR). SARS-CoV-2 antibodies were measured 28 days after the second vaccine dose. The RECOVAC LESS CoV-2 study was approved by the consortium's Institutional Review Board (IRB) (EudraCT:2021–001520-18).

Patient selection

For this nested analysis, we selected KTRs who were on maintenance immunosuppression with calcineurin inhibitors (CNIs) and/or mycophenolic acid and/or prednisolone, and vaccinated with two doses (100 µg) of the messenger RNA (mRNA)–based vaccine produced by Moderna (mRNA-1273) with an interval of 28 days according to the manufacturer's instructions.

The IRB of Leiden University Medical Center approved analysis on the impact of TDM in our KTRs (approval number: W2020.031). All proceedings were in accordance with the Declaration of Helsinki and Declaration of Istanbul on Organ Trafficking and Transplant Tourism. We used the modified STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for reporting on observational studies (**Table S1**).

Posttransplant outpatient care

The majority of KTRs are treated with triple immunosuppressive maintenance therapy (TMT), including a CNI (tacrolimus predominantly), mycophenolic acid, and prednisolone at our center. Maintenance prednisolone (5–7.5 mg once daily for both TMT and DMT) is not routinely withdrawn or tapered after transplantation. KTRs are typically tapered to dual immunosuppressive maintenance therapy (DMT) with CNI/prednisolone in case of clinical issues with opportunistic or recurrent infections, malignancies, etc. DMT with mycophenolic acid/prednisolone is considered in KTRs with a human leukocyte antigen (HLA)–identical living related kidney donor after 3 months. Mycophenolic acid dose is titrated to a mycophenolic acid AUC_{0-12h} between 30 and 45 mg·h/L for TMT, and between 60 and 90 mg·h/L for DMT throughout the whole posttransplant period in our center, based in part on the mycophenolic acid consensus paper.[13]

Mycophenolic acid exposure measurements

All KTRs undergo TDM by abbreviated AUC_{0-12h} measurements to quantify drug exposure to mycophenolic acid calculated by a validated limited sampling strategy based on samples drawn at 0, 1, 2, and 3 hours after administration of mycophenolic acid at our center. These AUC_{0-12h} measurements are routinely performed per protocol at 6 weeks, 6 months, and annually or biannually, as well as on indication by collecting venous blood samples or capillary dried blood spot kits. Technical information on the measurements can be found in the Supplemental Material (Text S1). Both mycophenolic acid AUC_{0-12h} and mycophenolic acid dose were validated manually by electronic record review, for both protocol and indication exposure measurements. Mycophenolic acid exposure was classified as missing if no measurement was available or in case mycophenolic acid dose was adjusted between exposure and antibody measurement.

Evaluation of seroconversion post vaccination

We analyzed blood samples for the presence of antibodies against the receptor binding domain (RBD) of the SARS-CoV-2 spike protein (immunoglobulin G (IgG) anti-RBD antibody) using an in-house anti-SARS-CoV-2 RBD IgG enzyme-linked immunosorbent assay (ELISA) (Sanquin, Amsterdam, The Netherlands).[15] This is an indirect ELISA using microtiter plates coated with RBD and detection by monoclonal mouse antihuman IgG coupled to horseradish peroxidase (HRP). We combined this assay with an in-house anti-SARS-CoV-2 total antibody nucleocapsid protein (NP) bridging ELISA, essentially as described, but using 0.3 µg/mL NP for coating and 7.5 ng/mL biotin-labeled NP for detection in half-area 96-well microtiter plates. Both antibodies against RBD and NP arise after a natural COVID-19 infection. In contrast, vaccination only induces antibodies to RBD, since NP is not part of any currently used vaccines. Combining these two tests allows us to discern an antibody response after vaccination from antibody titers due to previous COVID-19 infection. A participant was thus identified as diagnosed with previous COVID-19 if positive NP antibodies were observed or if the participant self-reported a previous COVID-19 infection. IgG anti-RBD antibody response is expressed in BAU/mL, after conversion according to the World Health Organization (WHO) international standard.[16] Cutoff values for seroconversion rates were set at ≥50 BAU/mL. This value has been established after analyzing the presence of IgG anti-RBD antibodies or total anti-NP antibodies in prepandemic blood samples, resulting in specificities of about 99% and 99.5% respectively, and a sensitivity of 95%.[17]

Statistical analysis

All statistical analyses were performed using R Statistics (version 4.1.0; R Foundation for Statistical Computing, Vienna, Austria) and RStudio

(version 1.4.1717; Posit Software, Boston, MA). Descriptive statistics were presented as numbers and percentages for categorical data. Continuous data were expressed in medians and interquartile ranges (IQRs) or means and standard deviations (SDs) with 95% confidence intervals (CIs) if applicable. We used the χ^2 (Chi-squared) test for categorical variables and Mann–Whitney *U*-test for continuous variables. Two-sided *P* values <0.05 were considered statistically significant.

The outcomes of interest were seroconversion (positive/negative) and height of IgG titer. We illustrated seroconversion rates using histograms and median IgG titers using box plots for the following immunosuppressive regimens: (i) TMT with CNI, mycophenolic acid, and prednisolone (standard CNI/mycophenolic acid/prednisolone); (ii) DMT with CNI and prednisolone (dual CNI/prednisolone); and (iii) DMT with mycophenolic acid and prednisolone (dual mycophenolic acid/prednisolone). Median AUC_{0-12h} exposure per daily mycophenolic acid dose is visualized using box plots.

We evaluated the association between mycophenolic acid exposure and seroconversion in KTRs for TMT and DMT by calculating odds ratios (ORs) and 95% CIs using multivariable logistic regression and probability plots. We adjusted for the following variables: age, gender, time after transplantation, previous COVID-19 infection(s), and serum creatinine. The association between mycophenolic acid AUC_{0-12h} and IgG titers was assessed using a correlation plot. Missing data were assumed to occur randomly and imputed 10 times using chained equations. The 10 imputed data sets were used to assess pooled odds ratios and confidence intervals. Three sensitivity analyses were performed in which (i) KTRs with missing antibody measurements and (ii) KTRs with a previous COVID infection were excluded to assess generalizability of the data (**Tables S2, S3**), and a final analysis in which time between mycophenolic acid AUC_{0-12h} and vaccination was included in the multivariable model (**Table S4**).

RESULTS

A total of 316 KTRs were on maintenance immunosuppressive therapy with a CNI and/or mycophenolic acid and/or prednisolone, and included in this analysis. **Table 1** shows the demographics of the cohort investigated. The median time between mycophenolic acid AUC_{0-12h} measurement and vaccination was 218 (IQR: 58–310) days. Twenty-three (7%) out of 316 KTRs had a previous infection with COVID-19. All patients were on a stable mycophenolate dose and in steady state. The median time after transplantation was 8 (IQR: 4–11) years and only one patient underwent vaccination at Year 1 post transplantation. None of the patients suffered from acute rejection in the year prior to vaccination. There were limited missing data on antibody titers (10%) and mycophenolic acid exposure (<1%).

Differences in characteristics between KTRs with and without seroconversion are shown in **Table 2**. After two vaccination doses, 162 (51%) out of 316 KTRs developed antibodies against SARS-CoV-2 and 21 (91%) out of 23 with a previous COVID-19 infection were seropositive. Age, gender, years after transplantation, and serum creatinine were not significantly different between KTRs with and without seroconversion. Mycophenolic acid dose, mycophenolic acid exposure, and immunosuppressive regimen were significantly different between KTRs with and without seroconversion. In mycophenolic acid–treated patients, the mean mycophenolic acid AUC_{0-12h} in KTRs with seroconversion was 29 mg·h/L (with a mean daily dose of 800 mg) vs. 39 mg·h/L (mean daily dose: 1,170 mg, $P < 0.001$) in KTRs without seroconversion. **Figure 1a,b** shows the percentage of KTRs with seroconversion and median IgG titer for different immunosuppressive regimens. Seroconversion rates for patients on TMT (45%) and DMT (40%) with mycophenolic acid were comparable and significantly lower than the rates in KTRs treated with a mycophenolic acid–free regimen consisting of DMT with CNI/prednisolone (77%; $P < 0.001$). Median IgG titer in KTRs treated with a mycophenolic acid–free regimen was significantly higher compared with KTRs on TMT and DMT with mycophenolic acid, 340 BAU/mL vs. 36 BAU/mL ($P < 0.001$).The median exposure to mycophenolic acid gradually increased with increasing total daily dose. **Figure S1** shows the median mycophenolic acid AUC_{0-12h} and IQRs for every total daily dose administered. Median mycophenolic acid exposure ranged from 30 mg·h/L (IQR: 27–36 mg·h/L) for 500 mg/day to 71 mg·h/L (IQR: 66–78 mg·h/L) for 2,500 mg/day.

Overall seroconversion rates ranged from 10% to 80%, when stratified by mycophenolic acid exposure and the three investigated maintenance regimens (**Figure 2a–c**). **Figure 2a** shows the overall relationship between mycophenolic acid exposure and seroconversion in all immunosuppressive maintenance regimens (patients on DMT with CNI/prednisolone have a mycophenolic acid AUC_{0-12h} of 0). **Figure 2b** shows the association between mycophenolic acid exposure and seroconversion in KTRs treated

Table 1 Patient characteristics

	Total	CNI/MPA/P	Dual CNI/P	Dual MPA/P	*P* value[a]
No. of patients	316	215	66	35	
Age, mean (SD)	58 (11)	57 (11)	62 (10)	59 (10)	0.002
Gender, male, No. (%)	191 (60%)	133 (62%)	37 (56%)	21 (60%)	ns
Years after transplantation, median (IQR)	7.7 (4–11)	6 (4–9)	9 (6–13)	13 (12–15)	<0.001
Antibody titer, median (IQR), BAU/mL	54 (7–428)	41 (6–204)	397 (121–1,586)	37 (5–81)	<0.001
Serum creatinine, mean (SD), mg/dL	1.42 (0.6)	1.38 (0.6)	1.53 (0.6)	1.46 (0.9)	ns
MPA daily dose, mean (SD), mg	980 (661)	1,138 (392)	—	1,857 (523)	<0.001
MPA AUC_{0-12h}, median (IQR), mg·h/L	34 (22–46)	35 (30–45)	—	64 (51–71)	<0.001

AUC_{0-12h}, 12-hour area under the curve; BAU, binding antibody units; CNI, calcineurin inhibitor; IQR, interquartile range; MPA, mycophenolic acid; ns, not significant; P, prednisolone; —, not applicable.
[a]Analyzed by *t*-test for means and nonparametric Mann Whitney *U*-test for medians.

Table 2 Characteristics of responders and nonresponders post vaccination

	No seroconversion	Seroconversion	*P* value[a]
No. of patients	154	162	
Age, mean (SD)	59 (11)	57 (11)	0.245
Gender, male, No. (%)	89 (58%)	102 (63%)	0.410
Years after transplantation, median (IQR)	7 (4–11)	8 (4–11)	0.416
Immunosuppressive regimen			<0.001
Standard CNI/MPA/P, No. (%)	118 (77%)	97 (60%)	
Dual CNI/P, No. (%)	15 (10%)	51 (31%)	
Dual MPA/P, No. (%)	21 (14%)	14 (9%)	
Antibody titer, median (IQR), BAU/mL	6 (1–22)	404 (142–1,627)	<0.001
Serum creatinine, mean (SD), mg/dL	1.38 (0.58)	1.46 (0.64)	0.213
MPA daily dose, mean (SD), mg	1,170 (597)	800 (671)	<0.001
MPA AUC_{0-12h}, median (IQR), mg·h/L[b]	39 (31–56)	29 (0–37)	<0.001

AUC_{0-12h}, 12-hour area under the curve; BAU, binding antibody units; CNI, calcineurin inhibitor; IQR, interquartile range; MPA, mycophenolic acid; ns, not significant; P, prednisolone.
[a]Analyzed by *t*-test for means and nonparametric Mann Whitney *U*-test for medians.
[b]Limited to patients on MPA.

with CNI/mycophenolic acid/prednisolone in which seroconversion rates ranged from 5% to 90%. The median mycophenolic acid AUC_{0-12h} exposures (including IQRs) for each of the total daily dose levels from Figure S1 have also been projected on the probability lines. Thirdly, Figure 2c shows the same relation in CNI-free KTRs treated with mycophenolic acid/prednisolone only. In KTRs with TMT, 50% of the participants seroconverted at a median mycophenolic acid AUC_{0-12h} of 31 mg·h/L. In contrast, 50% of KTRs with DMT seroconverted at a median mycophenolic acid AUC_{0-12h} of 57 mg·h/L. Total daily mycophenolate doses of 500 mg, 1,000 mg, and 1,500 mg resulted in a median mycophenolic acid exposure within therapeutic (consensus) range and were associated with seroconversion rates ranging between 38% and 55%. Higher daily doses gave a higher exposure with median AUC_{0-12h} measurements at 53 and 74 mg·h/L, and lower chances of seroconversion of 25% and 10%, respectively. The fit between the octiles and sextiles of mycophenolic acid AUC_{0-12h} and seroconversion as well as a density plot of all mycophenolic acid AUC measurements can be found in Figures S2, S3.

Overall mycophenolic acid exposure was associated with seroconversion both in KTRs with TMT and DMT (Table 3). Every 10 mg·h/L increase in mycophenolic acid AUC_{0-12h} resulted in an unadjusted OR of 0.89 (95% CI, 0.85–0.93; $P < 0.001$), and adjusted OR of 0.89 (95% CI, 0.85–0.93; $P < 0.001$) for seroconversion in KTRs on TMT. Unadjusted and adjusted ORs for KTRs on DMT were 0.86 (95% CI, 0.77–0.95; $P = 0.003$), and 0.87 (95% CI, 0.79–0.97; $P = 0.010$), respectively. Figure 2d shows the correlation between mycophenolic acid AUC_{0-12h} and IgG titer ($R = -0.44$, $P < 0.001$).

Sensitivity analyses in which we excluded KTRs with a previous COVID infection or imputed antibody titers (due to missing values) showed comparable results with respective adjusted ORs of 0.88 (95% CI, 0.84–0.93; $P < 0.001$) and 0.88 (95% CI, 0.83–0.92; $P < 0.001$), displayed in Tables S2 and S3. Multivariable analysis in which time between mycophenolic acid AUC_{0-12h} and vaccination was included also showed comparable results (Table S4).

DISCUSSION

In this study we found that the gold standard for drug exposure, AUC_{0-12h}, of mycophenolic acid was significantly associated with seroconversion rate and IgG titer in an exposure-dependent manner.

It is important to strive for an optimal humoral immune response with high antibody levels in KTRs for different reasons. First, a low level of antibodies still leave many KTRs unprotected despite seroconversion.[18] Second, a study reported that an antibody titer >1,000 BAU/mL was correlated with *in vitro* neutralization against the Omicron (BA.1) variant.[19] Third, the ongoing merge of new SARS-CoV-2 variants of concern are accompanied with reduced susceptibility for circulating antibodies.[19]

The overall seroconversion rate in our cohort (51%) was relatively high despite a relatively low incidence of 7% of previous COVID-19 infections. Seroconversion rates described in other transplantation cohorts range from 24% to 50% after two vaccinations.[12,20–24] Possible explanations for a higher seroconversion rate in our cohort could be that (i) we only included KTRs that received the Moderna vaccine, which has been shown to result in higher IgG titers than other vaccines, and (ii) the lower total daily dose of mycophenolate compared with other cohorts described in the literature (980 mg/day vs. 1,442 mg/day)[20] owing to routine TDM. The low incidence of COVID-19 was mainly a consequence of strict quarantine precautions taken by our population and government measures in the acute phase of the pandemic.

Our findings on the impact of mycophenolic acid exposure on seroconversion and antibody formation following vaccination are in line with previously published data.[10,21,25] It is, however, important to note that previous studies only used surrogate markers of mycophenolic acid exposure, complicating its applicability in clinical practice. Liefeldt *et al.* recently reported that inosine monophosphate dehydrogenase (IMPDH) activity was a significantly better predictor of seroconversion than mycophenolate dose.[20] Mycophenolic acid is an inhibitor of IMPDH, and the assessment

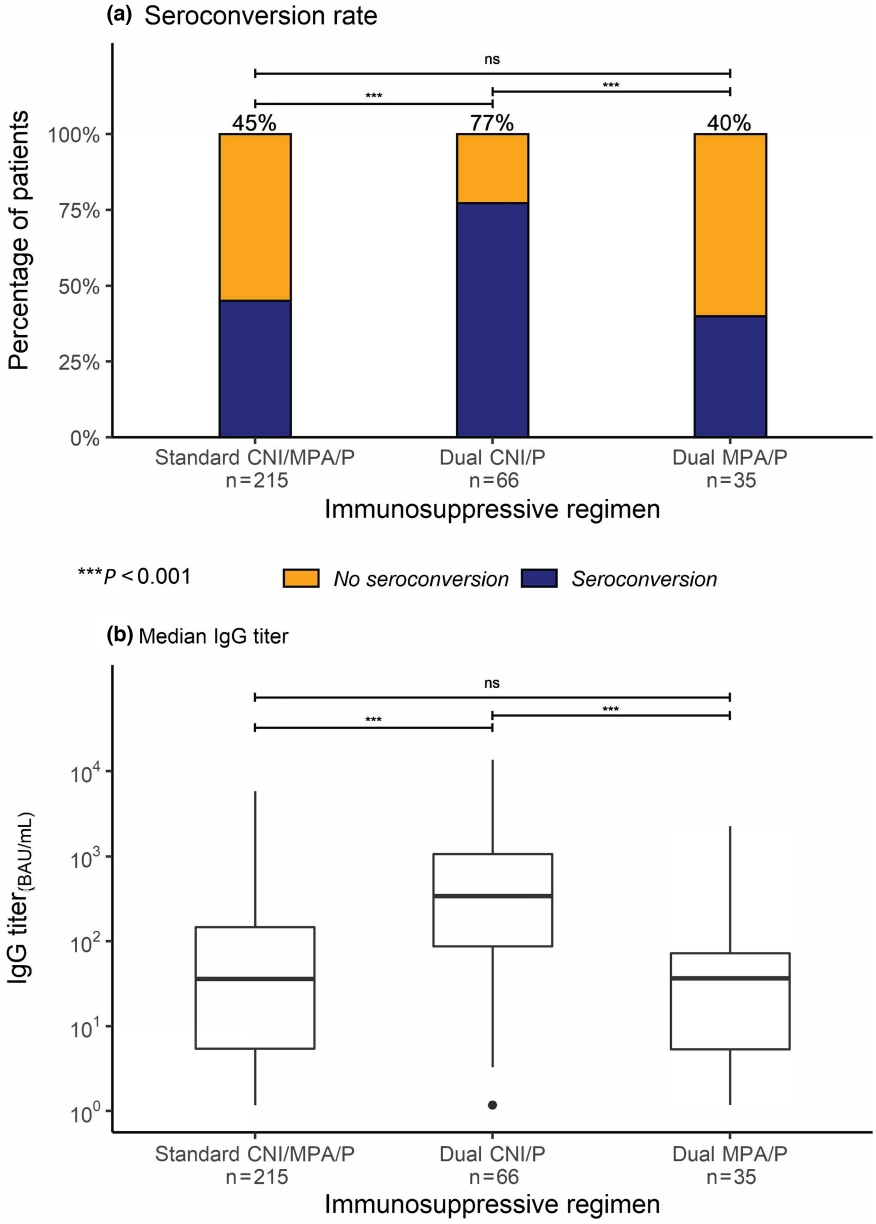

Figure 1 Seroconversion rate and median IgG titer per immunosuppressive regimen. (**a**) Overview of responders and nonresponders after two vaccination cycles for (i) standard CNI/MPA/P, (ii) dual CNI/P, and (iii) dual MPA/P. Percentages displayed are KTRs that have seroconverted. (**b**) Displays median and interquartile ranges for IgG titers per immunosuppressive regimen. Differences were assessed using the χ^2 test. BAU, binding antibody units; CNI, calcineurin inhibitor; IgG, immunoglobulin G; KTRs, kidney transplant recipients; MPA, mycophenolic acid; P, prednisolone; ns, not significant. ***P<0.001.

of IMPDH activity might also serve as a pharmacodynamic measure of the biological effect of mycophenolic acid.[26] However, measurement of IMPDH activity is not readily available in most transplant centers, complicating its use for this specific purpose. A recent study from Germany reported that mycophenolic acid trough levels were negatively associated with IgG titers, which is also suggestive of an exposure–response relationship.[12] However, trough levels, like daily dosages, do not adequately represent overall exposure for mycophenolic acid.[13,27] In contrast, we found an intimate exposure–response relationship between mycophenolic acid exposure, seroconversion rates, and IgG titers using the gold standard of unique AUC_{0-12h} measurements. To our knowledge, such a relationship has never been described previously for any vaccination in solid organ transplant recipients.

While benefits of administration of a third, fourth, or even fifth dose have already been described, we still think our results are of relevance for the field. Multiple studies report that a substantial proportion of patients (40–70%) remains seronegative with low IgG titers despite repeated boosting, making an additional intervention to improve antibody titers desirable.[4–8] Since our results support an intimate exposure–response relationship, a temporary dose reduction or complete discontinuation of mycophenolic acid prior to vaccination could perhaps be beneficial in terms of seroconversion and antibody formation for KTRs who show absent or low titers

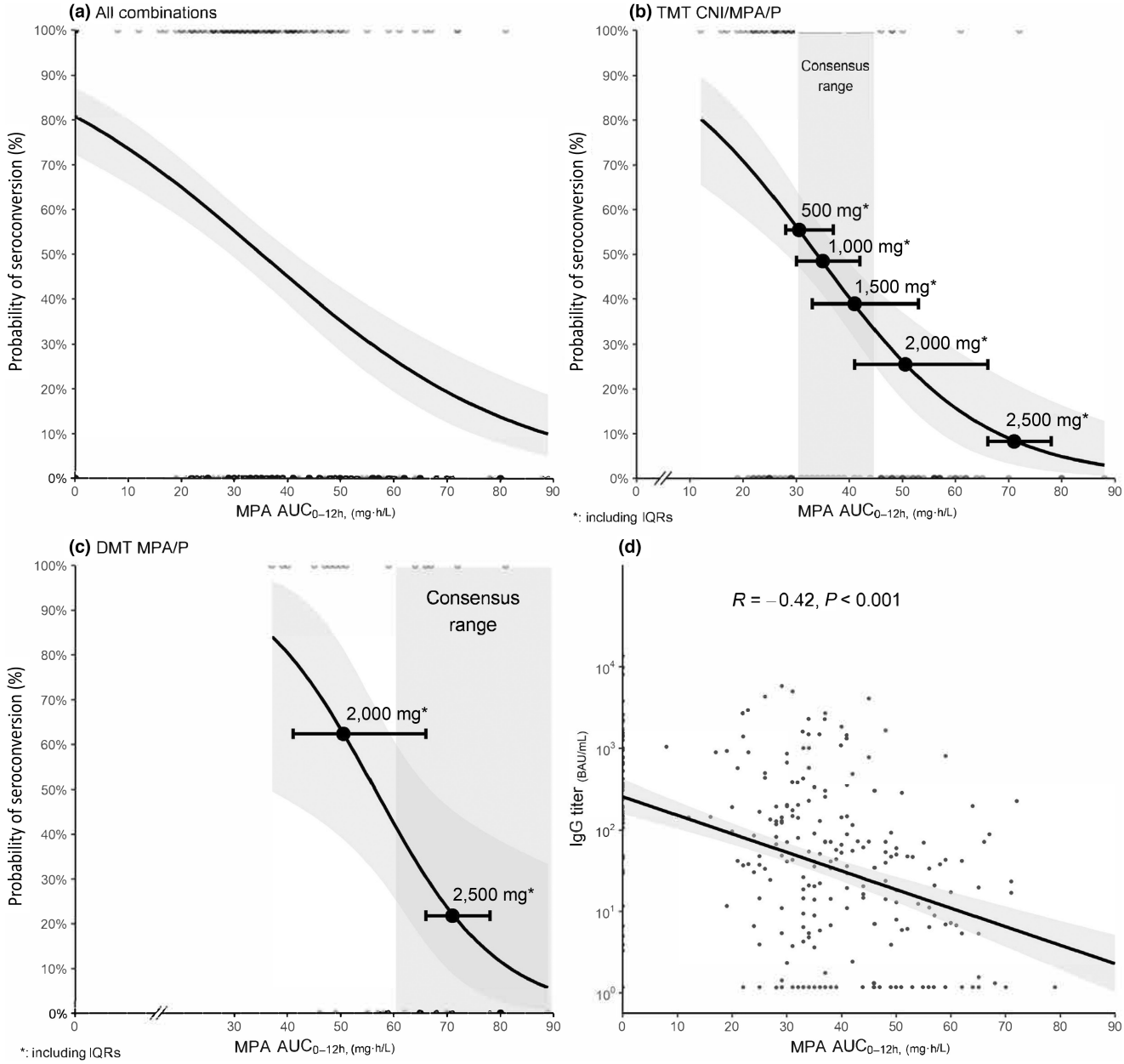

Figure 2 The concentration–effect relationship between MPA dose, MPA exposure, seroconversion rates, and IgG titers. (**a**) Shows the overall relationship between MPA exposure and seroconversion in all immunosuppressive maintenance regimens (in which patients on DMT with CNI/P have an MPA AUC_{0-12h} of 0 mg·h/L. (**b**) Shows the association in TMT with CNI/MPA/P. (**c**) Shows the same association in DMT with MPA/P. Median MPA AUC_{0-12h} and IQRs for each total daily dose are projected on the probability lines. Consensus range according to MPA consensus paper: TMT 30–45 mg·h/L and DMT 60–90 mg·h/L. (**d**) Shows the linear association between MPA AUC_{0-12h} and IgG titer. The y-axis has a logarithmic scale. AUC_{0-12h}, 12-hour area under the curve; BAU, binding antibody units; CNI, calcineurin inhibitor; DMT, dual maintenance therapy; IgG, immunoglobulin G; IQRs, interquartile ranges; MPA, mycophenolic acid; P, prednisolone; TMT, triple maintenance therapy.

after multiple vaccination doses. For KTRs on TMT, we found that a fixed mycophenolate dose of 2,000 mg/day often resulted in a mycophenolic acid AUC above the therapeutic consensus range (30–45 mg·h/L), low rates of seroconversion (10–40%), and low median IgG titers (40 BAU/mL, IQR: 2–221). In contrast, KTRs with a dose of 0 mg, 500 mg, and 1,000 mg/day had higher seroconversion rates varying between 50% to 80% and higher median IgG titers (89 BAU/mL, IQR: 11–670) while the latter two still led to exposure within the therapeutic consensus range. Therefore, some individuals may possibly benefit from a temporary discontinuation of mycophenolic acid. For KTRs on TMT with high immunological risk for rejection or graft loss, AUC-guided personalized dosing to a lower range might be a better strategy to balance between risks of allo-immunity and antibody formation. Multiple nonrandomized studies have recently described the positive effect of mycophenolic acid withdrawal on seroconversion rates and antibody titers, supporting such a strategy.[28–30] In contrast, a recent randomized trial showed no statistical significant difference in seroconversion

Table 3 Odds ratios for seroconversion for mycophenolic acid in KTRs on triple and dual maintenance therapy

		Odds ratios	
		Univariable	Multivariable[a]
MPA AUC_{0-12h} (per 10 mg·h/L increase)	CNI/MPA/P	0.89 (95% CI, 0.85–0.93; $P<0.001$)	0.89 (95% CI, 0.85–0.93; $P<0.001$)
	Dual MPA/P	0.86 (95% CI, 0.77–0.95; $P = 0.003$)	0.87 (95% CI, 0.79–0.97; $P = 0.010$)

AUC_{0-12h}, 12-hour area under the curve; CI, confidence interval; CNI, calcineurin inhibitor; KTR, kidney transplant recipient; MPA, mycophenolic acid; P, prednisolone.
[a]Adjusted for age, gender, time after transplantation, previous COVID infection (assessed by either positive nucleocapsid or questionnaire), and serum creatinine.

rate and IgG titers between KTRs in which mycophenolate was temporarily halted or continued, with seroconversion rates of 80% and 67% after three vaccination doses, respectively.[31] There are, however, two important limitations to mention. First, only 46 individuals were included in both arms, whereas the power calculation revealed a required group size of 71 individuals to achieve a power of 80% and a superiority margin of 5% with the assumption that patients with temporary discontinuation of mycophenolate had a superior response rate of 45% compared with an expected 20% response rate in patients that were continued on mycophenolate. This suggests that the study was underpowered to demonstrate a difference between both groups, making it difficult to draw firm conclusions on the effect of mycophenolate withdrawal. Second, mycophenolate was only halted 1 week before administration of the vaccine, which could be too short to allow recovery of the humoral immune response.

It is therefore of interest to prospectively explore the efficacy (seroconversion, IgG titers, breakthrough infections, hospitality, and mortality) and safety (allo-immunity) of reduction of mycophenolic acid exposure in larger KTR populations with different timing intervals, which is currently being investigated in international trials in both Israel (NCT04961229) and the United States (NCT05077254).

For selected KTRs on DMT with mycophenolic acid/prednisolone, a temporary switch to a regime with CNI/prednisolone might be a solution for the following two reasons. First, we found a 50% seroconversion rate and median IgG titers of 36 BAU/mL for mycophenolic acid/prednisolone at an AUC_{0-12h} of ±52 mg·h/L, which is already subtherapeutic according to the consensus range. Second, the previously published MECANO study showed that CNI/prednisolone was more efficacious in preventing rejection than mycophenolic acid/prednisolone, despite AUC_{0-12h}-controlled dosing.[32] Therefore, these KTRs could possibly benefit from switching from mycophenolic acid to a CNI since seroconversion rates and median IgG titers in this group are >70% and 340 BAU/mL if no contraindication for the use of a (short-term) CNI exists.

It is worth noting that the exposure–response relation between mycophenolic acid AUC_{0-12h} and antibody titers might also be present in (i) other patient populations that require maintenance therapy with mycophenolic acid for a different indication and (ii) other vaccinations due to the mechanism of action on B-cells. Mycophenolic acid blocks proliferation and differentiation of B cells into plasma cells, counteracts appropriate antibody responses, and suppresses immunoglobulin secretion from already activated B cells.[33,34] In support of this hypothesis, a small case series on 24 patients described seroconversion rates of up to 92% (vs. 65%) after mycophenolic acid discontinuation in patients with rheumatic disorders.[35] Moreover, several studies have previously linked mycophenolic acid use to suboptimal antibody responses after other vaccinations, including tetanus, pneumococci, and influenza.[36–38]

It is important to optimize antibody formation post vaccination given the limited alternative strategies available for vulnerable immunocompromised patients. Passive immunization with antibody-based therapies, such as casirivimab/imdevimab (REGEN-COV) and tixagevimab/cilgavimab (Evusheld), have shown efficacy in preventing severe infections in seronegative patients.[39–41] However, these studies were mostly conducted when the Wuhan and Delta variants were dominant. For Evusheld, a number needed to treat (NNT) of 22 was found to prevent a severe COVID-19 infection with hospitalization and/or mortality in individuals with an early COVID-19 infection.[40] In contrast, for pre-exposure prophylaxis, an NNT of 125 was documented to prevent one symptomatic infection.[41] New variants, such as Omicron variants (BA.2, BA.4, and BA.4), have reduced the effectiveness of these therapies, increasing the NNT and raising the issue of cost-effectiveness. Moreover, there is concern that widespread use of antibodies may lead to the emergence of resistant strains. Furthermore, direct antivirals such as Paxlovid, in particular the ritonavir component, may have severe interactions with maintenance immunosuppression and may thus lead to toxic levels while the benefit remains unclear.[42] Interestingly, conversion to a regimen with prednisolone, calcineurin inhibitor, and everolimus may be an interesting alternative strategy for individuals with a high immunological risk in which withdrawal of mycophenolic acid is undesirable, which is currently being investigated by the RECOVAC consortium.

There are several limitations in our study that could influence generalizability. First, we had missing data limited to 10% of antibody titers and <1% of mycophenolic acid exposure. However, we used multiple imputation to limit bias and performed additional sensitivity analysis (in KTRs without missing measurements) in which the association remained materially unchanged. Second, the observational nature of this study makes it difficult to make firm recommendations on adjustments on immunosuppression to increase seroconversion and antibody titers. Our data, however, strongly suggest causality because of a clear exposure–response relationship which provides a rationale and help to design randomized interventional trials in which efficacy and safety of temporary adjustment or stopping of mycophenolic acid is investigated. Third, mycophenolic acid exposure measurement did not always coincide with antibody measurement during the COVID pandemic in which social

distancing and lockdowns were in place. Nevertheless, we made sure to include this information in the multivariable models in which our results remained unchanged. Moreover, we only used AUC measurements of KTRs in steady state in which the drug dose remained unchanged to increase certainty regarding true drug exposure at the time of vaccination. Importantly, mycophenolic acid does not show great intrapatient variability in KTRs on steady state after several years post transplantation (our median coefficient of variation for mycophenolic acid AUC_{0-12h} is 21%—unpublished data—which is in line with current literature).[13] We think it is therefore justified to assume drug exposure was rather stable. Fourth, it could be seen as a limitation that we included patients with either cyclosporine A and tacrolimus given the different mycophenolic acid pharmacokinetics. This might pertain to the daily mycophenolate dose necessary to obtain a certain exposure but does not impact the association between mycophenolic acid AUC_{0-12h} and seroconversion or antibody titers. Moreover, a recent meta-analysis showed that exposure to calcineurin inhibitors did not seem to influence seroconversion rates in other cohorts.[10] We therefore do not expect that this will have impacted our results materially. Fifth, there is no data available on the impact of boosting on antibody titers and clinical outcomes (breakthrough infections, hospitalization, mortality) in this cohort. However, recent studies revealed that IgG levels correlate with protection from disease and that improving the antibody response is likely to be of importance to protect persistently seronegative patients from COVID-19, also for new variants of concern.[18,19,43,44] Sixth, our study only focused on KTRs that received the mRNA-based vaccine produced by Moderna, which may negatively impact generalizability. Finally, it is essential to note that this study was performed within a population on long-term maintenance treatment with an interval since transplantation of 8 (IQR: 4–11) years. Whether these findings are also applicable in KTRs within the first year of transplantation remains to be investigated.

In summary, this study demonstrates an intimate exposure–response relationship between gold standard mycophenolic acid exposure and antibody formation to support interventional studies investigating the impact of mycophenolic acid adjustments on antibody yield and subsequent clinical outcomes in addition to repeated boosting.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

In the last 3 years T.v.G. has received lecture fees and study grants from Chiesi and Astellas, in addition to consulting fees from Roche Diagnostics, Thermo Fisher, Vitaeris, CSL Behring, Astellas, and Aurinia Pharma. A.V.R. received lecture and consulting fees from Sandoz, Chiesi, Astellas, Hansa, CSL Behring, and Novartis in the past 3 years, all of which went to his employer LUMC. In all cases money has been transferred to hospital accounts, and none has been paid to their personal bank accounts. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

S.M., P.B., T.v.G., J.W.d.F., D.J.A.R.M., and A.P.J.d.V. wrote the manuscript. S.M., P.B., T.v.G., D.v.d.H., L.M., P.J.M.v.d.B., J.W.d.F., D.J.A.R.M., and A.P.J.d.V. designed the research. S.M., T.v.G., J.W.d.F., D.J.A.R.M., and A.P.J.d.V. performed the research. S.M., D.J.A.R.M., and A.P.J.d.V. analyzed the data.

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