Trough Level of Mycophenolic Acid Did Not Affect De Novo DSA Development in Kidney Transplantation

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ABSTRACT

Introduction. Mycophenolate mofetil has improved long-term outcomes of kidney transplantation. However, the impact of mycophenolic acid (MPA) trough level on the development of de novo donor-specific anti-HLA antibody (DSA) is unclear. We examined the relation between MPA trough level and de novo DSA development.

Method. We retrospectively studied 617 kidney recipients whose MPA trough level and de novo DSA data were available. All patients underwent primary kidney transplant from living donors from 2008 to 2014, and were chronically treated with a calcineurin inhibitor, mycophenolate mofetil, and +/- steroids. They were equally divided into 4 groups according to the mean trough level of MPA (mMPA) at 1 year post-transplantation: Group 1, mMPA < 2.14 ng/mL ($n = 152$); Group 2, mMPA 2.14-2.83 ng/mL ($n = 157$); Group 3, mMPA 2.83-3.57 ng/mL ($n = 153$); and Group 4, mMPA ≥ 3.57 ng/mL ($n = 155$). The groups were compared by incidence rate of de novo DSA, graft survival rate, and renal function.

Results. The incidence rates of de novo DSA were 33.3% in Group 1, 23.7% in Group 2, 22.9% in Group 3, and 30.3% in Group 4 ($P = .158$). Although there was no significant difference in graft survival rates, a significant difference of renal functions was noted: the higher the renal function, the lower the MPA trough level.

Conclusion. The mMPA trough level at 1 year post-transplantation was not statistically associated with the incidence rate of de novo DSA after kidney transplantation.

DONOR-SPECIFIC anti-HLA antibody (DSA) development is significantly associated with the occurrence of antibody-mediated rejection (AMR) [1–3]. AMR causes chronic active antibody-mediated rejection (CAAMR) and is difficult to control completely with current immunosuppressive regimens; long-term graft outcomes are therefore not favorable.

There are some treatments for AMR and CAAMR, such as double-filtration plasmapheresis, plasma exchange, steroid pulse, intravenous immunoglobulin, rituximab, and so on. However, none of these are established treatments for keeping CAAMR under control [4].

Mycophenolate mofetil (MMF) is an immunosuppressive drug that has recently become indispensable for immunosuppressive treatment following kidney transplantation; it has been used for kidney transplantations in Japan since 2000. It prevents the production of the guanosine nucleotides necessary for DNA synthesis, inhibits T and B cell proliferation, and acts as an immunosuppressant [5]. It is widely used with calcineurin inhibitors and steroids and has been the gold standard of immunosuppressive regimens in transplant maintenance for decades [6]. However, there have been few reports on the association between the trough level of mycophenolic acid (MPA), a bioactive metabolite of MMF, and the development of de novo DSA. We therefore studied the correlation between the two in kidney transplantation.

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0041-1345/19
https://doi.org/10.1016/j.transproceed.2019.03.078

Table 1. Patients' Backgrounds

	Average ± SD	Range	Median
Donor age (y)	56.7 ± 11.9	6–81	59
Recipient age (y)	47.8 ± 13.2	2–77	48
Dialysis period (y)	6.25 ± 7.6	0–35	2.74
Mean tacrolimus trough level (ng/mL)	4.70 ± 1.77	2–13	4.7
mMPA (μg/mL)	2.41 ± 1.10	.3–6.69	2.25
	Frequency		**%**
Donor sex: M/F	260/400		39.4/60.6
Recipient sex: M/F	420/258		62.0/38.1
Living/deceased	587/91		86.6/13.4
Primary/secondary/tertiary	639/36/3		94.3/5.3/.4
Preemptive	59		9.6
ABO-C/MM/I	372/118/188		54.9/17.4/27.7
Pancreatic kidney transplantation	25		4.1
DM	119		19.3
RXM induction	365		59.2
ATG induction	52		8.4
Preformed DSA	44		7.1

Abbreviations: ABO-C/MM/I, ABO-compatible/minor mismatch/incompatible; DM, diabetes mellitus; DSA, donor-specific anti-HLA antibody; mMPA, mean trough level of MPA; RXM, rituximab; SD, standard deviation.

PATIENTS AND METHODS

This is a retrospective cohort study. We reviewed 617 patients with end-stage renal disease who underwent kidney transplantation or pancreatic kidney transplantation between January 2008 and 2014, whose graft survival time was more than 1 year, and who had clinical data of their MPA trough level. MPA trough levels were measured by enzyme immunoassay. DSAs were measured using a Luminex assay and DSA positivity was defined as >500 mean fluorescence intensity. Table 1 shows the patients' backgrounds.

We divided the mean trough levels of MPA (mMPA) over 1 year after kidney transplant into 4 groups: G1 ($n=152$), $mMPA<2.14$; G2 ($n=157$), $2.14\leq mMPA<2.83$; G3 ($n=153$), $2.83\leq mMPA<3.57$; and G4 ($n=155$), $mMPA\geq 3.57$. Table 2 shows a comparison of the patient characteristics among Groups 1–4.

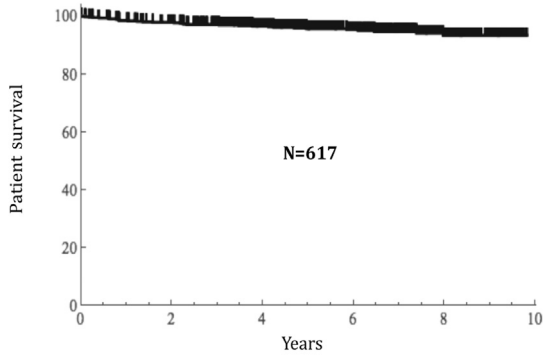
We statistically examined the correlation of frequencies of de novo DSA development with stratified trough levels of MPA. From 2008 to 2011, basiliximab (or rituximab, in the case of ABO incompatibility) were used as induction therapy. Maintenance immunosuppression therapy was performed with a combination of cyclosporine (CyA) or tacrolimus (Tac), MMF, and methylprednisolone (MP). If possible, MP was tapered off at approximately 14 days. From 2012 to 2014, rituximab or basiliximab (or antithymocyte globulin, for patients with high immunological risks) were used as induction therapy. During this period, maintenance immunosuppression was performed with a combination of CyA or Tac, everolimus, MMF, and MP. If possible, MP was tapered off in the same manner as the previous period. The dose of MMF was 2000 mg/d at the time of transplantation, reduced to 1000 mg/d at 6 months after transplantation, and maintained thereafter.

Table 2. Comparison of Each Group

	G1	G2	G3	G4	
n	152	157	153	155	*P*
Donor age (y) (average ± SD)	54.7 ± 11.2	57 ± 12.2	56.4 ± 12.6	58 ± 10.8	.00530
Recipient age (y) (average ± SD)	51.0 ± 12.7	49.4 ± 12.1	46.5 ± 11.7	44.1 ± 12.1	.000179
Dialysis period (y) (average ± SD)	7.21 ± 8.03	5.40 ± 6.82	7.21 ± 7.66	4.85 ± 6.73	.0637
MMF (μg/mL) (average ± SD)	1.29 ± .50	2.04 ± .53	2.68 ± .42	3.84 ± .90	< .00001
Donor sex: M (%)/F	371 (69)/17	55 (34)/105	71 (39)/113	23 (37)/39	.000123
Recipient sex: M (%)/F	27 (50%)/27	96 (59%)/67	125 (64%)/69	45 (69%)/20	.120
Deceased donor (%)	17 (15.9%)	17 (15.9%)	13 (12.4%)	14 (13.0%)	.822
Regraft (%)	7 (6.5%)	5 (4.67%)	10 (9.3%)	3 (2.8%)	.203
Pancreatic kidney transplantation (%)	5 (4.7%)	3 (2.8%)	5 (4.8%)	6 (5.6%)	.794
ABO-C/MM/I	59/23/25	50/24/33	62/14/29	63/18/27	.393
Preemptive (%)	7 (8.1%)	9 (12.2%)	11 (13.1%)	8 (9.6%)	.298
RXM induction (%)	53 (56.4%)	60 (61.9%)	59 (62.8%)	58 (63.0%)	.763
ATG induction (%)	5 (4.7%)	11 (10.4%)	13 (12.4%)	10 (9.3%)	.252
Steroid off (%)	36 (44.4%)	43 (53.1%)	31 (35.6%)	34 (40.0%)	.129
Preformed DSA (%)	3 (4.7%)	10 (6.4%)	9 (5.9%)	6 (3.9%)	.407

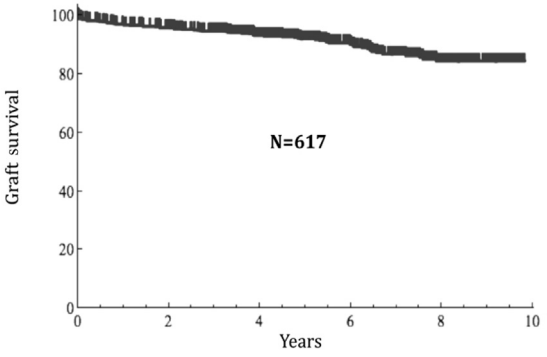
G1: $mMPA<2.14$; G2: $2.14\leq mMPA<2.83$; G3: $2.83\leq mMPA<3.57$; G4: $mMPA\geq 3.57$.

Abbreviations: ABO-C/MM/I, ABO-compatible/minor mismatch/incompatible; ATG, anti-thymocyte globulin; DSA, donor-specific anti-HLA antibody; MMF, mycophenolate mofetil; mMPA, mean trough level of mycophenolic acid; RXM, rituximab; SD, standard deviation.



Years	1	3	5	9
Patients at risk	610	607	388	42

Fig 1. The Kaplan-Meier curve for patient survival. The mean observation period was 5.59 ± 4.0 years.



Years	1	3	5	9
Grafts at risk	599	592	373	42

Fig 2. Kaplan-Meier curves for graft survival. The mean observation period was 5.59 ± 4.0 years.

This study was conducted according to the Declaration of Helsinki and the Japanese National Guidelines. Statistical analyses were performed using Mathematica version 10.1 (Wolfram Research, Champaign, Ill, United States). Continuous variables are expressed as mean values ± standard deviation. $P < .05$ was considered statistically significant.

RESULTS

The overall patient survival rates were 98.4% at 1 year, 97% at 3 years, 95.6% at 5 years, and 93.3% at 9 years (Fig 1). The graft survival rates were 97% at 1 year, 94.7% at 3 years, 92.2% at 5 years, and 84.5% at 9 years (Fig 2).

MMPA levels were almost constant at 2.41 ± 1.10 (μg/mL), regardless of the elapsed years (Table 1, Fig 3). The trough levels of CyA in each group were significantly different between groups ($P \leq .0001$), although there was no significant difference in the trough level of Tac ($P = .5055$) (Figs 4, 5).

There was no significant correlation of survival rate with mMPA among the 4 groups ($P > .05$) (Fig 6); however, the higher the estimated glomerular filtration rate, the lower the mMPA ($P < .0001$) (Fig 7).

Across the groups, the presence or absence of de novo DSA was confirmed in 537 cases. Of these, the numbers of de novo DSA-positive patients were: G1, 45 (33%); G2, 33 (24%); G3, 30 (23%); and G4, 40 (30%), respectively, with no significant differences between groups ($P = .1583$) (Fig 8).

DISCUSSION

Most immunosuppressive drugs should be managed with therapeutic drug monitoring (TDM) to maintain optimum immunosuppressive effects following the organ

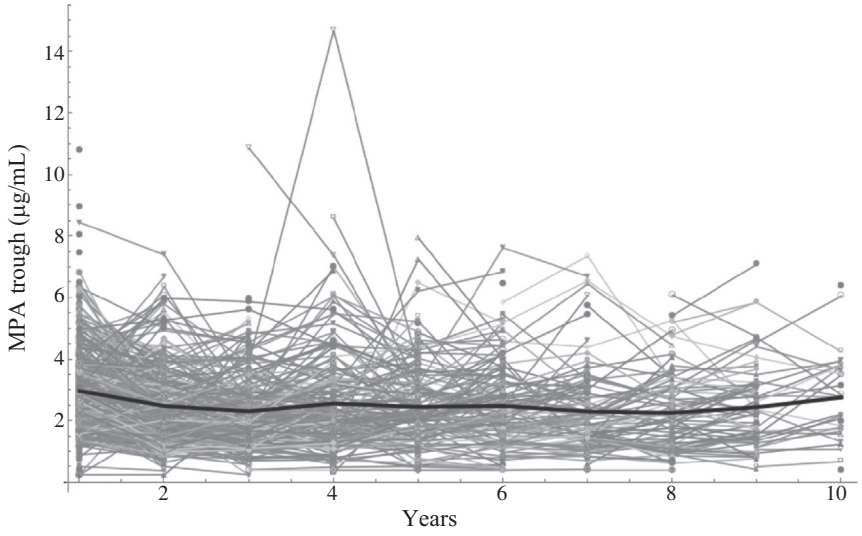
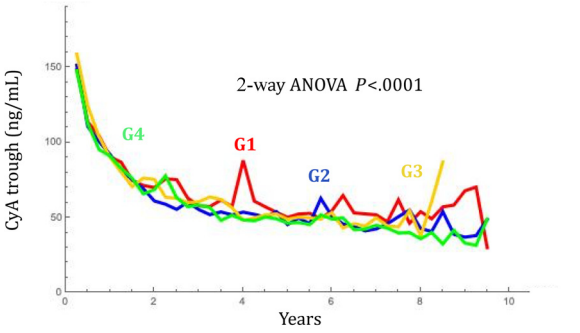


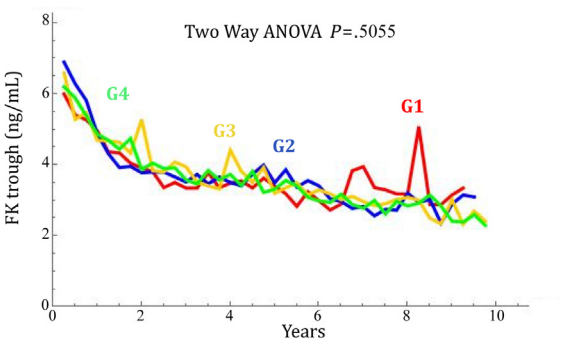
Fig 3. Changes in mMPA per year for all cases that have been engrafted for more than 1 year.



Years	1	3	5	9
Patients at risk				
G1	82	62	48	5
G2	75	59	38	4
G3	42	32	28	0
G4	40	29	18	3

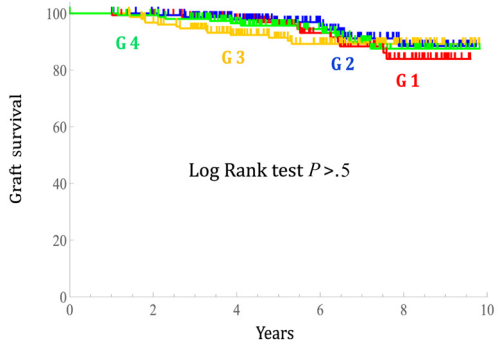
Fig 4. Comparison of CyA troughs among the 4 groups. G1, mMPA < 2.14 (n = 152); G2, 2.14 ≤ mMPA < 2.83 (n = 157); G3, 2.83 ≤ mMPA < 3.57 (n = 153); G4, mMPA ≥ 3.57 (n = 155).

transplantation. Calcineurin inhibitors are nephrotoxic, and a high concentration of any of them in the blood harms renal graft function [7]. It was reported that the trough level of Tac was related to the occurrence of rejection reactions and its variability contributed to the occurrence of DSA [8]. In our past research we obtained similar results: The lower the trough level of Tac, the higher the appearance of DSA [9]. It was also reported that MMF should be managed by therapeutic drug monitoring and its area under the concentration curve (AUC) and trough level should be kept within targets (AUC $_{0-12}$ 30–60 μg · h/mL and 1.0–3.0 μg/mL, respectively, analyzed with high-performance liquid chromatography-ultraviolet [10,11].



Years	1	3	5	9
Patients at risk				
G1	112	101	53	6
G2	123	106	67	6
G3	128	113	75	7
G4	139	128	76	8

Fig 5. Comparison of FK troughs among the 4 groups. G1, mMPA < 2.14 (n = 152); G2, 2.14 ≤ mMPA < 2.83 (n = 157); G3, 2.83 ≤ mMPA < 3.57 (n = 153); G4, mMPA ≥ 3.57 (n = 155).

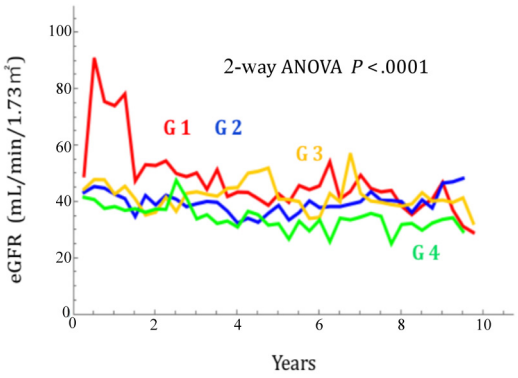


Years	1	3	5	9
Grafts at risk				
G1	152	140	86	12
G2	157	146	97	12
G3	153	135	90	9
G4	155	147	90	12

Fig 6. Comparisons of graft survival rates among the 4 groups. G1, mMPA < 2.14 (n = 152); G2, 2.14 ≤ mMPA < 2.83 (n = 157); G3, 2.83 ≤ mMPA < 3.57 (n = 153); G4, mMPA ≥ 3.57 (n = 155).

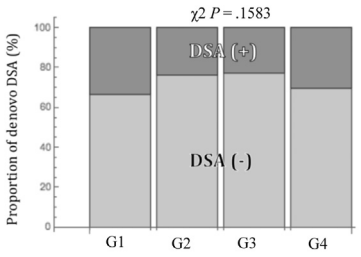
Filler et al reported that the lower MPA trough level, the higher the likelihood of the development of DSAs [12]. However, the present study showed no significant correlation between the trough level of MPA and the development of DSAs. Although there is no doubt that MMF is an effective immunosuppressant, its trough level may not be a good indicator of immunosuppression in kidney transplantation.

As shown in Figure 4, there was a statistically significant difference in the trough level of CyA between the groups. One explanation of this phenomenon is that it was due to



Years	1	3	5	9
Patients at risk				
G1	152	140	86	12
G2	157	146	97	12
G3	153	135	90	9
G4	155	147	90	12

Fig 7. Comparison of eGFR among the 4 groups. G1, mMPA < 2.14 (n = 152); G2, 2.14 ≤ mMPA < 2.83 (n = 157); G3, 2.83 ≤ mMPA < 3.57 (n = 153); G4, mMPA ≥ 3.57 (n = 155).



	G1(n=135)	G2(n=139)	G3(n=131)	G4(n=132)
DSA-poisitive	45(33%)	33(24%)	30(23%)	40(30%)
DSA-negative	90(67%)	106(76%)	101(77%)	92(70%)

Fig 8. Percentages of de novo DSA development in the 4 groups. G1, mMPA < 2.14 (n = 135); G2, 2.14 ≤ mMPA < 2.83 (n = 139); G3, 2.83 ≤ mMPA < 3.57 (n = 131); G4, mMPA ≥ 3.57 (n = 132).

the confounding factor of physicians who found low mMPA in their patients. As in G1, if mMPA was very low, the physician might have increased the dose of CyA to compensate. On the other hand, there was no significant difference in the trough levels of Tac between the groups (Fig 5). Therefore, the authors considered that the variability in the trough levels of CyA between the groups was not clinically significant.

Another phenomenon we think that we should be cautious in explaining was that the lower MPA trough group had higher estimated glomerular filtration rates—in other words, better renal function. We attribute this low mMPA to high renal function, because in our maintenance immunosuppression regimen, MMF was basically prescribed in a fixed dose of 1000 mg/d. It has been reported that glomerular filtration rate, and the AUCs of MPA and gluconate conjugate are inversely correlated in renal transplant patients [13,14].

In summary, the blood concentration of MPA and renal function in transplantation patients are negatively correlated. The blood concentration of mycophenolic acid is affected when renal function declines, so it is necessary to adjust the dose of MMF. However, this research has several limitations. First of all, this study is a retrospective observation. Intra-individual variability (diet, non-adherence, etc) and inter-individual variability (drug metabolism, regimen, immunologic risk, etc) were not considered sufficiently. In addition, the dose of MMF was basically fixed, and the difference in trough level depended on the difference in metabolic rate of MPA of each patient. Therefore, we need a prospective study of groups receiving different MMF doses in order to observe the correlation between different doses of MMF and different trough levels of MPA with the development of de novo DSA and graft function. Further research is needed.

CONCLUSION

There was no clear correlation between trough level of MPA and de novo DSA because the dose of MMF was roughly constant and was adjusted according to the risk of rejection.

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