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Clinical Implication of Mycophenolic Acid Trough Concentration Monitoring in Kidney Transplant Patients on a Tacrolimus Triple Maintenance Regimen: A Single-Center Experience

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF Jinsoo Rhu
ABD Kyo Won Lee
ACDE Hyojun Park
ADE Jae Berm Park
ABCDEF Sung Joo Kim
BC Gyu Seong Choi

Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea

Corresponding Author: Sung Joo Kim, e-mail: kmhyj.kim@samsung.com
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Background: This study was designed to analyze the clinical implications of mycophenolic acid trough concentration monitoring.

Material/Methods: We collected data of patients with mycophenolic acid trough concentration monitoring after their first kidney transplant between November 2006 and March 2015 who were prescribed tacrolimus, mycophenolate, and methylprednisolone. Analyses were performed on 3 periods: 1 month, 1 month to 1 year, and after 1 year post-transplantation. To analyze factors related to acute cellular rejection, logistic regression was used for 1 month, while Cox analysis was used during 1 month to 1 year and after 1 year post-transplantation.

Results: In the 145 patients receiving mycophenolate mofetil, mean tacrolimus trough ≥7.0 ng/mL (OR=0.177, CI=0.060–0.524, p=0.002) and mean mycophenolic acid trough ≥1.7 mg/L (OR=0.190, CI=0.040–0.896, p=0.036) were protective for rejection during 1 month. Mean mycophenolic acid trough ≥1.7 mg/L (HR=0.179, CI=0.040–0.806, p=0.025) and ≥0.7 mg/L (HR=0.142, CI=0.028–0.729, p=0.019) were related to better rejection-free survival during 1 month to 1 year and after 1 year, respectively. In 399 patients receiving enteric-coated mycophenolate sodium, mean tacrolimus trough ≥7.0 ng/mL (OR=0.258, CI=0.131–0.507, p<0.001) and mean mycophenolic acid trough ≥2.1 mg/L (OR=0.507, CI=0.264–0.973, p=0.041) were protective for rejection during 1 month. Mean mycophenolic acid trough ≥1.7 mg/L (HR=0.519, CI=0.289–0.932, p=0.028) and ≥0.7 mg/L (HR=0.208, CI=0.072–0.602, p=0.004) were related to better rejection-free survival during 1 month to 1 year and after 1 year, respectively.

Conclusions: Mycophenolic acid trough concentration monitoring can be useful in preventing acute cellular rejection in patients receiving tacrolimus, mycophenolate, and methylprednisolone.

MeSH Keywords: **Immunosuppression • Kidney Transplantation • Mycophenolic Acid • Tacrolimus**

Abbreviations: **KT** – kidney transplantation; **CNI** – calcineurin inhibitor; **MMF** – mycophenolate mofetil; **MPA** – mycophenolic acid; **MPAG** – 7-*O*-MPA-glucuronide; **EC-MPS** – enteric-coated mycophenolate sodium; **AUC** – area under the concentration-time curve; **HLA** – human leukocyte antigen; **BPAR** – biopsy-proven acute rejection; **BMI** – body mass index

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Background

In kidney transplantation (KT), multiple drugs are used for optimal immunosuppression with lower toxicities. Along with calcineurin inhibitors (CNIs; tacrolimus and cyclosporine A) and corticosteroids, mycophenolate (mycophenolate mofetil and enteric-coated mycophenolate sodium) is also a key component of the triple immunosuppressive regimen for maintenance.

Mycophenolate mofetil (MMF) is an inactive prodrug that is converted to its active metabolite, mycophenolic acid (MPA), by intestinal, liver, and plasma esterase, after absorption. MPA acts as a reversible inhibitor of inosine-5'-monophosphate dehydrogenase, which is an enzyme that functions in lymphocyte mitosis. MPA is metabolized to 7-*O*-MPA-glucuronide (MPAG) in the liver and intestine by UDP-glucuronyltransferase. MPAG is an inactive metabolite that is excreted through urine and bile. Some of the excreted MPAG in bile can be hydrolyzed by bacterial glucuronidase and reabsorbed as MPA though the enterohepatic recirculation [1].

Enteric-coated mycophenolate sodium (EC-MPS), which is a sodium salt of MPA, is another formulation that acts as a prodrug. It has been designed to delay the release of MPA until it reaches the small bowel, improving the MPA-related gastrointestinal adverse reaction. EC-MPS remains intact in the stomach, while it becomes highly soluble in the neutral pH of the small intestine [2].

Although MMF has a less variable pharmacokinetic profile than CNIs, research has shown that MMF possesses wide inter-patient and intra-patient variability over time [3]. These features are mainly due to its complex pharmacokinetics, which are influenced by kidney function, liver function, serum albumin level, alterations in absorption, and combinations with other drugs [4]. The pharmacokinetic profile of EC-MPS is different from that of MMF. While the maximum MPA plasma level occurs within 0.5 to 2 hours after MMF intake, EC-MPS needs 2 to 3 hours to reach its maximum level [1].

Monitoring methods are also a topic of interest. While monitoring the MPA AUC_{0-12} (area under the concentration-time curve) was significantly associated with clinical events [5–7], technical difficulties in utilization have been a barrier to its wide acceptance in clinical practice. Some investigators have published data on the efficacy of using limited sampling strategies or a single concentration such as the trough concentration. Such results have not been consistent among studies, showing the limited efficacies of these methods [7–12].

The transplantation society consensus meeting suggested that therapeutic drug monitoring of MPA can be of advantage in high-risk patients, patients with delayed graft function, or patients with immunosuppressive protocols excluding induction therapy or steroids or CNI or patients with CNI minimization [13]. In fact, MPA monitoring is only used in a few centers, whereas a few others only in case of unexpected rejection or adverse event [14].

Our center started monitoring plasma MPA trough concentration in 2006. After transplanting more than 2000 kidneys with 10 years of experience in MPA trough concentration monitoring, we designed this study to analyze the dose-concentration relationship of mycophenolate and MPA and the clinical implications of MPA trough concentration.

Material and Methods

Patients

We retrospectively reviewed data from our prospectively maintained KT database of Korean adult patients who underwent MPA trough concentration monitoring after their first KT at Samsung Medical Center between November 2006 and March 2015. Low-risk patients who received the triple maintenance regimen of tacrolimus, mycophenolate (either MMF or EC-MPS), and methylprednisolone were included. Exclusion criteria were as follows: patients who experienced desensitization prior to KT; patients who underwent induction therapy with daclizumab, alemtuzumab, rituximab, or more than 3 days of thymoglobulin; previous history of KT; multiorgan transplantation; ABO incompatible transplantation; positivity for donor-specific antibody; pediatric patients; cessation of main regimen within 1 month after KT; and other factors related to high-risk KT.

Immunosuppression

Patients received tacrolimus, mycophenolate, and methylprednisolone as a triple immunosuppressive regimen. Induction therapy was performed with either basiliximab or 3 days of thymoglobulin. For therapeutic monitoring of tacrolimus, the tacrolimus trough level was monitored and the dosage was adjusted to maintain a target concentration of 8 to 10 ng/mL during 1 month post-KT, 5 to 8 ng/mL during 1 month to 1 year, and 3 to 7 ng/mL afterward. Patients received either MMF or EC-MPS, and dosages were adjusted to maintain a target MPA trough concentration of 1.5 to 2.5 mg/L during 1 year post-KT [13,15]. Target concentration was lowered after 1 year post-KT to maintain above 1.0 mg/L. Methylprednisolone was tapered and withdrew as a protocol in average of 3 month post-KT.

Monitoring of MPA trough concentration was performed routinely alongside with tacrolimus trough concentration. Both whole blood tacrolimus trough concentration and plasma MPA trough concentration were monitored by high-performance

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liquid chromatography with tandem mass spectrometry. During admission for drug level adjustment, monitoring was performed several times. When patients visited the outpatient clinic, MPA trough concentration was measured along with other laboratory tests.

Data collection

Demographic data including gender, age at transplantation, and the height and weight of both donor and recipient were collected. Background information on the KT, such as mode of renal replacement therapy, time of renal replacement, cause of renal failure, human leukocyte antigen (HLA) mismatch, donor serum creatinine, donor-recipient relationship, cause of cadaveric donor death, panel reactive antibody status, and CMV status were collected. Data on episodes of acute cellular rejection based on Banff criteria, episodes of gastrointestinal complications (diarrhea and gastritis), episodes of cytopenia (anemia, neutropenia, and thrombocytopenia), episodes of infection (BK virus, cytomegalovirus, pneumonia, urinary tract infection, influenza, invasive fungal infection, *Pneumocystis jiroveci* infection, tuberculosis, and other infections), graft failures, and deaths were collected. Data on the daily dosages of MMF and EC-MPS, tacrolimus trough concentrations, and MPA trough concentrations were collected.

Data analysis

As the target level of tacrolimus and MPA trough concentration changes with time after KT, analyses were performed separately based on the 3 different follow-up periods: within 1 month, 1 month to 1 year, and after 1 year post-KT. The mean dosage of mycophenolate (MMF or EC-MPS), mean MPA trough levels, and tacrolimus trough levels were calculated using Excel (Microsoft, Redmond, WA). Mean dosages were calculated to determine the mean daily exposure to the drug from the date of KT to the time point of interest. For example, the mean dosage of mycophenolate during 1 month to 1 year was calculated using data from 1 month post-KT to 1 year post-KT. A paired t-test was used to analyze the changes in mean MPA trough levels and tacrolimus trough levels between the consecutive follow-up periods. Simple linear regression was used to analyze the correlation between the dosage of mycophenolate (MMF or EC-MPS) and the mean MPA trough levels of each follow-up period, while calculating the intercept and coefficient.

To analyze the risk factors for BPAR within 1 month post-KT, multivariable logistic regression analysis was performed to identify the best fit model, whereas the multivariable Cox proportional hazard ratio was used to analyze factors related to BPAR between 1 month to 1 year post-KT and after 1 year post-KT. For the Cox regression analyses of 2 periods, BPAR was set as an end point. Cessation of tacrolimus, cessation of mycophenolate for longer than 3 months, and cessation of MPA monitoring were censored in the analysis. The analyses for risk factors of BPAR were performed separately for MMF and EC-MPS.

For the analysis, continuous variables were divided into 2 groups. Recipient and donor age was divided by 40 years of age. Both recipient and donor body mass index (BMI) were classified as greater than or less than 21 kg/m^2. HLA status was classified as matched or mismatched. Mean tacrolimus trough levels were divided based on whether they were greater than or less than 7 ng/mL, 5 ng/mL, and 5 ng/mL for 1 month, 1 month to 1 year, after 1 year post-KT, respectively. Mean MPA trough levels were divided on the point where they showed the highest sensitivity and specificity for predicting BPAR. Consequently, patients receiving MMF were divided based on 1.7 mg/L for 1 month, 1.7 mg/L for 1 month to 1 year, and 0.7 mg/L for after 1 year, while patients receiving EC-MPS were divided based on 2.1 mg/L for 1 month, 1.7 mg/L for 1 month to 1 year, and 0.7 mg/L for after 1 year.

P-values <0.05 were used to indicate statistical significance. Statistical analyses were performed with SPSS 18.0 (SPSS Inc., Chicago, IL). This study was approved by the Institutional Review Board of Samsung Medical Center (IRB No. 2016-12-056).

Results

Table 1 summarizes the demographic information, immunosuppressive features, and clinical outcomes of the patients. We included 555 patients, including 334 males (60.2%) and 221 females (39.8%). The mean recipient age and donor age were 47.25±12.48 years and 45.00±14.88 years, respectively. Diabetic nephropathy (n=101, 18.2%), IgA nephropathy (n=80, 14.4%), and hypertensive nephropathy (n=70, 12.6%) were the 3 most common causes of renal failure. HLA was matched in 70 patients (12.6%), while the mean number of HLA mismatches was 2.89±1.57 per patient. The mean donor serum creatinine level was 1.26±0.98 mg/dL. There were 225 cases (40.5%) that were a living-related relationship, while 107 (19.3%) were living-unrelated cases. There were 223 (40.2%) cadaveric donors, and the most common cause of cadaveric donor death was cerebrovascular accident (n=115, 51.6%), followed by hypoxic brain damage (n=54, 24.2%), trauma (n=44, 19.7%), and others (n=10, 4.5%). MMF was used in 145 patients (26.1%), while EC-MPS was used in 399 patients (71.9%). Eleven patients (2.0%) switched from MMF to EC-MPS or from EC-MPS to MMF. Basiliximab was used in 438 patients (78.9%), while thymoglobulin was used in 116 patients (20.9%) for 3 days.

BPAR occurred in 192 patients (34.6%). Diarrhea and gastritis occurred in 38 patients (6.8%) and 47 patients (8.5%),

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Table 1. Patient demographics, immunosuppressive regimens, and clinical outcomes of low risk kidney transplantation patients who received a triple maintenance regimen of tacrolimus, mycophenolic acid, and methylprednisolone.

Factors	No. patients or Mean ± SD	Percentages (%)
Age (years), mean ±SD	47.25±12.48	
Sex (M/F)	334/221	60.2/39.8
BMI(kg/m^2), mean ±SD	23.03±3.54	
Renal replacement therapy		
Hemodialysis	378	68.1
Peritoneal dialysis	90	16.2
No dialysis	87	87
Time on renal replacement (days), mean ±SD	1563±1438	
Underlying kidney disease		
Diabetic nephropathy	101	18.2
IgA nephropathy	80	14.4
Focal segmental glomerulosclerosis	9	1.6
Other glomerulonephritis	66	11.9
Polycystic kidney disease	27	4.9
Hypertensive nephropathy	70	12.6
Others	29	5.2
Unknown	173	31.2
HLA-A, HLA-B, HLA-DR mismatches (mm), n		
0 mm	70	12.6
1 mm	33	5.9
2 mm	89	16.0
3 mm	155	27.9
4 mm	125	22.5
5 mm	70	12.6
6 mm	13	2.3
HLA mm per patient, mean ±SD	2.89±1.57	
Donor age (years), mean ± SD	45.00±14.88	
Donor sex (M/F)	308/247	55.5/45.5
Donor BMI, mean ± SD	24.26±3.43	
Donor serum creatinine(mg/dL), mean ± SD	1.26±0.98	
Donor-recipient relationship, n		
Living-related	225	40.5
Living-unrelated	107	19.3
Cadaveric donor	223	40.2
Cause of cadaveric donor death, n		
Cerebrovascular accident	115	51.6
Trauma	44	19.7
Hypoxic brain damage	54	24.2
Other	10	4.5
Panel reactive antibody		
0%	451	81.3
1–49%	77	13.9
≥50%	15	2.7
Donor/receptor CMV status, n		
Positive/positive	504	90.8
Positive/negative	14	2.5
Negative/positive	21	3.8
Negative/negative	0	0.0

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Table 1 continued. Patient demographics, immunosuppressive regimens, and clinical outcomes of low risk kidney transplantation patients who received a triple maintenance regimen of tacrolimus, mycophenolic acid, and methylprednisolone.

Factors	No. patients or Mean ± SD	Percentages (%)
Drug		
Mycophenolate mofetil	145	26.1
Enteric-coated mycophenolate sodium	399	71.9
Switching from either drug	11	2.0
Induction therapy		
Basiliximab	438	78.9
Thymoglobulin only up to 3 days	116	20.9
None	1	0.2
Biopsy proven Rejection	192	34.6
Within 1 month	76	13.7
1 month to 1 year	63	11.4
After 1 year	53	9.5
Gastrointestinal complication		
Diarrhea	38	6.8
Gastritis	47	8.5
Cytopenia		
Anemia	4	0.7
Neutropenia	88	15.9
Thrombocytopenia	1	0.2
Infection		
BK virus	229	41.3
Cytomegalovirus	260	46.8
Pneumonia	34	6.1
Urinary tract injection	79	14.2
Influenza	6	1.1
Invasive fungal infection	3	0.5
Pneumocystis jiroveci	1	0.2
Tuberculosis	11	2.0
Others	115	20.7
MPA monitor duration (months), mean ±SD	34.23±20.84	
Number of tacrolimus tests, median (IQR)		
Within 1 month	17 (6)	
1 month to 1 year per month	1.90 (1.82)	
After 1 year per month	1.29 (1.37)	
Number of MPA tests, median (IQR)		
Within 1 month	5 (2)	
1 month to 1 year per month	1.36 (0.45)	
After 1 year per month	0.82 (0.61)	
Graft failure	25	4.5
Death	8	1.4

SD – standard deviation; BMI – body mass index; HLA – human leukocyte antigen; CMV – cytomegalovirus; MPA – mycophenolic acid.

respectively. Most episodes of cytopenia were neutropenia (n=88, 15.9%), while there were 4 anemias and 1 thrombocytopenia. The most common infections were cytomegalovirus (n=260, 46.8%) and BK polyoma virus (n=229, 41.3%). Mean MPA trough monitoring duration was 34.23±20.84 months. Graft failure occurred in 25 patients (4.5%), and death occurred in 8 patients (1.4%).

Mean trough concentrations of tacrolimus and MPA

The mean tacrolimus trough levels were 8.37±1.56, 7.39±1.37, and 6.40±1.38 ng/mL during the follow-up periods of 1 month, 1 month to 1 year, and after 1 year post-KT, respectively. (Table 2) As the target levels decreased from 8 to 10 ng/mL during 1 month post-KT to 5 to 8 ng/mL during 1 month to 1

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Table 2. Mean trough levels of FK and MPA categorized by treatment period of 1 month, 1 month to 1 year, and >1 year analyzed by paired t-test.

Drug levels by period	Drug levels	p-value
Tacrolimus trough, mean ±SD (ng/mL)		
Within 1 month	8.37±1.56	
1 month to 1 year	7.39±1.37	<0.001
After 1 year	6.40±1.38	<0.001
MPA trough, mean ±SD (mg/L)		
Within 1 month	2.15±1.51	
1 month to 1 year	2.46±1.29	<0.001
After 1 year	2.35±1.31	<0.001

SD – standard deviation

Table 3. Mean dosages and mean plasma trough levels of mycophenolic acid, which were prescribed with mycophenolate mofetil (N=145) and enteric-coated mycophenolate sodium (N=399).

	Periods	Dosage (mg)	MPA trough (mg/L)	r^2	β*	p-Value
MMF (N=145)	Total	1002.29	1.89	0.063	0.620	0.002
	Within 1 month	1369.71	1.34	0.014	0.387	0.161
	1 month to 1 year	1085.20	2.02	0.106	0.829	<0.001
	After 1 year	868.29	1.98	0.125	1.028	<0.001
EC-MPS (N=399)	Total	713.63	2.59	0.032	1.022	<0.001
	Within 1 month	1017.10	2.45	0.000	0.086	0.861
	1 month to 1 year	751.69	2.56	0.062	1.548	<0.001
	After 1 year	608.71	2.22	0.060	1.488	<0.001

* Coefficient was calculated after the MPA trough level was multiplied by 1,000. MMF – mycophenolate mofetil; EC-MPS – enteric-coated mycophenolate sodium; MPA – mycophenolic acid.

year post-KT and to 3 to 7 ng/mL afterward, the mean tacrolimus trough levels decreased. Paired t-tests showed significant differences in mean tacrolimus trough levels between 1 month and 1 month to 1 year post-KT and between 1 month to 1 year and after 1 year post-KT (p<0.001, both).

Mean MPA trough levels were 2.15±1.51, 2.46±1.29, and 2.35±1.31 ng/mL during 1 month, 1 month to 1 year, and after 1 year post-KT, respectively. The mean MPA trough level was the highest during 1 month to 1 year post-KT, and a paired t-test showed that the differences were statistically significant between the consecutive periods (p<0.001, both).

Correlation between mycophenolate dosage and mean MPA trough levels

Table 3 summarizes the correlation between dosage and trough levels using simple linear regression analyses. The coefficient was calculated after the MPA trough level was multiplied by 1,000. A total of 8 separate analyses were performed in patients receiving MMF and patients receiving EC-MPS for 1 month, 1 month to 1 year, after 1 year post-KT, and the total follow-up period. The correlations between mean MMF or EC-MPS dosages and mean MPA trough levels during the total period were significant (p=0.002 and p<0.001, respectively). However, there was no correlation between the 2 in patients receiving MMF or patients receiving EC-MPS during 1 month (p=0.161 and p=0.861, respectively). Between 1 month and 1 year and after 1 year post-KT, there were significant correlations between mean dosages and mean trough levels in patients receiving MMF (p<0.001, both) and patients receiving EC-MPS (p<0.001, both). In the 145 patients receiving MMF, the coefficient increased from 0.829 to 1.028. However, in the 399 patients receiving EC-MPS, the coefficient slightly decreased from 1.548 to 1.488. The coefficients were calculated after the mean MPA trough concentration was multiplied by 1000. Figure 1 illustrates the linear regression curve of each treatment period for each group.

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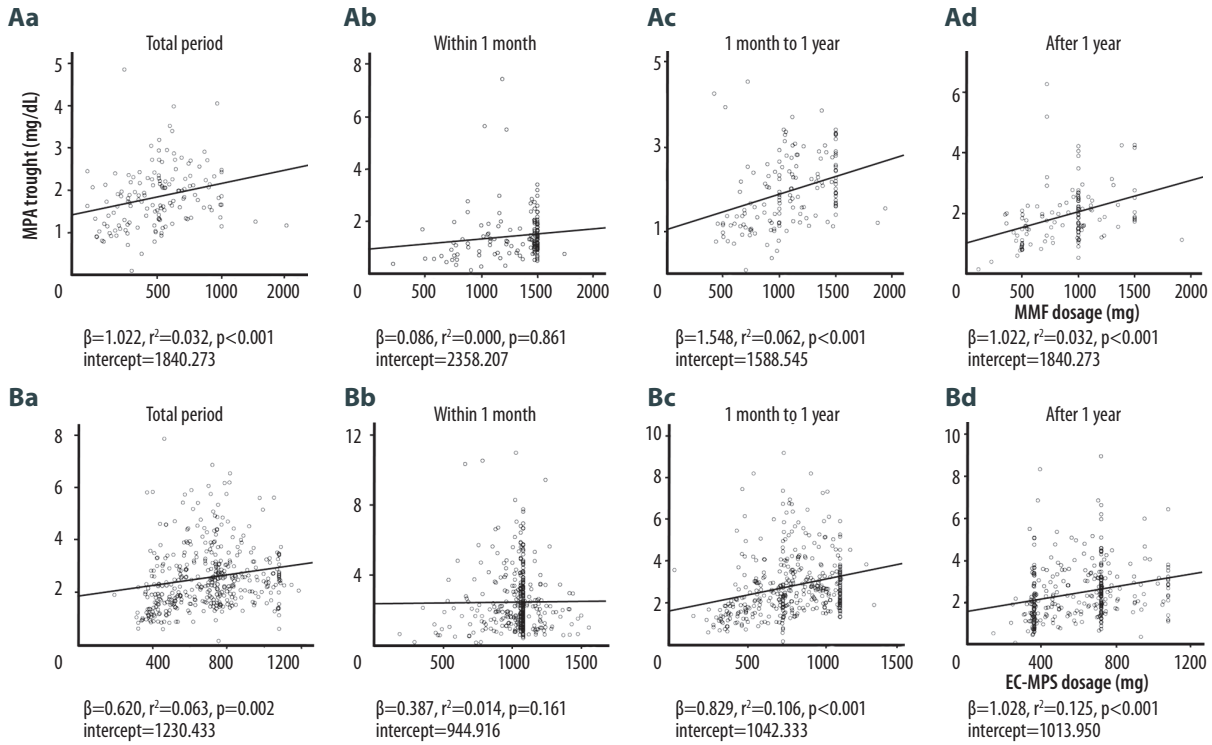

Figure 1. The correlations between mean MMF or EC-MPS dosages and mean MPA trough levels analyzed by simple linear regression. (**Aa**) patients receiving MMF showed a significant relationship during the total time period (p=0.002). (**Ab**) While the correlation was not significant within 1 month (p=0.161), (**Ac, Ad**) 1 month to 1 year and >1 year post-KT showed significant correlations (p<0.001, both). (**Ba**) patients receiving EC-MPS also showed a significant relationship during the total period (p=0.002). (**Bb**) There was no correlation within 1 month (p=0.161), (**Bc, Bd**) but 1 month to 1 year and >1 year post-KT showed a significant correlation (p<0.001, both).

Table 4. Multivariable logistic regression models for BPAR within 1 month after kidney transplantation in mycophenolate mofetil users (N=145) and enteric-coated mycophenolate sodium users (N=399).

Independent variable			Multivariable logistic regression			
			No.	OR	95% CI	p-value
MMF (N=145)	Induction with 3 days of thymoglobulin		32			0.004
	Induction with basiliximab		112	11.524	2.175–61.046	
	Mean tacrolimus trough (ng/mL)	<7.0	34			0.002
		≥7.0	110	0.177	0.060–0.524	
	Mean MPA trough (mg/L)	<1.7	108			0.036
		≥1.7	36	0.190	0.040–0.896	
EC-MPS (N=399)	Mean tacrolimus trough (ng/mL)	< 7.0	57			<0.001
		≥7.0	342	0.258	0.131–0.507	
	Mean MPA trough (mg/L)	<2.1	221			0.041
		≥2.1	178	0.507	0.264–0.973	

BPAR – biopsy-proven acute rejection; MMF – mycophenolate mofetil; EC-MPS – enteric-coated mycophenolate sodium; MPA – mycophenolic acid.

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Table 5. Cox proportional hazard model of the risk factors for BPAR during 1 month to 1 year in mycophenolate mofetil users (N=119) and enteric-coated mycophenolate sodium users (N=346).

			No.	Univariable			Mutivariable		
				HR	95% CI	p	HR	95% CI	p
MMF (N=119)	Recipient age	<40	38			0.230			
		≥40	81	0.513	0.172–1.526				
	Donor age	<40	44			0.305			
		≥40	75	1.965	0.541–7.141				
	Recipient BMI	<21	40			0.064			
		≥21	79	6.870	0.893–52.861				
	Donor BMI	<21	22			0.817			
		≥21	97	1.194	0.265–5.390				
	HLA-A,B,DR mismatch >0		102	0.848	0.188–3.828	0.830			
	Induction with 3 days of thymoglobulin		29			0.619			
	Induction with basiliximab		89	0.742	0.228–2.409				
	Mean tacrolimus trough (ng/mL)	<5.0	7			0.544			
		≥5.0	112	22.127	0.001–4859				
	Mean MPA trough (mg/L)	<1.7	62			0.025			
		≥1.7	57	0.179	0.040–0.806				
EC-MPS (N=346)	Recipient age	<40	93			0.157			
		≥40	253	1.686	0.818–3.474				
	Donor age	<40	114			0.008	2.525	1.179–5.407	0.017
		40	232	2.768	1.297–5.905				
	Recipient BMI	<21	104			0.050	1.912	0.926–3.948	0.080
		≥21	242	2.062	1.000–4.249				
	Donor BMI	<21	54			0.071			
		≥21	292	2.937	0.914–9.445				
	HLA-A,B,DR mismatch	>0	296	2.726	0.848–8.766	0.092			
	Induction with 3 days of thymoglobulin		74			0.010	0.598	0.327–1.095	0.096
	Induction with basiliximab		272	0.459	0.255–0.827				
	Mean tacrolimus trough (ng/mL)	<5.0	6			0.660			
		5.0	340	0.641	0.089–4.647				
	Mean MPA trough (mg/L)	<1.7	80			0.009	0.519	0.289–0.932	0.028
		≥1.7	266	0.458	0.256–0.820				

BPAR – biopsy-proven acute rejection; MMF – mycophenolate mofetil; EC-MPS – enteric-coated mycophenolate sodium; BMI – body mass index; HLA – human leukocyte antigen; MPA – mycophenolic acid.

BPAR within 1 month post-KT

Multivariable logistic regression analyses with a backward likelihood ratio test was performed to identify the best fit model for predicting BPAR within 1 month. In patients receiving MMF (N=145), induction therapy with basiliximab (OR=11.524, CI=2.175-61.046, p=0.004) was associated higher risk compared to thymoglobulin, while mean tacrolimus trough concentration ≥7.0 ng/mL (OR=0.177, CI=0.060–0.524, p=0.002) and mean MPA trough concentration ≥1.7 mg/L (OR=0.190, HR=0.040–0.896, p=0.036) were associated with lower risk of BPAR. In patients receiving EC-MPS (N=399), mean tacrolimus trough concentration ≥7.0 ng/mL (OR=0.258, CI=0.131–0.507, p<0.001) and mean MPA trough concentration ≥2.1 mg/L were associated with lower risk of BPAR (Table 4).

BPAR during 1 month to 1 year post-KT

In patients receiving MMF (N=119), univariable Cox proportional hazard model showed that only mean MPA trough concentration ≥1.7 mg/L was associated with lower risk of BPAR (HR=0.179, CI=0.040-0.806, p=0.025). In patients receiving EC-MPS (N=346), multivariable Cox proportional hazard model showed that mean MPA trough concentration ≥1.7 mg/L was associated with lower risk of BPAR (HR=0.519, CI=0.289-0.932, p=0.028), while donor age ≥40 years was associated

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Table 6. Cox proportional hazard models of the risk factors for BPAR after 1 year post-kidney transplantation in mycophenolate mofetil users (N=95) and enteric-coated mycophenolate sodium users (N=268).

			No.	Univariable			Mutivariable		
				HR	95% CI	p	HR	95% CI	p
MMF (N=95)	Recipient age	<40	29			0.602			
		≥40	66	1.364	0.424–4.385				
	Donor age	<40	36			0.053			
		≥40	59	4.423	0.980–19.957				
	Recipient BMI	<21	38			0.847			
		≥21	57	0.901	0.312–2.599				
	Donor BMI	<21	16			0.312			
		≥21	79	26.116	0.047–14628				
	HLA-A,B,DR mismatch	>0	81	1.828	0.237–14.075	0.563			
	Induction with 3 days of thymoglobulin		24			0.724			
	Induction with basiliximab		70	0.810	0.251–2.608				
	Mean tacrolimus trough (ng/mL)	<5.0	15			0.570			
		≥5.0	80	0.690	0.192–2.479				
	Mean MPA trough (mg/L)	<0.7	6			0.019			
		≥0.7	89	0.142	0.028–0.729				
EC-MPS (N=268)	Recipient age	<40	74			0.635			
		≥40	194	1.202	0.561–2.575				
	Donor age	<40	99			0.970			
		≥40	169	0.987	0.505–1.932				
	Recipient BMI	<21	89			0.039	0.463	0.240–0.893	0.022
		≥21	179	0.506	0.265–0.966				
	Donor BMI	<21	45			0.412			
		≥21	223	1.491	0.574–3.872				
	HLA-A,B,DR mismatch	>0	224	2.134	0.734–6.207	0.164			
	Induction with 3 days of thymoglobulin		48			0.772			
	Induction with basiliximab		220	0.878	0.364–2.120				
	Mean tacrolimus trough (ng/mL)	<5.0	52			0.226			
		≥5.0	216	1.799	0.695–4.654				
	Mean MPA trough (mg/L)	<0.7	11			0.009	0.208	0.072–0.602	0.004
		≥0.7	257	0.249	0.088–0.708				

BPAR – biopsy-proven acute rejection; MMF – mycophenolate mofetil; EC-MPS – enteric-coated mycophenolate sodium; BMI – body mass index; HLA – human leukocyte antigen; MPA – mycophenolic acid.

with higher risk of BPAR (HR=2.525, CI=1.179–5.407, p=0.017). In both groups, mean tacrolimus trough concentration did not yield any difference in BPAR-free survival (Table 5).

BPAR after 1 year post-KT

In patients receiving MMF (N=95), univariable Cox proportional hazard model showed that only mean MPA trough concentration ≥0.7 mg/L was associated with lower risk of BPAR (HR=0.142, CI=0.028–0.729, p=0.019). In patients receiving EC-MPS (N=268), multivariable Cox proportional hazard model showed that mean MPA trough concentration ≥0.7 mg/L was associated with lower risk of BPAR (HR=0.208, CI=0.072-0.602, p=0.004), while recipient BMI ≥21 kg/m^2 was associated with higher risk of BPAR (HR=0.463, CI=0.240–0.893, p=0.022). Mean tacrolimus trough concentration did not yield any difference in BPAR-free survival for both groups (Table 6).

Figures 2 and 3 shows the survival curves of patients receiving MMF and patients receiving EC-MPS, respectively, divided into 2 groups based on a mean MPA trough level of 1.7 mg/L between 1 month to 1 year and 0.7 mg/L after 1 year post-KT.

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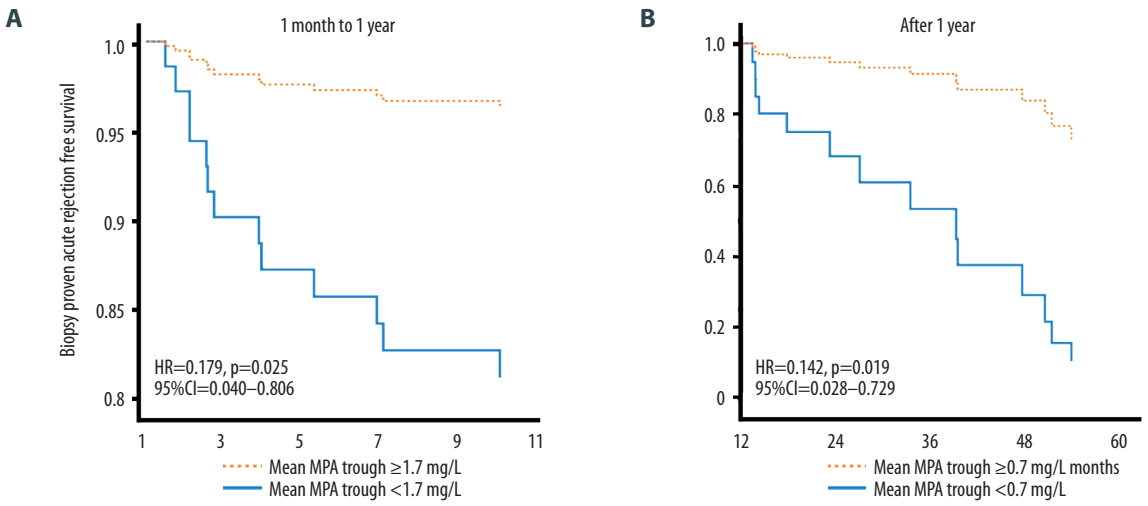

Figure 2. Univariable Cox analyses show that (**A**) patients receiving MMF with a mean MPA trough level under 1.7 mg/L demonstrate a significant risk of BPAR-free survival compared to patients with mean MPA trough level over 1.7 mg/L during 1 month to 1 year, and mean MPA trough level under 0.7 mg/L demonstrate a significant risk of BPAR-free survival compared to patients with mean MPA trough level over 0.7 mg/ (**B**) >1 year after kidney transplantation.

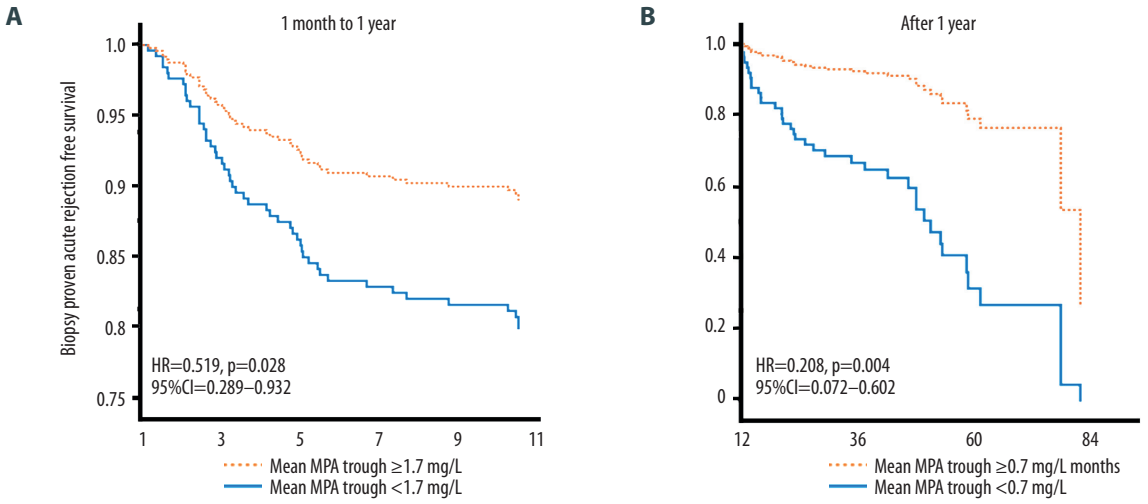

Figure 3. Multivariable Cox analyses show that patients receiving EC-MPS with a mean MPA trough level under 1.7 mg/L demonstrate a significant risk of BPAR-free survival compared to patients with mean MPA trough level over 1.7 mg/L during (**A**) 1 month to 1 year, and mean MPA trough level under 0.7 mg/L demonstrate a significant risk of BPAR-free survival compared to patients with mean MPA trough level over 0.7 mg/ (**B**) >1 year after kidney transplantation.

Discussion

This study shows the correlations between mean mycophenolate dosage and mean MPA trough concentrations and between mean MPA trough concentration and BPAR. Mycophenolate has become one of the most important drugs in KT. However, the optimal management for patients receiving mycophenolate has not yet been established [5,6]. Due to its high inter- and intra-patient variability, the need for MPA monitoring has been suggested by many transplant specialists. While AUC_{0-12} monitoring stands as the best monitoring measure [5,6], applying it in the real world is unrealistic for many clinicians. The effort to determine clinical significance with a limited sampling strategy or single point concentrations was intended to foster the use of this application and to provide quality management for the patients [8–11]. Although published studies with trough concentration monitoring showed conflicting results [16–21], our center started monitoring MPA trough concentrations in

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November 2006. Over the past 10 years, we have accumulated a great deal of data on MPA trough monitoring. Our study included a large number of patients and was designed as a multivariable study for each follow-up period, taking into account the influence of patient-related factors and immunosuppressants such as tacrolimus, basiliximab, and thymoglobulin.

Some institutions still use a fixed-dose regimen of mycophenolate, which was the regimen that was created when MMF was first utilized. Many studies have shown high variability of MPA concentrations during a fixed-dose regimen [3]. Although our study showed significant relationships between mean mycophenolate dosages and MPA concentrations, there are several obstacles to predicting clinical outcomes without monitoring blood levels. First, there were no significant relationships between the mean dosages and mean MPA trough concentrations during a 1 month follow-up period in patients receiving MMF and patients receiving EC-MPS (p=0.161 and p=0.861, respectively). Second, the coefficient changed with the follow-up period in patients receiving MMF and patients receiving EC-MPS (β=0.829 to 1.028 and β=1.548 to 1.488, respectively), indicating that the same dosage does not guarantee the same concentration at different time points. Finally, the relationship is rather weak in patients receiving MMF and patients receiving EC-MPS (r^2=0.106 to 0.125 and r^2=0.062 to 0.060 after 1 month post-KT, respectively), emphasizing the high variability. The changing relationship between dosages and MPA concentrations is likely to be related to the changes in clearance of MPA that occur over time [22].

Another interesting finding is that our results, calculated from South Korean patients, differed from other studies. The mean dosages of mycophenolate needed to reach the target level were relatively lower than in the previous studies with other ethnicities [8].

As shown in Table 2, mean tacrolimus and MPA trough levels differed significantly during each period, which is why we analyzed risk factors related to BPAR by 2 statistical methods over 3 different periods. During the first month, mean tacrolimus trough level ≥7.0 ng/mL in patients receiving MMF and patients receiving EC-MPS (OR=0.117, CI=0.060-0.524, p<0.001 and OR=0.258, CI=0.131–0.507, p<0.001, respectively) and a mean MPA trough level ≥1.7 mg/L in patients receiving MMF (OR=0.190, CI=0.040-0.896, p=0.036) and ≥2.1 mg/L in patients receiving EC-MPS (OR=0.507, CI=0.264–0.973, p=0.041) had a protective effect on BPAR. Induction with basiliximab was a risk factor compared to thymoglobulin (OR=11.524, CI=2.175–61.046, p=0.004) only in patients receiving MMF.

Between 1 month to 1 year post-KT, mean MPA trough level ≥1.7 mg/L was significantly related to better BPAR-free survival in patients receiving MMF (HR=0.179, CI=0.040–0.806, p=0.025) and patients receiving EC-MPS (HR=0.519, CI=0.289–0.932, p=0.028). Mean tacrolimus trough level ≥5.0 ng/mL was not a significant factor in patients receiving MMF (p=0.544) and patients receiving EC-MPS (p=0.660). Since only 7 patients (5.9%) receiving MMF and 6 patients (1.7%) receiving EC-MPS failed to achieve a target tacrolimus trough level of 5.0 ng/mL, it was challenging to obtain a significant relationship with clinical outcome.

After 1 year post-KT, a mean MPA trough concentration ≥0.7 mg/L (HR=0.142, CI=0.028–0.729, p=0.019) was the only factor related to BPAR-free survival in patients receiving MMF. During the same period, mean MPA trough concentration ≥0.7 mg/L (HR=0.208, CI=0.072–0.602, p=0.004) showed significant relationship to better BPAR-free survival in patients receiving EC-MPS.

The insignificance of mean tacrolimus trough concentration on BPAR-free survival was likely due to the fact that the vast majority of patients achieved the target level. Therefore, the mean MPA trough level is likely to be important in patients who achieve a proper mean tacrolimus trough level. Although our data showed the clinical significance of mean tacrolimus trough level only during the 1 month postoperative period, the importance of tacrolimus in maintenance cannot be ignored.

The mean MPA trough concentration of 0.7 mg/L may appear too low to apply as a safety level. However, the 0.7 mg/L was calculated in the period after 1 year post-KT, when patients are mostly stabilized and the goal was to keep the trough concentration over 1.0 mg/L. In fact, in patients receiving EC-MPS, mean MPA trough concentration ≥1.0 mg/L (HR=0.333, CI=0.150–0.740, p=0.007) and ≥1.3 mg/L (HR=0.465, CI=0.232–0.935, p=0.032) were also significantly related to better BPAR-free survival in a multivariable analysis (Supplementary data). Since we designed the study to divide the patients on the point where they showed the highest sensitivity and specificity, we selected 0.7 mg/L to be used as a dividing point for the long-term follow-up period.

The retrospective nature of our study was a limitation in controlling potential confounding factors. We tried to overcome the limitation by including only low-risk KT patients who underwent induction therapy with thymoglobulin or basiliximab and a triple immunosuppressive regimen with tacrolimus, mycophenolate, and corticosteroids. By excluding intermediate-to-high-risk patients who underwent desensitization, our finding can be only applicable to patients with low risk who underwent induction therapy and triple immunosuppression.

In addition, multivariable regression analysis was performed. Another limitation of this study is that MPA trough concentration can be variable, especially in EC-MPS [7,16–18]. However, practical issues still exist in monitoring the MPA exposure, and

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based on the results of our study, the clinical implication of MPA trough concentration of EC-MPS should be reconsidered.

The fact that we only used mean MPA trough concentration as a variable may be another weakness. Mean MPA level reflects the overall exposure to the drug, while instant exposures are less relevant. Some might criticize that low- or over-exposure is the one related with rejection or toxicities. However, proving that certain adverse effect in a certain period is related to a single cause is difficult. KT patients are influenced by numerous factors including different kind of drugs are prescribed simultaneously. On the other hand, mean trough concentrations, both tacrolimus and MPA, can be an indicator that the patient's immune was suppressed sufficiently or insufficiently. Therefore, we suggest that monitoring mycophenolate exposure by MPA trough concentration can potentially be helpful.

Clinical fields are all different throughout different countries. The cost for MPA trough concentration is not expensive in our country, while the cost can be the biggest obstacle in others. In these situations, we suggest that therapeutic drug monitoring can be of benefit in patients at high risk for rejection, or patients who are undergoing immunosuppressant change. Adjusting the drug dosage can be another point where MPA monitoring can be beneficial. Study derived from Opticept trial suggested that patients with high- and low-weight can be at risk of over- or underexposure unless doses are adjusted [23].

Conclusions

Mean mycophenolic acid trough concentration showed a significant relationship to acute cellular rejection in patients receiving mycophenolate mofetil and patients receiving enteric-coated mycophenolate sodium. Therefore, mycophenolic acid trough concentration monitoring has a potential benefit in avoiding acute rejection throughout the post-transplantation period in patients treated with a tacrolimus, mycophenolate, and methylprednisolone triple immunosuppressive regimen, and this requires further investigation for specifying the usage.

Conflict of interest

None.

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