Limited Sampling Strategy for the Estimation of Mycophenolic Acid and its Acyl Glucuronide Metabolite Area under the Concentration-Time Curve in Japanese Lung Transplant Recipients

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ABSTRACT - **Purpose:** The dose of mycophenolate mofetil (MMF) used to prevent rejection after lung transplantation is often adjusted based on the 12-hour area under the concentration-time curve (AUC_{0-12}) of mycophenolic acid (MPA). A limited sampling strategy (LSS) is useful to define the pharmacokinetic (PK) profiles of MPA and mycophenolic acid acyl glucuronide (AcMPAG). Therefore, this study aimed to design a LSS based on multiple linear regression for estimating the AUC_{0-12} of MPA and AcMPAG at the minimum blood sampling points in Japanese lung transplant patients with concomitant tacrolimus. **Methods:** Forty-five lung transplantation recipients were enrolled in a PK study of MPA, mycophenolic acid glucuronide (MPAG), and AcMPAG. The plasma MPA, MPAG, and AcMPAG concentrations were determined just before and at 0.5, 1, 2, 4, 8, and 12 hours after dosing. The AUC_{0-12} of MPA and AcMPAG was calculated using a linear trapezoidal rule from the plasma concentration of each blood sampling time. LSS was used to develop models for estimated AUC in the model group (n = 23) and was evaluated in the validation group (n = 22). **Results:** The best three time-point equation was $4.04 + 1.64 \cdot C_1 + 3.08 \cdot C_4 + 5.17 \cdot C_8$ for MPA, and $-0.13 + 3.01 \cdot C_1 + 3.51 \cdot C_4 + 5.74 \cdot C_8$ for AcMPAG. The prediction errors (PE) and the absolute prediction errors (APE) were within the clinically acceptable ± 5% and 15% range, respectively (MPA: PE = 2.00%, APE = 11.66%, AcMPAG: PE = 0.98%, APE = 14.69%). The percentage of estimated AUC_{0-12} within ± 15% of the observed AUC_{0-12} was 77.27% for MPA and 81.82% for AcMPAG. **Conclusion:** LSS using three time-point (C_1, C_4, and C_8) provides the most reliable and accurate simultaneous estimation of the AUC_{0-12} of MPA and AcMPAG in Japanese lung transplant patients.

INTRODUCTION

Mycophenolate mofetil (MMF) is rapidly hydrolyzed *in vivo* to the immunosuppressant mycophenolic acid (MPA), which reversibly inhibits inosine 5′-monophosphate dehydrogenase, an enzyme involved in the *de novo* synthesis of guanosine in lymphocytes (1, 2). Subsequently, MPA is predominantly metabolized to a pharmacologically inactive mycophenolic acid glucuronide (MPAG) and pharmacologically active mycophenolic acid acyl glucuronide (AcMPAG) by uridine diphosphate glucuronosyltransferase (3). On the other hand, MPAG is hydrolyzed back to MPA during enterohepatic recirculation, and its contribution to the total MPA exposure is approximately 40% (4).

MMF is administered in combination with a calcineurin inhibitor, such as tacrolimus or cyclosporine, and steroid to reduce the risk of rejection after lung transplantation (5). Several studies have reported that the 12-hour area under the concentration-time curve (AUC_{0-12}) of MPA is a useful pharmacokinetic parameter for predicting clinical efficacy and rejection (6, 7). Therefore, the dose of MMF is often adjusted based on the AUC_{0-12} of MPA.

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Recently, Zegarska et al. have been reported that AcMPAG concentrations in liver transplant recipients is related to the development of bacterial infection (8). In addition, Yoshimura et al. have been reported that the cutoff values of AcMPAG AUC_{0-24} for successful gastrointestinal acute graft-versus-host disease prevention in hematopoietic stem cell transplant patients were 15.6 μg·hr/mL (9). Accordingly, therapeutic drug monitoring (TDM) of AcMPAG is considered important in ensuring the safety and effectiveness of MMF treatment in both clinical practice and research.

The continuous measurement of MPA and AcMPAG AUC_{0-12} based on multiple blood sampling points increases the patient's burden. For this reason, a limited sampling strategy (LSS) estimating the AUC_{0-12} based on a limited number of blood samples is essential for defining the PK profiles of MPA and AcMPAG. However, LSS that simultaneously evaluates the AUC_{0-12} of MPA and AcMPAG has not been reported.

This study aimed to design a LSS based on multiple linear regression for estimating the AUC_{0-12} of MPA and AcMPAG at the minimum blood sampling points in Japanese lung transplant patients with concomitant tacrolimus.

MATERIALS AND METHODS

Patients

This study was a single-center prospective study, was performed in Tohoku University Hospital from December 2016 to December 2017. The inclusion criteria were as follows: age ≥18 years, after lung transplantation, use of MMF, and the ability and willingness to provide written informed consent. We did not set the exclusion criteria. The chronic diseases that lead to lung transplantation were lymphangioleiomyomatosis (n=16), interstitial pneumonia (n=9), pulmonary hypertension (n=7), bronchiectasis (n=3), Eisenmenger syndrome (n=3), pulmonary emphysema (n=2), diffuse panbronchiolitis (n=2), cystic fibrosis (n=1), and other pulmonary disease (n=2). Forty-five transplant recipients were enrolled in a study investigating the pharmacokinetics of MPA, MPAG, and AcMPAG. This study protocol was approved by the Ethics Committee of Tohoku University Graduate School of Medicine (approval number: 2017-1-096). All patients were provided written informed consent.

Immunosuppression regimen

All patients received MMF, tacrolimus, and prednisolone as a basic triple immunosuppressive regimen in lung transplantation. MMF (CellCept®; Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) was administered by intubation from the day 2 after surgery, and it was switched to oral administration after tube was withdrawn. MMF was administered at 1000 mg/day (body weight < 60 kg) or 1500 mg/day (body weight ≥ 60 kg). The dose of MMF was adjusted so that the white blood cell count was 4000 or more. The dose was reduced if abdominal symptoms were present. Tacrolimus was adjusted to maintain a target concentration of 10 to 14 ng/mL within 6 months after transplantation, 9 to 13 ng/mL from 7 months to 1 year after transplantation, and 8 to 10 ng/mL afterward. Prednisolone was orally administered at 1 mg/kg/day after transplantation, and then tapered to a fixed maintenance dose of 5 mg/day by 6 months after transplantation.

Assay of plasma MPA, MPAG, and AcMPAG concentrations

MPA was purchased from Sigma-Aldrich (Tokyo, Japan), MPAG, AcMPAG, and MPA-$^2H_3$ (internal standard, IS) were from Toronto Research Chemicals (Toronto, Ontario, Canada). The plasma concentrations of MPA, MPAG, and AcMPAG was measured as previously described, with modifications (10, 11). Briefly, blood collected in heparin tubes was centrifuged at 1580 × *g* for 10 minutes to separate the plasma. To stabilize AcMPAG, 10% acetic acid was added to 20 μL per milliliter of plasma (11). Thirty-microliters of acidified plasma were mixed with 30 μL of 1 μg/mL MPA-$^2H_3$ dissolved in acetonitrile for use as an internal standard substance. In addition, 120 μL of acetonitrile was added to denatured proteins and centrifuged at 15,000 × *g* for 5 minutes. To prepare a sample for injection, 200 μL of water was added to 100 μL of this supernatant. For LC/ESI-MS/MS analysis, a LCMS-8050 triple quadrupole mass spectrometer coupled with a Nexera X2 UHPLC system (Shimadzu, Kyoto, Japan) was used. Nexera X2 UHPLC consisted of a vacuum degasser, two solvent delivery systems, an autosampler, and a column oven. Chromatographic separation was achieved using an Inertsil C8-3 column (150 × 2.1 mm i.d., 3 μm, GL Sciences, Tokyo, Japan), which was maintained at 40°C. The mobile phase consisted of solution A (0.1% formic acid in water) and solution B (0.1% formic acid in acetonitrile), which formed the following gradient: 45% B (0–2 min); 45–70% B (2–5.4 min); 45% B (5.4–6.5 min). The flow rate of the mobile phase was 0.3 mL/min. The

LCMS-8050 was equipped with an electrospray ionization source operating in positive and negative ion detection mode. During selected reaction monitoring, the *m/z* transitions 321.25→303.25, 495.15→319.15, 495.15→319.15, and 324.30→306.30 monitored MPA, MPAG, AcMPAG, and MPA-$^2H_3$, respectively. Three controls (0.15, 1.5, 15 µg/mL for MPA, 1.2, 12, 120 µg/mL for MPAG, and 0.03, 0.3, 3 µg/mL for AcMPAG, respectively) were used for quality control. Linearity was achieved with a correlation coefficient (R^2) > 0.995 (0.1 to 20 µg/mL for MPA, 0.8 to 160 µg/mL for MPAG, and 0.02 to 4 µg/mL for AcMPAG, respectively). The intra- and inter-day precisions were less than 14.2%, and accuracy was ± 11.2%.

Pharmacokinetic analyses and AUC_{0-12} calculation

The plasma concentrations of MPA, MPAG, and AcMPAG were determined just prior to dosing and 0.5, 1, 2, 4, 8, and 12 hours after administration. The pharmacokinetic parameters and AUC_{0-12} of MPA, MPAG, and AcMPAG were analyzed by a non-compartmental model from the plasma concentration of each blood sampling time (Phoenix WinNonlin software Version 7.0 [Certara USA, Inc., Princeton, NJ, USA]).

STATISTICAL ANALYSIS

According to the previous report (12-14), the patients were randomly assigned to two groups at a 1: 1 ratio: the model group (n = 23) and the validation group (n = 22). An AUC_{0-12} prediction formula was derived using multiple regression analysis with the model group AUC_{0-12} as a dependent variable and the plasma concentration at each blood sampling time as an explanatory variable. A maximum of three concentrations were used for the clinically convenient limited sampling strategy. In the validation group, the predictive performance of the LSS was analyzed with linear regression, correlation coefficient (*r*), prediction error (PE) (%), absolute prediction error (APE) (%), and the percentage of estimated AUC within ± 15% of the observed AUC as previous reported (15). The two error parameters were calculated using the following equations:

$$\text{PE (\%)} = \frac{100}{n} \cdot \frac{\Sigma(AUC_{predicted} - AUC_{observed})}{AUC_{observed}}$$

$$\text{APE (\%)} = \frac{100}{n} \cdot \frac{\Sigma(|AUC_{predicted} - AUC_{observed}|)}{AUC_{observed}},$$

where n is the number of patients. According to the previous report (15-17), the acceptable percentage limits of PE and APE were defined to be ± 5% and 15%, respectively. The Bland–Altman test was used to evaluate the agreement between the observed and estimated AUC, and the fixed range was defined as the mean ± 1.96SD. Continuous variables (expressed as mean ± SD) were compared using the *t* test, and categorical variables were compared using the Fisher's exact test. The significance level was set at $P < 0.05$. We used SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA) for statistical analysis.

RESULTS

Forty-five patients (18 males and 27 females) were included in this study and the patients were randomly assigned to two groups at a 1: 1 ratio: the model group (n = 23) and the validation group (n = 22). Patient characteristics are summarized in Table 1. The mean age was 44.4 ± 11.6 years and the mean body weight was 51.5 ± 11.6 kg. There were no significant differences in sex, laboratory test results, concomitant medications affecting the pharmacokinetics of MPA (proton pump inhibitor, magnesium oxide, ciprofloxacin), MMF dose, pharmacokinetic parameters of MPA and metabolites, tacrolimus dose, or the post-transplant period between the model group and the validation group.

The mean plasma concentration-time profiles of MPA, MPAG, and AcMPAG in the model and validation groups are shown in Figure 1. These curves followed a similar tendency, whereby the peak was reached after 2 hours for MPA and 4 hours for MPAG and AcMPAG. Among these compounds, MPA and AcMPAG are related to clinical outcomes; therefore, we focused on both MPA and AcMPAG.

Table 1. Baseline characteristics of patients

	All Patients (n = 45)	Model Group (n = 23)	Validation Group (n = 22)	*P*
Age (yr)	44.4 ± 11.6	45.5 ± 11.6	43.1 ± 11.8	0.497
Sex (Male/Female)	18/27	8/15	10/12	0.236
Body weight (kg)	51.5 ± 11.6	53.2 ± 12.6	49.6 ± 10.3	0.298
Serum creatinine (mg/dL)	1.01 ± 0.34	1.03 ± 0.40	0.99 ± 0.27	0.684
Blood urea nitrogen (mg/dL)	19.4 ± 7.0	19.3 ± 7.8	19.5 ± 6.2	0.927
Estimated glomerular filtration rate (mL/min/1.73 m^2)	60.9 ± 23.2	58.5 ± 20.5	63.4 ± 26.0	0.487
γ-Glutamyltransferase (U/L)	31.3 ± 18.3	28.9 ± 21.8	33.8 ± 13.8	0.371
Alanine aminotransferase (U/L)	12.9 ± 7.1	11.3 ± 5.0	14.6 ± 8.6	0.128
Aspartate aminotransferase (U/L)	16.6 ± 4.4	15.7 ± 2.9	17.6 ± 5.4	0.137
Total protein (mg/dL)	6.29 ± 0.51	6.27 ± 0.55	6.31 ± 0.48	0.779
Serum albumin (mg/dL)	3.96 ± 0.36	3.97 ± 0.39	3.95 ± 0.33	0.891
White blood cell count ($\times 10^3/\mu L$)	6.38 ± 2.14	6.23 ± 2.17	6.54 ± 2.16	0.633
Neutrophil count ($\times 10^3/\mu L$)	4.30 ± 1.86	4.16 ± 1.89	4.45 ± 1.86	0.602
Concomitant medications				
Proton pump inhibitor	43	22	21	1.000
Magnesium oxide	11	5	6	1.000
Ciprofloxacin	1	1	0	1.000
MMF dose (range) (mg/day)	789 ± 402 (250-1500)	826 ± 415 (250-1500)	750 ± 393 (250-1500)	0.532
MPA AUC_{0-12} (μg·hr/mL)	32.2 ± 11.8	32.8 ± 13.0	31.5 ± 10.6	0.713
Dose-normalized MPA AUC_{0-12} [(μg·hr/mL)/g]	51.8 ± 35.6	43.1 ± 13.4	60.9 ± 48.5	0.098
MPA T_{max} (hr)	2.53 ± 2.00	2.09 ± 1.90	3.00 ± 2.05	0.128
MPA C_{max} (μg/mL)	6.98 ± 3.50	7.03 ± 3.76	6.94 ± 3.29	0.931
MPAG AUC_{0-12} (μg·hr /mL)	404 ± 288	457 ± 349	350 ± 199	0.210
Dose-normalized MPAG AUC_{0-12} [(μg·hr/mL)/g]	617 ± 516	556 ± 259	681 ± 692	0.423
MPAG T_{max} (hr)	3.53 ± 1.87	3.13 ± 1.29	3.95 ± 2.28	0.147
MPAG C_{max} (μg/mL)	49.7 ± 34.0	53.4 ± 39.3	45.9 ± 27.8	0.469
AcMPAG AUC_{0-12} (μg·hr/mL)	3.23 ± 2.60	3.32 ± 2.56	3.14 ± 2.69	0.819
Dose-normalized AcMPAG AUC_{0-12} [(μg·hr/mL)/g]	5.78 ± 8.41	4.39 ± 3.34	7.23 ± 11.50	0.262
AcMPAG T_{max} (hr)	3.41 ± 2.21	3.11 ± 1.66	3.73 ± 2.68	0.355
AcMPAG C_{max} (μg/mL)	0.55 ± 0.39	0.55 ± 0.39	0.54 ± 0.41	0.987
Tacrolimus dose (mg/day)	2.81 ± 1.74	3.04 ± 1.63	2.56 ± 1.85	0.356
Tacrolimus trough concentration (ng/mL)	9.12 ± 2.07	9.41 ± 2.35	8.82 ± 1.73	0.345
Post-transplant period (day)	1460 ± 934	1404 ± 983	1518 ± 899	0.686

Continuous variables (expressed as mean ± SD) were compared using the *t* test, and categorical variables were compared using the Fisher's exact test. MMF: mycophenolate mofetil, MPA: mycophenolic acid, MPAG: mycophenolic acid glucuronide, AcMPAG: mycophenolic acid acyl glucuronide.

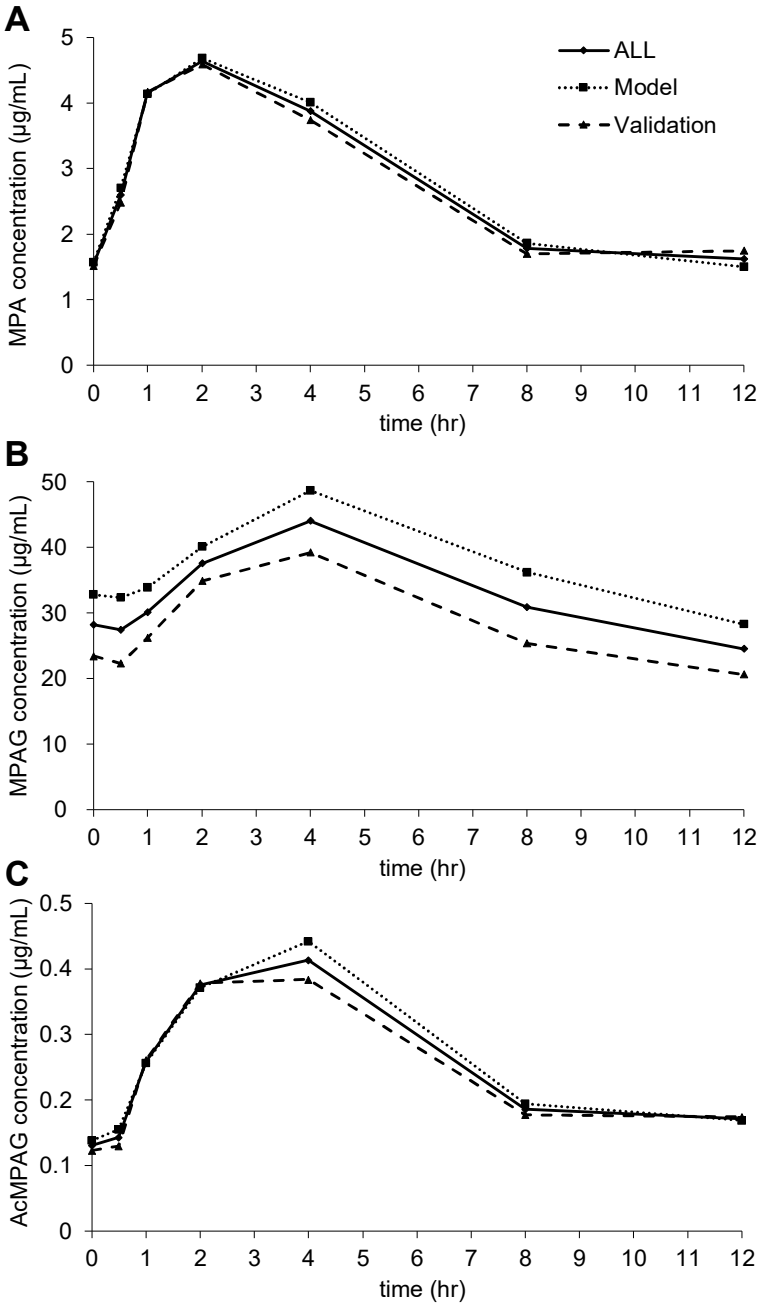

Figure 1. Mycophenolic acid (MPA) (A), mycophenolic acid glucuronide (MPAG) (B), and mycophenolic acid acyl glucuronide (AcMPAG) (C) concentration-time profiles.

Multiple linear regression analyses of the MPA and AcMPAG $AUC_{0–12}$ are shown in Table 2. The highest correlation coefficient between MPA AUC_{0-12} and plasma MPA concentrations was at C_1, C_4, and C_8 for three time points (MPA AUC_{0-12} = 4.04 + 1.64·C_1 + 3.08·C_4 + 5.17·C_8, r = 0.923, P < 0.001). Conversely, the highest correlation coefficient between AcMPAG AUC_{0-12} and plasma AcMPAG concentrations was obtained at three time points, C_2, C_4, and C_{12} (AcMPAG AUC_{0-12} = 0.28 + 1.96·C_2 + 3.44·C_4 + 4.64·C_{12}, r = 0.990, P < 0.001)

Table 2. Predictive performance of three concentration limited sampling strategies developed for MPA and AcMPAG

Sampling time (hr)	Equation	*r*	*P*	PE (%)	APE (%)	Within ± 15%*
		MPA				
1,4,8	$4.04 + 1.64 \cdot C_1 + 3.08 \cdot C_4 + 5.17 \cdot C_8$	0.923	<0.001	2.00	11.66	77.27
2,4,8	$4.42 + 1.64 \cdot C_2 + 2.86 \cdot C_4 + 4.96 \cdot C_8$	0.895	<0.001	0.82	11.27	68.18
2,4,12	$6.40 + 1.70 \cdot C_2 + 2.92 \cdot C_4 + 4.48 \cdot C_{12}$	0.885	<0.001	6.61	14.86	59.09
1,4,12	$5.32 + 1.76 \cdot C_1 + 2.98 \cdot C_4 + 5.49 \cdot C_{12}$	0.843	<0.001	8.47	17.43	54.55
0.5,2,12	$10.82 + 0.68 \cdot C_{0.5} + 1.37 \cdot C_2 + 9.12 \cdot C_{12}$	0.831	<0.001	12.20	18.17	54.55
0,2,12	$9.54 + 3.65 \cdot C_0 + 1.45 \cdot C_2 + 7.17 \cdot C_{12}$	0.829	<0.001	11.29	18.16	45.45
0,2,4	$4.91 + 3.06 \cdot C_0 + 2.03 \cdot C_2 + 3.39 \cdot C_4$	0.820	<0.001	2.48	16.35	63.64
0.5,4,12	$11.97 + 0.46 \cdot C_{0.5} + 2.14 \cdot C_4 + 7.34 \cdot C_{12}$	0.809	<0.001	11.75	20.36	50.00
0.5,2,4	$5.12 + 0.68 \cdot C_{0.5} + 2.11 \cdot C_2 + 3.98 \cdot C_4$	0.796	<0.001	1.36	17.61	54.55
0,4,12	$12.82 + 0.86 \cdot C_0 + 2.05 \cdot C_4 + 6.93 \cdot C_{12}$	0.788	<0.001	12.32	21.11	45.45
1,2,12	$11.35 + 0.8 \cdot C_1 + 0.97 \cdot C_2 + 9.08 \cdot C_{12}$	0.785	<0.001	13.87	20.06	54.55
		AcMPAG				
2,4,12	$0.28 + 1.96 \cdot C_2 + 3.44 \cdot C_4 + 4.64 \cdot C_{12}$	0.990	<0.001	7.38	14.84	68.18
1,4,12	$0.12 + 2.43 \cdot C_1 + 3.89 \cdot C_4 + 5.03 \cdot C_{12}$	0.976	<0.001	6.89	17.50	59.09
0,4,12	$0.26 + 1.70 \cdot C_0 + 3.62 \cdot C_4 + 7.19 \cdot C_{12}$	0.975	<0.001	2.47	22.10	45.45
0.5,4,12	$0.23 + 0.98 \cdot C_{0.5} + 3.86 \cdot C_4 + 7.23 \cdot C_{12}$	0.975	<0.001	1.42	20.55	45.45
2,4,8	$0.09 + 2.24 \cdot C_2 + 3.07 \cdot C_4 + 5.27 \cdot C_8$	0.974	<0.001	1.53	12.81	72.73
0,2,4	$-0.12 + 5.84 \cdot C_0 + 2.76 \cdot C_2 + 3.60 \cdot C_4$	0.969	<0.001	0.81	19.64	59.09
2,8,12	$0.59 + 2.13 \cdot C_2 + 6.51 \cdot C_8 + 3.92 \cdot C_{12}$	0.966	<0.001	14.84	25.23	45.45
4,8,12	$0.24 + 3.15 \cdot C_4 + 4.12 \cdot C_8 + 5.21 \cdot C_{12}$	0.965	<0.001	0.50	21.61	45.45
0.5,2,4	$-0.12 + 2.53 \cdot C_{0.5} + 2.80 \cdot C_2 + 4.53 \cdot C_4$	0.959	<0.001	-0.31	21.45	63.64
1,2,4	$0.06 + 1.59 \cdot C_1 + 2.08 \cdot C_2 + 4.67 \cdot C_4$	0.956	<0.001	6.42	25.97	59.09
1,4,8	$-0.13 + 3.01 \cdot C_1 + 3.51 \cdot C_4 + 5.74 \cdot C_8$	0.955	<0.001	0.96	14.87	77.27

*Percentage of estimated AUC within ± 15% of the observed AUC. MPA: mycophenolic acid, AcMPAG: mycophenolic acid acyl glucuronide, PE: prediction error, APE: absolute prediction error.

Considering the combination of the three time-point models, it was two models (C_1, C_4, C_8 and C_2, C_4, C_8) that PE and APE were clinically acceptable within ± 5% and 15%, respectively.

Moreover, the percentage of estimated $AUC_{0\text{-}12}$ within ± 15% of the observed $AUC_{0\text{-}12}$ was higher in model (C_1, C_4, C_8) than in model (C_2, C_4, C_8). Therefore, the LSS of MPA and AcMPAG with the most convenient and conventional sampling times were for C_1, C_4, and C_8 (MPA $AUC_{0\text{-}12} = 4.04 + 1.64 \cdot C_1 + 3.08 \cdot C_4 + 5.17 \cdot C_8$, AcMPAG $AUC_{0\text{-}12} = -0.13 + 3.01 \cdot C_1 + 3.51 \cdot C_4 + 5.74 \cdot C_8$). In the

validation group, the PE and APE of this models were within the clinically acceptable range (MPA: PE = 2.00%, APE = 11.66%, AcMPAG: PE = 0.98%, APE = 14.69%). The percentage of estimated AUC within ± 15% of the observed AUC was 77.27% for MPA and 81.82% for AcMPAG. The correlation between the measured and estimated $AUC_{0\text{-}12}$ of MPA and AcMPAG for LSS in Figure 2 also suggested a good estimation (MPA: $r = 0.923$, $P < 0.001$, AcMPAG: $r = 0.955$, $P < 0.001$). The Bland–Altman analysis revealed a mean bias of 2.00% for MPA $AUC_{0\text{-}12}$ and 0.98% for AcMPAG. Only three plotted differences exceeded the fixed range of the mean ± 1.96 SD in the estimation using C_1, C_4, and C_8 (Figure 3).

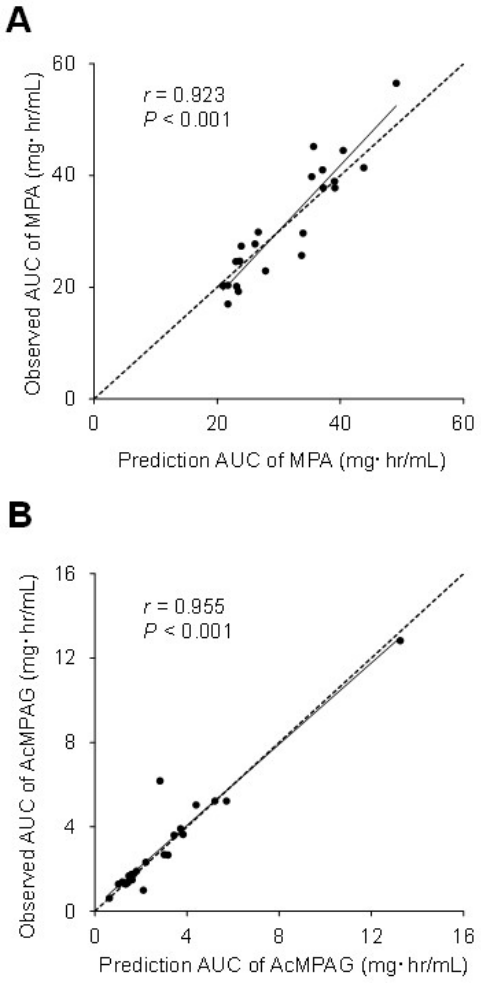

Figure 2. Correlation between the measured and estimated $AUC_{0\text{-}12}$ of MPA (A) and AcMPAG (B) using three sampling points (C_1, C_4, and C_8) for limited sampling strategies (MPA $AUC_{0\text{-}12} = 4.04 + 1.64 \cdot C_1 + 3.08 \cdot C_4 + 5.17 \cdot C_8$, AcMPAG $AUC_{0\text{-}12} = -0.13 + 3.01 \cdot C_1 + 3.51 \cdot C_4 + 5.74 \cdot C_8$).

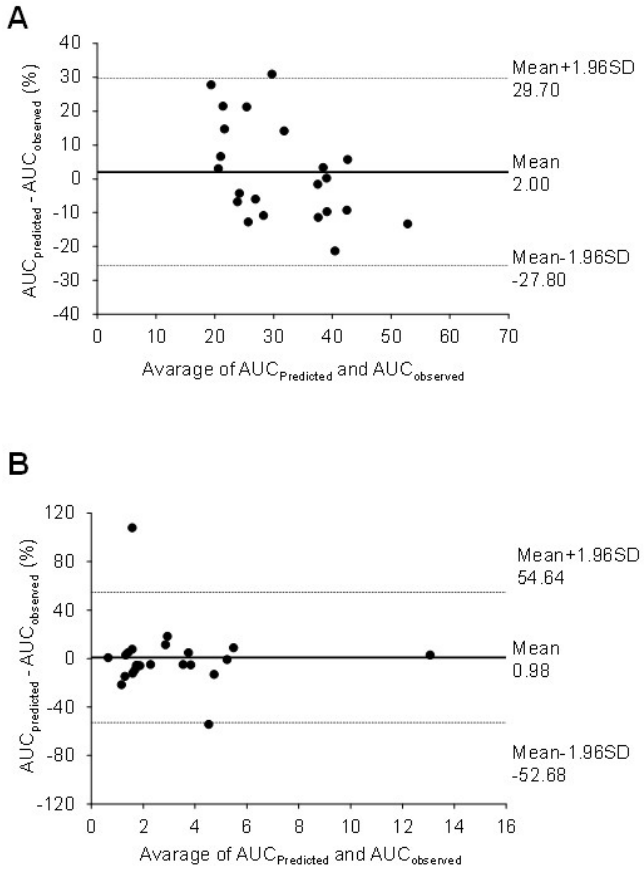

Figure 3. Bland–Altman plot for the validation group of MPA (A) and AcMPAG (B) using the three time-point (C_1, C_4, and C_8) equation (MPA $AUC_{0\text{-}12} = 4.04 + 1.64 \cdot C_1 + 3.08 \cdot C_4 + 5.17 \cdot C_8$, AcMPAG $AUC_{0\text{-}12} = -0.13 + 3.01 \cdot C_1 + 3.51 \cdot C_4 + 5.74 \cdot C_8$). The line represents the mean bias and the dashed lines represent ± 1.96 standard deviation (SD) of the mean bias.

When the trough concentration was included at blood sampling points within 4 hours, the blood sampling points with the greatest correlations were (C_0, C_2, C_4) (MPA $AUC_{0\text{-}12} = 4.91 + 3.06 \cdot C_0 + 2.03 \cdot C_2 + 3.39 \cdot C_4$, $r = 0.820$, $P < 0.001$, AcMPAG $AUC_{0\text{-}12} = -0.12 + 5.84 \cdot C_0 + 2.76 \cdot C_2 + 3.60 \cdot C_4$ $r = 0.955$, $P < 0.001$). The PE of this models was within the clinically acceptable range (MPA: 2.43%, AcMPAG: 0.98%), but the APE was exceeded 15% (MPA: 16.32%, AcMPAG: 19.87%).

DISCUSSION

In this study, we showed that the $AUC_{0\text{-}12}$ can be predicted with high accuracy by LSS using plasma MPA and AcMPAG concentrations following MMF administration in Japanese lung transplantation

patients.

Both immunoassays and chromatographic methods are available for therapeutic drug monitoring of MPA. Although immunoassays are widely used in clinical laboratories due to ease of adopting such methods on automated analyzers, immunoassay such as a particle-enhanced turbidimetric inhibition immunoassay (PETINIA) shows cross-reactivity with AcMPAG. In fact, we have revealed an average positive bias of 26.3% in the PETINIA compared to that with LC-MS/MS in lung transplant patients (18). Furthermore, MPAG and AcMPAG can only be measured by LC-MS/MS. Therefore, plasma MPA, MPAG, and AcMPAG concentrations were quantified by LC-MS/MS in this study.

The pharmacokinetics of MPA and its metabolites are reported to be affected by the administration of concomitant drugs, and differ between lung and heart transplant patients (19-23). Therefore, the development of the LSS of MPA and AcMPAG should consider concomitant medication as well as the transplanted organ. Proton pump inhibitor or magnesium oxide reduces the solubility of MMF and decreases the drug exposure of mycophenolic acid (24, 25). In addition, ciprofloxacin reduces plasma MPA concentration because of noncompetitive inhibition of deconjugation of MPAG by intestinal β-glucuronidase (26). However, there were no significant differences in baseline characteristics of patients including concomitant drugs between the model group and the validation group. The mean MPA, MPAG, and AcMPAG concentration–time profiles of all patients, the model group, and the validation group followed the same trend.

The primary enzymes involved in MPA glucuronidation are uridine-diphosphate glucuronosyltransferase (UGT) 1A9, 2B7, 1A8, and 1A7 (27). In addition, UGT1A9 -275T>A and -2152C>T polymorphism that affect the pharmacokinetics of MPA have been reported in Caucasians (28). Nevertheless, maximum MPA concentration, time to reach the maximum MPA concentration, AUC ratio MPAG/MPA, and AUC ratio AcMPAG/MPA were similar to those previously reported (29).

Considering the correlation coefficient, PE, APE, and the percentage of estimated AUC within ± 15% of the observed AUC, the most convenient and conventional sampling times were obtained with the three time-point (C_1, C_4, and C_8) equations. Therefore, the three time-point (C_1, C_4, and C_8) equations were developed (MPA $AUC_{0\text{-}12}$ = 4.04 + $1.64 \cdot C_1 + 3.08 \cdot C_4 + 5.17 \cdot C_8$, $r = 0.923$, $P < 0.001$, AcMPAG $AUC_{0\text{-}12}$ = -0.13 + $3.01 \cdot C_1 + 3.51 \cdot C_4 + 5.74 \cdot C_8$, $r = 0.955$, $P < 0.001$). Compared to previous reports (30, 31), the three time-point equations developed in this study have a relatively better predictive performance (MPA: PE = 2.00%, APE = 11.66%, AcMPAG: PE = 0.98%, APE = 14.69%). Moreover, about 80% of the estimated AUC was within ± 15% of the observed AUC.

LSS in lung transplantation patients was previously reported by Ting et al (12). Those authors developed LSS by two time points (C_0 and C_2) in the same number of patients for cyclosporine and tacrolimus in combination; however, the correlation coefficient of the two time points (C_0 and C_2) was 0.699 in this study, and a good correlation was not observed (data not shown). Cyclosporine inhibits the biliary transporter ATP-binding cassette, subfamily C, member 2 (ABCC2), resulting in reduced enterohepatic re-circulation of MPAG/MPA (18-20), but tacrolimus has no such effect. For this reason, pharmacokinetics of MPA, MPAG, and AcMPAG are significantly different between cyclosporine and tacrolimus (29). All patients received tacrolimus, suggesting that the influence of C_8 or C_{12} derived from the enterohepatic re-circulation of MPAG/MPA was greater than that reported by Ting et al (2006). Tacrolimus is used more commonly after lung transplantation due to its effect at reducing the risk of bronchiolitis obliterans syndrome and low levels of rejection as well as control of persistent rejection (32, 33). Therefore, the formula used here to estimate $AUC_{0\text{-}12}$ may be useful to adjust the dose of MMF in patients treated with tacrolimus after lung transplantation.

Blood sampling 8 hours after MMF administration is not practical because it restrains outpatients' activities for a long time. The same multiple regression method was performed with the variables within 2 hours post-dose (C_0, $C_{0.5}$, C_1, and C_2), but the correlation coefficient of the three time points was not sufficient (data not shown). When the trough concentration is included at blood sampling points within 4 hours, there was a good correlation with the three time-point equation (C_0, C_2, and C_4) (MPA: $r = 0.820$, $P < 0.001$, AcMPAG: $r = 0.969$, $P < 0.001$). However, the APE exceeded 15%, and should therefore be carefully evaluated. Recently, we developed a LC-MS/MS method for the quantification of MPA, MPAG, and AcMPAG in dried blood spot samples (10). The dried blood spot method makes it possible to collect blood sample 8 hours after MMF administration without restraining

the outpatients. Therefore, application of the dried blood spot method to outpatients may permit TDM using three-point blood sampling (C_1, C_4, and C_8).

This study has some limitations that should be considered. All cases were Japanese lung transplant patients taking tacrolimus, and it is not evaluated by other races. In additon, no collected datapoint between 4 and 8 hours after MMF intake potentially related to the enterohepatic re-circulation of MPAG/MPA was not collected. Moreover, the PK profiles were obtained at approximately 4 years post-transplant, and genetic polymorphism for MPA and AcMPAG metabolism were not analyzed. We also need to consider using population PK modeling to make AUC estimation based on limited sampling more robust and enhance its prediction performance.

CONCLUSION

We established a formula to estimate the $AUC_{0\text{-}12}$ of MPA and AcMPAG by LSS in Japanese lung transplant patients with concomitant tacrolimus. The best three time-point equation was $4.04 + 1.64 \cdot C_1 + 3.08 \cdot C_4 + 5.17 \cdot C_8$ for MPA, and $-0.13 + 3.01 \cdot C_1 + 3.51 \cdot C_4 + 5.74 \cdot C_8$ for AcMPAG. It could be a useful tool to utilized for clinical practice and research.

CONFLICT OF INTEREST

No conflicts of interest to disclose.

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ABBREVIATIONS

MMF: mycophenolate mofetil
MPA: mycophenolic acid
MPAG: mycophenolic acid glucuronide
AcMPAG: mycophenolic acid acyl glucuronide
$AUC_{0\text{-}12}$: 12-hour area under the concentration-time curve
TDM: therapeutic drug monitoring
LSS: limited sampling strategy
PE: prediction error
APE: absolute prediction error
UGT: uridine-diphosphate glucuronosyltransferase

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