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Estimation of Mycophenolic Acid Area Under the Curve With Limited-Sampling Strategy in Chinese Renal Transplant Recipients Receiving Enteric-Coated Mycophenolate Sodium

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Background: The enteric-coated mycophenolate sodium (EC-MPS), whose active constituent is mycophenolic acid (MPA), has been widely clinically used for organ transplant recipients. However, its absorption is delayed due to its special designed dosage form, which results in difficulty to monitor the exposure of the MPA in patients receiving the EC-MPS. This study was aimed at developing a relatively practical and precise model with limited sampling strategy to estimate the 12-hour area under the concentration–time curve ($AUC_{0-12\ h}$) of MPA for Chinese renal transplant recipients receiving EC-MPS.

Methods: A total of 36 Chinese renal transplant recipients receiving the EC-MPS and tacrolimus were recruited in this study. The time point was 2 weeks after the transplantation for all the patients. The MPA concentrations were measured with enzyme-multiplied immunoassay technique for 11 blood specimens collected predose and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 hours after the morning dose of EC-MPS. The measured AUC was calculated with these 11 points of MPA concentrations with the linear trapezoidal rule. Limited sampling strategy was used to develop models for estimated AUC in the model group (n = 18). The bias and precision of different models were evaluated in the validation group (n = 18).

Results: C_4 showed the strongest correlation with the measured AUC. The best 3 time point equation was $6.629 + 8.029 \times C_0 + 0.592 \times C_3 + 1.786 \times C_4$ ($R^2 = 0.910$; $P < 0.001$), whereas the best 4 time point equation was $3.132 + 5.337 \times C_0 + 0.735 \times C_3 + 1.783 \times C_4 + 3.065 \times C_8$ ($R^2 = 0.959$; $P < 0.001$). When evaluated in the validation group, the 4 time point model had a much better performance than the 3 time point model: for the 4 time point model: $R^2 = 0.873$, bias = 0.505 [95% confidence interval (CI), −10.159 to 11.170], precision = 13.370 (95% CI, 5.186–21.555), and 77.8% of estimated AUCs was within 85%–115% of the measured AUCs; for the 3 time point model: $R^2 = 0.573$, bias = 6.196 (95% CI, −10.627 to 23.018), precision = 21.286 (95% CI, 8.079–34.492), and 50.0% of estimated AUCs was within 85%–115% of the measured AUCs.

Conclusions: It demanded at least 4 time points to develop a relatively reliable model to estimate the exposure of MPA in renal transplant recipients receiving the EC-MPS. The long time span needed restricted its application, especially for the outpatients, but it could be a useful tool to guide the personalized prescription for the inpatients.

Key Words: enteric-coated mycophenolate sodium, mycophenolic acid, pharmacokinetics, limited sampling strategy, renal transplantation

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INTRODUCTION

Mycophenolic acid (MPA), the active constituent of mycophenolate mofetil (MMF) and enteric-coated mycophenolate sodium (EC-MPS), plays an important role in the triple immunosuppressant regimen in renal transplant recipients.[1] MMF has an excellent short- and long-term efficacy, which has been clinically proven for years.[2] However, for some patients, MMF would cause unfavorable side effects like gastrointestinal complications, which might lead to the early dose reduction and even discontinuation of MMF. As a result, the very low MPA exposure would increase the risk of rejection and graft loss.[3] It was demonstrated that 720 mg EC-MPS delivered bioequivalent mean MPA exposure compared with 1000 mg

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Y. Jia, M. Xu, and T. Zhu conceived and designed the study. B. Peng analyzed the data and wrote the draft. Y. Jia revised the manuscript. L. Li, J. Wang, X. Wang, G. Qi, R. Rong, L. Wang, and J. Qiu provided the patients' data. All authors read and approved the final manuscript.

The authors declare no conflict of interest.

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TABLE 1. The Baseline Characteristics of the Patients

	All Patients, N = 36	Model Group, n = 18	Validation Group, n = 18	*P*
Age, yr	39.00 ± 11.33	37.39 ± 12.13	40.61 ± 10.58	0.402
Sex				0.423
Male	28	15	13	
Female	8	3	5	
Body weight, kg	59.34 ± 9.44	62.06 ± 10.09	61.77 ± 11.59	0.938
Serum creatinine, μmol/L	172.74 ± 140.55	179.89 ± 187.85	166.30 ± 89.70	0.840
Estimated glomerular filtration rate, mL/min	62.60 ± 29.73	68.80 ± 35.21	57.02 ± 24.33	0.404
Blood urea nitrogen, mmol/L	11.61 ± 8.00	10.37 ± 8.60	12.72 ± 7.01	0.538
Alanine aminotransferase, IU/L	63.74 ± 55.64	40.56 ± 38.80	84.60 ± 61.91	0.084
Aspartate aminotransferase, IU/L	25.21 ± 11.84	23.00 ± 12.14	27.20 ± 11.85	0.456
Serum albumin, g/L	38.37 ± 3.13	38.78 ± 3.07	38.80 ± 3.30	0.603
Fasting glucose, mmol/L	5.03 ± 1.26	4.67 ± 1.10	5.35 ± 1.36	0.249
Tacrolimus doses, mg/d	6.57 ± 1.31	6.69 ± 1.26	6.44 ± 1.38	0.575
Tacrolimus trough concentration, ng/mL	7.17 ± 2.34	7.47 ± 2.69	6.91 ± 2.08	0.618
EC-MPS doses, mg/q12 h	600.00 ± 121.70	600.00 ± 123.48	600.00 ± 123.48	1.000
MPA-$AUC_{0-12\ h}$, $mg \cdot h \cdot L^{-1}$	43.04 ± 16.56	45.94 ± 19.10	40.15 ± 13.48	0.301
MPA-C_{max}, mg/L	10.71 ± 7.83	11.72 ± 9.71	9.70 ± 5.44	0.446
MPA-T_{max}, h	4.82 ± 2.78	4.75 ± 2.58	4.89 ± 3.04	0.883

All values are expressed as mean ± SD except for sex.
AUC, area under the curve.

MMF.[4,5] Due to the specially designed dosage form, EC-MPS delays the release of MPA in the small intestine, reducing the gastrointestinal burden and alleviating the gastrointestinal complications, and thus, it might lower the risk of rejection and graft loss because of the insufficient MPA exposure.[6–9]

The 12-hour area under the concentration–time curve ($AUC_{0-12\ h}$) of MPA is regarded as the best parameter to reflect the exposure of the drug.[10] The $AUC_{0-12\ h}$ is tightly related to the clinical outcomes after the transplantation and the risk of side effects, and that is why, therapeutic drug monitoring (TDM) is needed.[11] A range between 30 and 60 $mg \cdot h \cdot L^{-1}$ of $AUC_{0-12\ h}$ has been recommended for renal transplant recipients, and the drug dosage should be adjusted through TDM to achieve the best clinical outcomes.[12–14] However, more than 10 samples at different time points over 12 hours are needed to calculate the accurate $AUC_{0-12\ h}$ with the regular method and that would be impractical for both patients and staff of the hospital.[15] Therefore, limited sampling strategy (LSS) is a relatively feasible and practicable method for TDM of MPA.

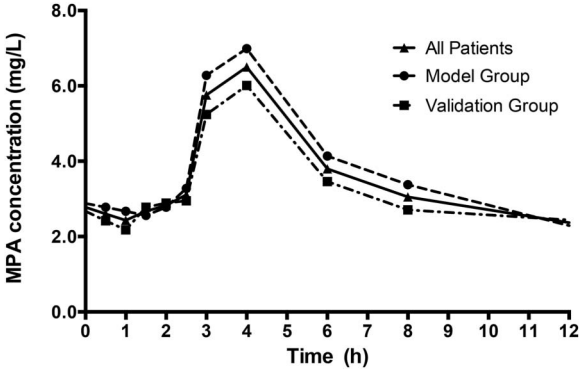

FIGURE 1. The mean MPA concentration–time profiles of all patients, model group, and validation group.

In the past decades, it has been proven that LSS could provide a good estimation of the MPA $AUC_{0-12\ h}$ for MMF, and this technique has been applied in many transplant centers.[16] But when it comes to the EC-MPS, its special dosage form causes a more unpredictable absorption profile than MMF, and the huge variability makes the difficulty for drug concentration monitoring.[17] Although several studies investigated the application of LSS for EC-MPS, there was still a paucity of data, especially for the Chinese population.[9,14–16,18–22]

The development of a model that balances precision and practicability to estimate the MPA exposure of EC-MPS is highly clinically appreciated. This study is aimed to search for the best equation with the least time points based on the profiles of Chinese renal transplant recipients receiving EC-MPS and tacrolimus.

METHODS AND MATERIALS

Patients

A total of 36 renal transplant patients were recruited for this study from 3 transplant centers: 19 patients from Zhongshan Hospital, Fudan University, Shanghai; 9 patients from Changzheng Hospital, Second Military Medical University, Shanghai; and 8 patients from Shanghai First People's Hospital, Shanghai Jiao Tong University, Shanghai. All the patients were adults (>18 years) receiving the first-time living-related renal transplantation, and an informed consent was obtained before participation. All donors had a documented linear blood relationship with the recipients, and the

TABLE 2. The EC-MPS Pharmacokinetic Profiles of the Patients

Time Points, h	All Patients, N = 36			Model Group, n = 18			Validation Group, n = 18		
	Mean ± SD	Median	Range	Mean ± SD	Median	Range	Mean ± SD	Median	Range
0	2.78 ± 1.47	2.40	0.90–7.00	2.88 ± 1.45	2.60	1.30–6.70	2.67 ± 1.53	2.20	0.90–7.00
0.5	2.60 ± 1.84	2.20	0.40–11.20	2.78 ± 2.33	2.20	1.10–11.20	2.42 ± 1.20	2.20	0.40–4.50
1	2.43 ± 1.82	2.05	0.40–11.50	2.67 ± 2.40	2.00	0.70–11.50	2.18 ± 0.98	2.20	0.40–3.70
1.5	2.67 ± 2.15	2.15	0.30–12.70	2.56 ± 1.47	2.15	0.70–5.70	2.78 ± 2.71	2.10	0.30–12.70
2	2.83 ± 2.58	2.15	0.30–14.80	2.78 ± 1.87	2.15	0.70–8.50	2.89 ± 3.20	1.95	0.30–14.80
2.5	3.11 ± 2.52	2.45	0.20–12.40	3.28 ± 2.43	2.40	0.70–9.90	2.95 ± 2.66	2.65	0.20–12.40
3	5.76 ± 6.80	3.55	0.20–36.50	6.28 ± 8.43	3.70	0.90–36.50	5.24 ± 4.85	3.55	0.20–16.50
4	6.50 ± 6.92	4.55	0.20–35.60	6.99 ± 8.18	4.55	1.10–35.60	6.01 ± 5.57	4.90	0.20–24.50
6	3.80 ± 2.77	2.90	0.20–14.80	4.14 ± 3.30	3.05	1.10–14.80	3.46 ± 2.15	2.70	0.20–7.90
8	3.05 ± 1.80	2.60	0.60–7.70	3.38 ± 2.01	2.90	0.60–7.70	2.71 ± 1.56	2.15	0.90–6.40
12	2.37 ± 1.25	2.35	0.30–7.00	2.29 ± 1.00	2.30	0.30–4.50	2.44 ± 1.49	2.35	0.80–7.00

All values are the concentrations of the MPA (in milligrams/liter).

transplantation was carried out in accordance with the Declaration of Helsinki and with the 2007 Chinese "Regulation on Human Organ Transplantation." Exclusion criteria included retransplantation, combined transplantation, ABO blood incompatibility transplantation, pregnancy, allergy to EC-MPS, severe liver/lung/heart diseases, active infection, tumor, or mental diseases. The immunosuppressant regimen was tacrolimus + EC-MPS + prednisone. The initial dose of EC-MPS was 720 mg every 12 hours, and it was adjusted based on the doctors' experience and patients' clinical manifestations. The study design was approved by the ethics committee of Zhongshan Hospital, Fudan University (No. B2012-109). The baseline characteristics of patients are shown in Table 1.

MPA Detection and AUC Calculation

At 2 weeks after the transplantation, blood specimens (2 mL each time) were collected predose and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 hours after the morning dose of EC-MPS. All the specimens were stored at 4°C overnight and were analyzed with enzyme-multiplied immunoassay technique (EMIT) in the clinic laboratory of the Zhongshan Hospital. The measured AUC was calculated with these 11 points of MPA concentrations with the linear trapezoidal rule.

Statistical Analysis

The patients were randomly divided into 2 groups: the model group and the validation group. The randomization was performed by a computer treatment assignment in which randomized numbers were generated with the equation RAND(). LSS was used to build equations with the data of the model group. The MPA concentration of each time point was analyzed with linear regression, and the correlation coefficient (R^2), standard error (SE) of estimation, residual and its mean square (ME), and P value were calculated separately. Considering precision and the clinical practicability, multiple linear regression with the method "forward" was done using MPA concentrations of 3 and 4 time points. Besides R^2, prediction error (PE)% or bias, and absolute PE % or precision were introduced to evaluate the equations.[15,23] Among them, a precision that was less than 15% was considered clinically acceptable.[24] Bias and precision were defined as follows:

$$\text{PE\% or bias}: \frac{100}{n} \times \sum \left(\frac{AUC_{estimated} - AUC_{measured}}{AUC_{measured}} \right).$$

$$\text{(Absolute PE)\% or precision}: \frac{100}{n} \times \sum \left(\frac{|AUC_{estimated} - AUC_{measured}|}{AUC_{measured}} \right).$$

To further assess the external consistency of the equations, they were validated in the validation group. Time points of

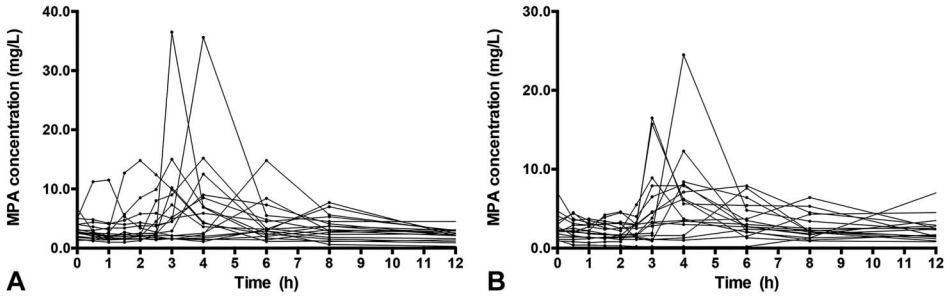

FIGURE 2. The pharmacokinetic profiles of the model group (A) and the validation group (B).

TABLE 3. Univariate Correlation Between the MPA-C at Each Time Point and the $AUC_{0-12\ h}$

Time Points, h	Equation	R^2	Adjusted R^2	SE of Estimation	Residual (ME)	*P*
0	$27.618 + 6.366 \times C_0$	0.234	0.187	17.230	4750.105 (296.882)	0.042
0.5	$38.413 + 2.703 \times C_{0.5}$	0.109	0.053	18.590	5529.640 (345.603)	0.181
1	$38.557 + 2.768 \times C_1$	0.121	0.066	18.466	5455.654 (340.978)	0.158
1.5	$29.399 + 6.457 \times C_{1.5}$	0.246	0.199	17.099	4678.232 (292.390)	0.036
2	$34.402 + 4.153 \times C_2$	0.166	0.114	17.986	5176.183 (323.511)	0.094
2.5	$37.109 + 2.693 \times C_{2.5}$	0.118	0.062	18.498	5474.875 (342.180)	0.164
3	$44.209 + 0.275 \times C_3$	0.015	−0.047	19.547	6113.035 (382.065)	0.631
4	$33.515 + 1.777 \times C_4$	0.580	0.553	12.767	2607.789 (162.987)	<0.001
6	$34.875 + 2.673 \times C_6$	0.213	0.164	17.465	4880.395 (305.025)	0.054
8	$31.784 + 4.183 \times C_8$	0.193	0.142	17.692	5008.067 (313.004)	0.068
12	$21.256 + 10.757 \times C_{12}$	0.318	0.276	16.258	4229.333 (264.333)	0.015

AUC, area under the curve; ME, mean square.

the equations were used as independent variables for multiple regression with the data of the validation group, and correlation coefficients were calculated. The equations from model group were used to calculate the estimated AUC, and bias and precision were also applied as mentioned above. The Bland–Altman test was used to evaluate the agreement between the measured AUC and estimated AUC, and the fixed range was defined as mean ± 1.96 SD. Correlation between estimated and measured AUC was depicted with scatter diagram.

Continuous variables (expressed as mean ± SD) were compared using the *t* test, and categorical variables were compared using the χ^2 test. A value of $P < 0.05$ was considered to be statistically significant. Statistical analysis was performed using SPSS 18.0 for Windows (SPSS, Inc, Chicago, IL).

RESULTS

The baseline characteristics of the patients are listed in Table 1. The age, sex, body weight, the function of liver, the tacrolimus doses, and tacrolimus trough concentration showed no significant difference between the 2 groups. The estimated glomerular filtration rates were 68.8 ± 35.2 for the model group and 57.0 ± 24.3 for the validation group, which also showed no significant difference ($P = 0.404$).

The average dose of EC-MPS for all patients was 600.0 ± 121.7 mg every 12 hours, and the measured MPA $AUC_{0-12\ h}$ was 43.04 ± 16.56 $mg \cdot h \cdot L^{-1}$. The mean MPA C_{max} was 10.7 ± 7.8 mg/L, and the maximum MPA concentration occurred at 4.8 ± 2.8 hours after the morning dose of EC-MPS, which was in accordance with the delayed absorption of the drug. The mean MPA concentration of each group is depicted in Figure 1. The curves present the same trend, where they rise sharply between 2.5 and 3 hours and reach the top at 4 hours (about 6 mg/L) and then decrease gently after 6 hours. There is only 1 apex obviously observed in the curves, but within 3–5 hours, they all keep at the peak level.

Details of pharmacokinetic profiles are shown in Table 2 and Figure 2. Although the mean MPA concentration of each group presents the same trend, the individuals are in great variability.

The correlations between MPA concentration at each time point and the measured AUC are shown in Table 3. The concentration at 4 hours has the strongest correlation with the full AUC ($R^2 = 0.580$; adjusted $R^2 = 0.553$; SE = 12.767; residual = 2607.789; $P < 0.001$). Others have poor estimation for full AUC.

Using the stepwise multiple linear regression, equations consisting of 3 and 4 time points were developed with the highest correlation coefficient (Table 4). For 3 time points, the equation is $6.629 + 8.029 \times C_0 + 0.592 \times C_3 + 1.786 \times C_4$ ($R^2 = 0.910$; adjusted $R^2 = 0.891$; SE = 6.317; residual = 558.589; $P < 0.001$); for 4 time points, it is $3.132 + 5.337 \times C_0 + 0.735 \times C_3 + 1.783 \times C_4 + 3.065 \times C_8$ ($R^2 = 0.959$; adjusted $R^2 = 0.946$; SE = 4.444; residual = 256.748; $P < 0.001$).

As shown in Table 5, when evaluated in the validation group, the 4 time point model has a much better performance than the 3 time point model [the 4 time point model: R^2 = 0.873, bias = 0.505 (95% CI, −10.159 to 11.170), precision = 13.370 (95% CI, 5.186–21.555), and 77.8% of estimated AUCs

TABLE 4. Multiple Linear Regression Analysis of the $AUC_{0-12\ h}$

Model	Equation	R^2	Adjusted R^2	SE of Estimation	Residual (ME)	*P*
1	$6.629 + 8.029 \times C_0 + 0.592 \times C_3 + 1.786 \times C_4$	0.910	0.891	6.317	558.589 (39.899)	<0.001
2	$3.132 + 5.337 \times C_0 + 0.735 \times C_3 + 1.783 \times C_4 + 3.065 \times C_8$	0.959	0.946	4.444	256.748 (19.750)	<0.001

AUC, area under the curve; ME, mean square.

TABLE 5. Predictive Performance of LSS

Model	R^2	Bias (95% CI)	Precision (95% CI)	Within 85%–115%*
1	0.910	3.157 (−6.443 to 12.757)	13.081 (6.013–20.149)	72.22
2	0.959	1.460 (−3.877 to 6.796)	7.173 (3.228–11.118)	83.33
Model	**R^2**	**Bias (95% CI)**	**Precision (95% CI)**	**Within 85%–115%***
1	0.573	6.196 (−10.627 to 23.018)	21.286 (8.079–34.492)	50.00
2	0.873	0.505 (−10.159 to 11.170)	13.370 (5.186–21.555)	77.78

*Compared with the $AUC_{measured}$ of each group.
Bias, prediction error; precision, absolute prediction error.

was within 85%–115% of the measured AUCs; for the 3 time point model: R^2 = 0.573, bias = 6.196 (95% CI, −10.627 to 23.018), precision = 21.286 (95% CI, 8.079–34.492), and 50.0% of estimated AUCs was within 85%–115% of the measured AUCs. In the Bland–Altman test, only 1 plotted difference exceeds the fixed range of the mean ± 1.96 SD in each model, but model 2 has a much better internal consistency (Fig. 3). The correlation between estimated and measured AUC in Figure 4 and the mountain plot of models 1 and 2 in Figure 5 also suggest a better estimation of model 2.

DISCUSSION

In this study, the pharmacokinetic profiles of the Chinese renal transplant recipients receiving EC-MPS were analyzed with LSS using EMIT. Compared with high-performance liquid chromatography, which was considered as the "gold standard" for drug concentration measurement, EMIT was reported for higher results. However, EMIT had a good linear correlation with high-performance liquid chromatography, making it a practical method for drug monitoring.[25–28] More importantly, due to the high efficiency, convenience, and automation of EMIT, it has been more widely used in clinical setting. In this study, all samples were tested with EMIT, which should be considered when adjusting drug doses.

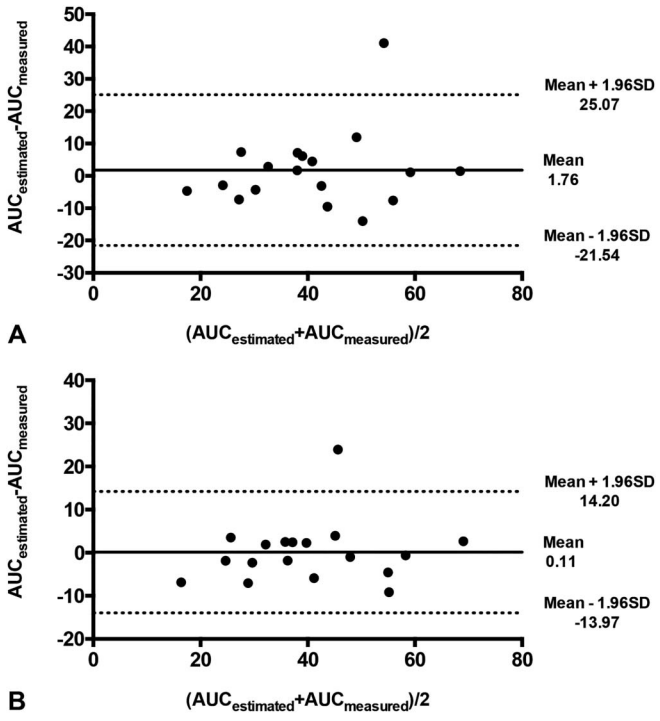

FIGURE 3. The Bland–Altman plot for the validation group using model 1 (A) and model 2 (B). It evaluated the agreement between the measured AUC and estimated AUC, and the fixed range was defined as mean ± 1.96 SD.

In accordance with the previous studies, large interindividual variability was shown in MPA exposure.[20,29] MPA is metabolized by the UDP-glucuronosyltransferase (UGT) to a major metabolite, 7-O-glucuronide, and 7-O-glucuronide undergoes biliary excretion into the intestine via multidrug resistance protein 2 and solute carrier organic anion transporter.[30] A series of studies have proven that the genetic polymorphisms of *UGT* and *SLCO* influence the pharmacokinetics of MPA.[31] Food intake time, types of food ingestion, and gastric emptying may also have influence on it.[9] Although for EC-MPS drug concentration monitoring is more difficult, it still highlights the value and importance of TDM. Various studies have shown the relationship between the MPA AUC and the risk of rejection and side effects. With TDM, the patients could receive personalized prescription.

Despite the interindividual variability, it shared the same trend within each group. The concentration of MPA increased sharply between 2.5 and 3 hours postdose, and the maximum occurred about 4 hours after the oral administration (peak time point: 4 hours postdose and mean MPA-T_{max}: 4.8 hours postdose), which was in accordance with the majority of the individual profiles of patients. It was delayed compared with a previous study, which has reported that the median time to maximum MPA concentration was 2.0 hours.[32] This result might reflect the metabolic characteristics of EC-MPS in Chinese population. There was only 1 obvious peak in the curves of our study. This may be due to the limitations of LSS. Within the range of 3–6 hours postdose, the inadequate sample points may conceal the second peak, which was also seen in other research.[15]

The results of univariate correlation analysis suggested that the predose MPA concentration was poor at predicting the systemic exposure of MPA (R^2 = 0.187). Only the concentration at 4 hours postdose, which was also the peak of the concentration, had a relatively higher correlation, but it

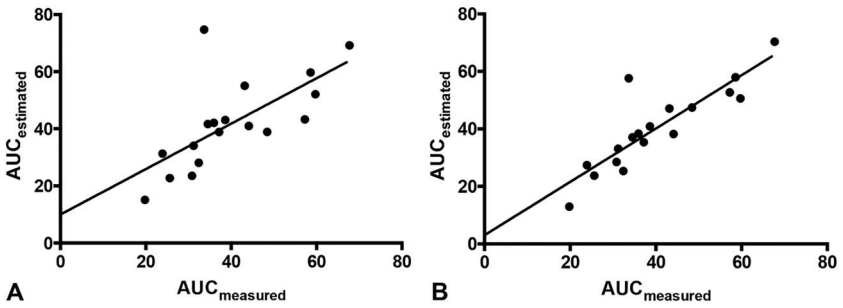

FIGURE 4. The correlation between estimated AUC and measured AUC calculated with model 1 (A) and model 2 (B).

was still not reliable enough ($R^2 = 0.553$). As a result, 3 time point (C_0, C_3, and C_4) and 4 time point (C_0, C_3, C_4, and C_8) equations were developed. Although the frequency of sampling was acceptable, the time span needed was still impractical for outpatients. In our study, the same multiple regression method was performed with the variables within 2 hours postdose (C_0, $C_{0.5}$, C_1, $C_{1.5}$, and C_2) so that it might have better practicability, but all parameters were excluded except $C_{1.5}$ because of high P value ($P > 0.10$, data not shown). Fleming et al[15] did the same effort, but the R^2 for the early 4 points within 2 hours was only 0.292. De Winter et al and Pawinski et al also wanted to develop an equation using the time points within 3 hours postdose, but they failed because of biased and imprecise results.[16,33] When C_8 was added into the 3 time point equation in our study, it had a much better performance in the validation group. Because the reported enterohepatic recirculation was about 3–12 hours postdose and caused the second peak of MPA, the C_8 was related to this phenomenon so that it greatly increased the precision of the equation.[2,32] It is the pharmacokinetic characteristics of EC-MPS that decreases the convenience for TDM, especially for the outpatients.

A series of studies with similar parameters to evaluate the equations are shown in Table 6.[9,15,16,19,22,33] It is in accordance with our results that a relatively feasible and precise equation should contain time points at a later stage. Compared with the results in Table 6, the equations developed in our study have a relatively better predictive performance, especially for the 4 time point equation, whose bias and precision for the validation group were 0.505 and 13.370, respectively. Considering that the SE of estimation was only 4.444, and about 80% of the estimated AUC was within 85%–115% of the measured AUC, this equation could be the reference for the treatment of inpatients in clinical setting.

This study has a number of limitations that should be considered. All cases were from a Chinese population, and the genetic polymorphism for MPA metabolism was not analyzed. Blood samples were stored at 4°C overnight for the practicability. This could cause a measurement error. The number of sampling time points for the measured AUC is limited, especially between 2 and 4 hours postdose, in which the MPA concentration increased sharply. Renal function of kidney transplant recipient achieves a stable condition mostly 2 weeks after the transplantation, and the pharmacokinetics of EC-MPS is relatively stable at this time point as well. Additionally, inappropriate MPA exposure in early stage after the kidney transplantation correlated to allograft rejection, infection, and myelosuppression; thus, MPA pharmacokinetic monitoring at an early stage after the kidney transplantation is of great importance. Based on these considerations, we chose 2 weeks after transplantation for the study time point. However, beyond 2 weeks after the transplantation, there are still important changes in dose-corrected MPA exposure, which could potentially change the overall performance of the currently obtained 4 point LSS at other (later) time points after the transplantation. Further validation is needed before LSS calculated by this predictive equation can be applied to patients receiving long-term EC-MPS. Moreover, the relatively small number of patients involved in this study amplified the bias. The

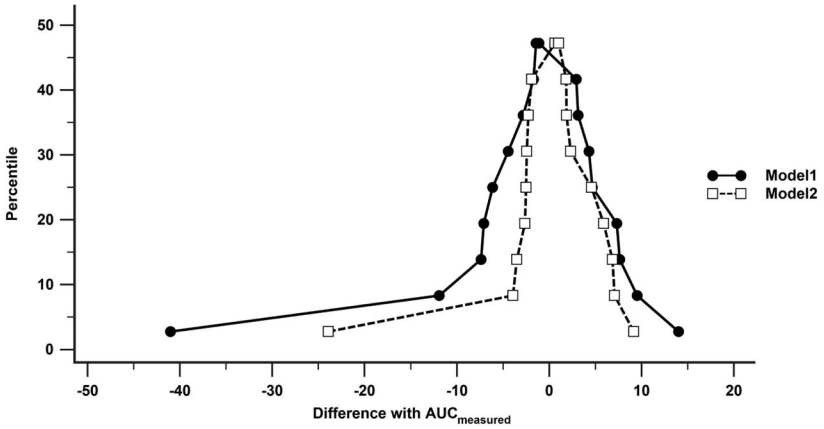

FIGURE 5. The mountain plot of models 1 and 2.

TABLE 6. Equations Developed With LSS for Patients Receiving EC-MPS

Reference	No. Patients	Organ	Posttransplant Time	Immunosuppressive Regimen	No. Sampling
Pawinski et al[16]	69	Kidney	196 mo	Tac, EC-MPS	9
	6	Liver			
Fleming et al[15]	18	Kidney	1 and 5.5 mo	Tac, EC-MPS, Pred	17
Capone et al[22]	52	Kidney	6–254 mo	CsA, EC-MPS, Methylpred	10
De Winter et al[33]	109	Kidney	197–7694 d	CsA, EC-MPS	13
				non-CsA, EC-MPS	
Yao et al[9]	38	Kidney	7.6 ± 1.0 d	Tac, EC-MPS, Pred	10
Fructuoso et al[19]	71	Kidney	13.5–79.5 mo	Tac, EC-MPS	13

Reference	Equations	R^2 of Model	R^2 of Validation	Bias	Precision
Pawinski et al[16]	$17.28 + 0.89 \times C_1 + 1.76 \times C_3 + 6.09 \times C_9$	NR	0.824	6.32 ± 25.75	27.45 ± 29.89
	$8.53 + 1.09 \times C_1 + 1.07 \times C_2 + 1.65 \times C_3 + 3.59 \times C_6$	NR	0.898	3.32 ± 18.26	14.05 ± 11.89
Fleming et al[15]	$8.09 + 2.53 \times C_{1.5} + 0.44 \times C_{2.5} + 1.36 \times C_{3.5} + 6.45 \times C_6$	0.950	0.558	2.92	17.23
Capone et al[22]	$22.906 + 3.880 \times C_0 + 1.117 \times C_1 + 7.527 \times C_8$	0.812	NR	3.78 ± 13.81	10.95 ± 9.00
De Winter et al[33]	$36.536 + 1.642 \times C_{0.5} + 0.569 \times C_{1.5} + 0.905 \times C_2$	0.42	0.33	−1.0	24.0*
	$19.801 + 1.827 \times C_{0.5} + 1.111 \times C_1 + 1.429 \times C_2$	0.69	0.31	0.4	14.5*
Yao et al[9]	$15.09 + 1.05 \times C_{1.5} + 1.8 \times C_4 + 4.18 \times C_6$	0.902	NR	5.9	16.7
	$10.44 + 0.7 \times C_1 + 1.22 \times C_2 + 1.75 \times C_4 + 4.36 \times C_6$	0.941	NR	2.7	13.5
Fructuoso et al[19]	$15.99 + 0.87 \times C_1 + 0.68 \times C_2 + 7.85 \times C_4$	0.843	0.714	−0.214	7.477
	$11.15 + 0.68 \times C_1 + 0.45 \times C_{1.5} + 0.57 \times C_2 + 8.16 \times C_4$	0.888	0.760	−1.481	7.683

*Calculated as root mean squared prediction error.
Bias, prediction error; NR, not reported; precision, absolute prediction error; Tac, tacrolimus; Pred, prednisone; Methylpred, methylprednisone; CsA, cyclosporine A.

models should thus be further tested with larger patient groups in more centers.

CONCLUSIONS

In summary, we developed optimal equations for the estimation of full MPA $AUC_{0-12\ h}$ in the Chinese renal transplant recipients receiving EC-MPS and tacrolimus. The best 3 time point equation was $6.629 + 8.029 \times C_0 + 0.592 \times C_3 + 1.786 \times C_4$, whereas the best 4 time point equation was $3.132 + 5.337 \times C_0 + 0.735 \times C_3 + 1.783 \times C_4 + 3.065 \times C_8$. The latter one exhibited much better estimation ability than the former one. The long time span needed restricted its application, especially for the outpatients, but it could be a useful tool to guide the personalized prescription for the inpatients.

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