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Original Article

Estimation of optimal minimum dosage of mycophenolate mofetil in children with various glomerular diseases

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Abstract: Background: Mycophenolate mofetil (MMF), a common immunosuppressant and a 2-ethyl ester derivative of mycophenolic acid (MPA), has exerted therapeutic efficacy in several kidney diseases. Here, we aimed to investigate the variation of MMF exposure in children with different glomerular diseases and estimate the optimal minimum dosage of MMF. Materials & methods: The serum MPA, the active compound of MMF, was measured in 57 children with primary nephrotic syndrome, Henoch Schönlein purpura nephritis, primary IgA nephropathy, or lupus nephritis. The MPA area under the curve over a 12 hour period (MPA-$AUC_{0\text{-}12\ h}$) was calculated and its associations with the patients' clinical features were analyzed. Receiver operating characteristic curves were performed to explore the optimal minimum MMF dosage. Results: The average of MPA-$AUC_{0\text{-}12\ h}$ was 36.22 (14.38-91.47) μg·h/mL. MAP-$AUC_{0\text{-}12\ h}$ of 39/57 patients was more than the target value (30 μg·h/mL). The dose-normalized MPA-$AUC_{0\text{-}12\ h}$ was associated with plasma albumin but not with other clinical features. The ROC curves analyses indicated that area under the curve (AUC) in PNS was small (0.560) with a low sensitivity (28.6%) when evaluating the optimal minimum MMF dosage. In patients with HSPN, LN, or IgAN, the AUC was 0.829 with 64.0% sensitivity and 100% specificity and an optimal minimum MMF dosage of 588.2 mg/m^2 was obtained. Conclusions: MPA-$AUC_{0\text{-}12\ h}$ in PNS varied drastically in regard of MMF dose and should be monitored timely to adjust the MMF dosage while 588.2 mg/m^2 might be used as an optimal minimum dosage of MMF in children with LN, HSPN, or IgAN.

Keywords: Area under curve, children, glomerular disease, mycophenolic acid

Introduction

Mycophenolate mofetil (MMF) is a common immunosuppressant and a 2-ethyl ester derivative of mycophenolic acid (MPA). MMF is metabolized to MPA, which suppresses inosine monophosphate dehydrogenase, inhibiting *de novo* synthesis of guanine and selectively blocking T & B lymphocyte proliferation *in vivo* [1, 2]. Due to its treatment efficacy and limited liver and kidney toxicity, MMF is now one of the standard immunosuppressive reagents for kidney [3], liver, and heart transplantation worldwide. It is used to prevent acute organ rejection both in adult and pediatric patients.

Recent reports [4, 5] have confirmed the efficacy of MMF in several kidney diseases, such as primary nephrotic syndrome [6, 7], focal segmental glomerulosclerosis [8], and lupus nephritis [9]. MMF has also been recommended in the 2012 KDIGO Guidelines [10]. MMF usage enables the reduction or withdrawal of steroids reducing their toxicity in the acute phase. MMF is also suitable for long-term treatment as it does not cause nephrotoxicity, which is frequently observed after cyclosporine or tacrolimus administration in these patients.

The pharmacokinetics of MMF's main metabolite, mycophenolic acid (MPA), is complex. MPA glucuronide (MPAG) undergoes enterohepatic circulation resulting in a secondary MPA concentration peak at 6-12 hours after MMF administration, which may account for approximately 10%-60% of the total MPA area under curve (AUC).

MPA is highly protein bound (97%-99%) and only the unbound, free fraction (fMPA) is phar-

macologically active. More importantly, pharmacokinetic parameters of MPA, MPAG, and acyl glucuronide of MPA have shown high (approximately 10-fold) inter- and intra-patient variability in both adults and children. The differences are apparent, especially in children younger than 10 years of age and in adults [11]. To date, it remains unclear whether the target exposure is the same in children and adults, especially as the target MPA AUC values in patients with autoimmune diseases has not yet been established [12].

At present, several studies monitoring MPA-AUC after organic transplantation have been reported, but there is scant literature on MPA-AUC in children with glomerular disease. This study analyzed the MPA of children with glomerulus disease who were administered only corticosteroids and MMF in an attempt to discern possible factors responsible for the variability in MPA exposure in these patients and estimate the optimal minimum dosage of MMF.

Materials and methods

Subjects

The study enrolled 57 children who were hospitalized in the Department of Nephrology, The Children's Hospital of Zhejiang University school of Medicine (Hangzhou, China) from September 2014 to June 2015. The children's guardians gave written informed consent for the participation of their children in the study.

The inclusive criteria were: (1) a clinical diagnosis of steroid sensitive but frequently relapsing (or steroid dependent) nephrotic syndrome, Henoch Schönlein purpura nephritis (HSPN), primary IgA nephropathy (IgAN), or lupus nephritis (LN), excluding secondary hepatitis B virus-associated glomerulonephritis, ANCA associated vasculitis, or hereditary factors; all patient presented with nephrotic syndrome at diagnosis; (2) no serious gastrointestinal diseases; (3) age of the onset of disease from 1 to 16 years; (4) estimated glomerular filtration rate > 90 ml/min; (5) no combined use of any other immunosuppressive agent except steroids; and (6) no combined use of aluminum or magnesium gastric mucosa protecting agents or other reagents such as the substrate of uridine diphosphate glucuronosyltramsferase (UGT) [13].

The children with PNS or LN were treated with 2 mg/kg/d prednisone or 0.8 mg/kg/d methylprednisolone tablets to remit the proteinuria firstly. Then all patients including HSPN and IgAN were treated with 1 mg/kg/d prednisone orally every morning combined with MMF treatment. Cell Cept (Shanghai Roche Pharmaceuticals Ltd) was administered orally twice daily at a maximum dose of 1200 mg/m^2/d.

Methods

A three-point abbreviated pharmacokinetic profile for MPA was performed during 0.5~1 month after MMF therapy, based on MPA plasma concentrations before oral intake of MMF (C0), and at 30 min (C0.5) and 2 hours (C2) thereafter. Peripheral blood specimens measuring approximately 2-mL were collected and stored in EDTA-anticoagulant tubes, and the plasma was separated by low-speed centrifugation at 3000 r/min for 5 min. The MPA plasma concentration was determined by the enzyme-multiplied immunoassay technique (EMIT). The reagent and samples were handled strictly in accordance with the manufacturer's instructions for the relevant instruments and reagents. The instrument used in this study was a Viva-E drug concentration analyzer (Siemens, German). The reagents included the EMIT® 2000MPA special kit with supporting standards and controls (Siemens Healthcare Diagnostics).

The MPA-AUC$_{0\text{-}12\,h}$ was calculated with a three-point abbreviated formula using the blood MPA concentrations at 0 hours, 0.5 hours, and 2 hours after dosing: MPA-AUC$_{0\text{-}12\,h}$ = 7.75 + (6.49 * C0) + (0.76 * C0.5) + (2.43 * C2) [7, 14].

At the same time, the plasma albumin, creatinine, GPT, hemoglobin, white blood cell count, and sodium, potassium, and calcium concentrations before dosing were also measured. All measurements were carried out within 12 hours.

Statistical analysis

The data were analyzed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Testing data whether is accord with normal distribution and transform the abnormal distribution data to normal by data conversion. The continuous data were expressed as mean ± standard deviation ($\bar{X}$ ± s), and the means between the groups were compared using the *t*-test. The categorical data were analyzed using the χ^2 test. A P value < 0.05 was considered statistically significant.

Table 1. Basic clinical features of the children patients with steroid sensitive but frequently relapsing (or steroid dependent) nephrotic syndrome, Henoch Schönlein puerperal nephritis, primary IgA nephropathy, and lupus nephritis

Parameters	Median/N
Total	57
Gender (Male/Female)	30/27
Clinical diagnosis	
PNS	23
HSPN	20
IgAN	7
LN	7
Age (years)	8.3 (3.8-14.9)
Body weight (Kg)	27 (15.5-57.5)
MMF dose (mg/m^2)	595 (266-899)
MMF dose (mg/kg)	21.7 (10.4-36.8)
MPA-AUC$_{0\text{-}12\ h}$ (μg·h/mL)	35.0 (14.4-91.5)

Abbreviations: PNS, primary nephrotic syndrome; HSPN, Henoch Schönlein purpura nephritis; IgAN, primary IgA nephropathy; LN, lupus nephritis; MMF, Mycophenolate mofetil.

Receiver operating characteristics (ROC) curves were performed to investigate the optimal minimum MMF dose achieving target value of MPA-AUC (30 μg·h/mL) during treatment [15, 16]. Grouped data were compared by the analysis of variance.

Results

Baseline patient characteristics

Clinical and biochemical characteristics of the 57 patients recruited for the study were summarized in **Table 1**. There were 23 cases (40.4%) with PNS, 20 cases (35.1%) with HSPN, seven cases (12.3%) with IgAN, and seven cases (12.3%) with LN enrolled in the study.

Measurement of MPA-AUC$_{0\text{-}12\ h}$

The C0, C0.5, and C2 of all the 57 patients were detected and the MPA-AUC$_{0\text{-}12\ h}$ was calculated (**Table 2**). The MPA-AUC$_{0\text{-}12\ h}$ ranged from 14.38 μg·h/mL to 91.47 μg·h/mL with an average value of 36.22. MAP-AUC$_{0\text{-}12\ h}$ of 39 patents was more than the target value (68.4%, 30 μg·h/mL) [15, 16]. Then MPA-AUC$_{0\text{-}12\ h}$ was normalized by MMF dose per body surface area or MMF dose per total body weight to analyze the effects of individual difference on MPA exposure variation during treatment of MMF. The dose/body surface area-normalized MPA-AUC$_{0\text{-}12\ h}$ was 38.6±15.5 μg·h/mL/600 mg/m^2 and the coefficient of variation was 40.2%. The dose/total body weight-normalized MPA-AUC$_{0\text{-}12\ h}$ was 1.75±0.73 μg·h/mL/mg/kg and the coefficient of variation was 41.7%.

Correlations of dose-normalized MPA-AUC$_{0\text{-}12\ h}$ with clinical parameters in various glomerular diseases

To reveal the factors that effecting dose-normalized MPA-AUC$_{0\text{-}12\ h}$ (normalized by dose/body surface area or dose/total body weight), its associations with age, weight, gender, serum GPT, creatinine, blood urea nitrogen, white blood cell count, hemoglobin level, pH value, plasma concentration of sodium, potassium, and calcium. The results suggested that only serum albumin was associated with dose-normalized MPA-AUC$_{0\text{-}12\ h}$ (**Figure 1** and **Table 3**). In addition, the patients were also grouped by clinical diagnosis type, gender, and age and then their dose-normalized MPA-AUC$_{0\text{-}12\ h}$ was compared. Significant difference of dose-normalized MPA-AUC$_{0\text{-}12\ h}$ was still not found among the patients stratified by disease type, gender, or age (**Table 4**).

Estimation of optimal minimum dosage of MMF in children with various glomerular diseases

There were 28 children among the 57 cases with a lower MPA-AUC$_{0\text{-}12\ h}$ (< 30 μg·h/mL). Next, we tried to estimate MMF dosage (dose/body surface area) before treatment that could achieve targeted value of MPA-AUC$_{0\text{-}12\ h}$ (30 μg·h/mL), although high variance existed in the dose-normalized MPA-AUC$_{0\text{-}12\ h}$. The ROC curves analyses suggested the area under the curve (AUC) in total children was 0.630 with 51.3% sensitivity and 77.8% specificity (**Table 5**, minimum MMF dose = 617.3 mg/m^2). Considering the albumin was correlated dosed-normalized MPA-AUC$_{0\text{-}12\ h}$, the patients were grouped by 30 g/L albumin and the AUC in patients with albumin < 30 g/L was 0.616 and that in patients with albumin ≥ 30 g/L was 0.684. In addition, although no significant difference of dose-normalized MPA-AUC$_{0\text{-}12\ h}$ among different disease type was identified, a trend of difference between PNS and HSPN groups was observed ($P < 0.1$). Then the ROC curves were also per-

Table 2. Pharmacokinetic profile of MPA during MMF treatment in children with various glomerular diseases

Case No.	C0 (μg/mL)	C0.5 (μg/mL)	C2 (μg/mL)	MPA-AUC$_{0\text{-}12\ h}$ (μg/mL)	Adjusted MPA-AUC[1] (μg•h/mL/600 mg/m^2)	Adjusted MPA-AUC[2] (μg•h/mL/mg/kg)
1	0.96	6.1	1.94	23.33	22.89	0.96
2	0.47	1.18	1.76	15.97	12.08	0.57
3	6.59	39.66	4.45	91.47	61.03	2.49
4	2.44	5.73	2.89	34.96	34.82	1.94
5	0.1	11.92	0.92	19.69	20.80	0.84
6	0.19	17.85	3.15	30.20	50.38	2.05
7	0.22	2.56	1.34	14.38	14.67	0.83
8	0.65	2.21	8.45	34.18	38.56	1.64
9	2.21	17.6	3.21	43.27	37.12	1.90
10	3.7	11.93	3.53	49.41	39.13	1.58
11	1.38	13.48	2.08	32.01	36.10	1.54
12	3.02	5.76	6.57	47.69	42.06	2.19
13	0.93	24.3	3.56	40.90	28.47	1.25
14	1.23	12.31	1.51	28.76	35.46	1.52
15	0.87	2.81	7.66	34.15	27.04	1.09
16	1.04	2.33	4.6	27.45	35.13	1.46
17	0.88	24.27	1.46	35.45	35.74	1.68
18	0.72	28.68	2.04	39.18	37.84	2.08
19	0.56	7.39	1.39	20.38	23.84	1.02
20	3.53	10.45	14.14	72.96	69.46	3.11
21	0.92	21.51	0.85	32.13	22.82	0.96
22	4.08	0.89	2.57	41.15	64.20	2.74
23	1.09	16.84	2.28	33.16	42.02	2.04
24	1.43	25.74	1.43	40.07	43.51	1.84
25	1.75	15.36	5.15	43.30	43.30	2.02
26	2.6	30.36	3.95	57.30	69.44	2.98
27	2.25	14.04	4.58	44.15	36.29	1.81
28	1.92	15.15	3.28	39.70	54.78	2.38
29	0.46	6.17	2.15	20.65	25.89	1.12
30	1.32	18.66	2.15	35.72	38.80	1.64
31	1.27	29.7	2.02	43.47	33.91	1.45
32	1.34	5.96	2.4	26.81	44.82	1.93
33	0.46	5.02	2.14	19.75	18.13	0.75
34	4.02	4.97	2.52	43.74	70.86	3.50
35	2.12	26.31	1.4	44.91	36.37	1.80
36	0.62	5.35	1.48	19.44	38.87	1.81
37	1.3	1.82	8.02	37.06	57.14	2.30
38	1.3	35.94	3.44	51.86	61.82	3.25
39	0.75	5	1.08	19.04	20.28	0.86
40	1.2	11.53	2.47	30.30	24.55	1.21
41	0.48	13.97	1.38	24.84	21.31	1.09
42	1.88	6.42	3.21	32.63	22.52	0.98
43	0.43	3.92	0.97	15.88	18.90	0.88
44	1.25	2.52	2.92	24.87	56.11	2.39
45	2.16	5.16	8.06	45.28	67.91	3.17
46	1.48	19.56	2.16	37.47	46.99	2.02

47	1.15	10.15	3.13	30.53	47.63	2.04
48	1.03	10.11	2.76	28.83	28.13	1.29
49	2.65	25.86	5.58	58.16	62.81	3.10
50	0.86	1.19	2	19.10	15.96	0.69
51	2.59	20.16	3.91	49.38	47.80	2.17
52	0.58	8.34	1.56	21.64	23.50	1.00
53	2.12	18.6	2.29	41.21	38.32	2.06
54	3.12	14.82	3.51	47.79	41.87	1.72
55	2.1	8.11	4.14	37.60	30.01	1.47
56	1.17	14.06	2.24	31.47	27.57	1.13
57	2.73	31.02	3.74	58.13	51.13	2.66

0, C0.5, and C2: MPA plasma concentrations before oral intake of MMF (C0), and at 30 min (C0.5) and 2 hours (C2). MPA-AUC, mycophenolic acid area under curve exposure; Adjusted MPA-AUC[1], MPA-$AUC_{0\text{-}12\ h}$ normalized by dose/body surface area; Adjusted MPA-AUC[2], MPA-$AUC_{0\text{-}12\ h}$ normalized by dose/total body weight.

formed in subgroups stratified by disease type. The results indicated that AUC in PNS was smallest (0.560, MMF minimum dosage = 819.4 mg/m^2). Its specificity was 100%, however, the sensitivity was low (28.6%). In patients with LN, HSPN, or IgAN, the AUC was 1.000, 0.797, and 0.750, respectively. After including the patients with HSPN, LN or IgAN, the AUC was 0.829 with 64.0% sensitivity and 100% specificity and an optimal minimum MMF dosage of 588.2 mg/m^2. All the 18 patients treated with ≥ 588.2 mg/m^2 MMF achieved target value of MPA-$AUC_{0\text{-}12\ h}$ and only seven patient among the 16 patients treated with < 588.2 mg/m^2 MMF achieved the target value. The above results revealed that MPA-$AUC_{0\text{-}12\ h}$ in PNS varied drastically in regard of MMF dose and MPA-$AUC_{0\text{-}12\ h}$ should be monitored timely to adjust the MMF dosage. On the contrary, 588.2 mg/m^2 might be used as an optimal minimum dosage of MMF in children with LN, HSPN, or IgAN. MMF dose per total body weight was also used to estimate the optimal minimum dosage of MMF with the same method above (**Table 6**). However, almost all the area under the ROC were smaller than that in evaluating MMF dose per total surface area in overall or in subgroups stratified by disease type or albumin level. The results suggested that using MMF dose per total body weight to estimate the optimal minimum MMF dosage was not the preferred choice.

Discussion

Many studies have investigated the variation in MMF exposure in patients who have received organ transplants, but few studies have reported on MMF exposure in children with glomerular disease. In the present study, we evaluated the MPA-$AUC_{0\text{-}12\ h}$ children with four types of glomerular disease by limited sample strategy and investigated its association with clinical parameters of the patients. And we estimated the optimal minimum MMF dosage when achieving target value of MPA-$AUC_{0\text{-}12\ h}$.

In view of the complexity of MPA pharmacokinetics, it is impossible to confirm whether one particular therapeutic drug monitoring (TDM) strategy for dosage prediction and monitoring is more effective than any other. Although studies have been performed regarding the effect of MPA pharmacokinetic parameters on MPAG or fMPA, MPA-$AUC_{0\text{-}12\ h}$ is considered the standard criterion for monitoring MPA as it is a reflection of exposure to the drug over the entire dosing period [17]. However, in the clinical setting, measuring the full AUC from 0-12 hours is impractical as it requires several blood samples over the 12 hour dosing interval. It is also expensive and difficult to perform in children.

To date, a large number of predictive formulas for measuring MPA-AUC have been developed using limited sampling strategies, however, it is not possible to conclude whether one particular TDM strategy for dose prediction and monitoring is more effective than another [14, 18-22]. Full 12-hour plasma AUCs are considered the best marker for drug exposure [17], however, it is now widely recognized that the three-point or four-point method for evaluating MPA exposure is reasonable and acceptable in both adults and children [7, 23-27].

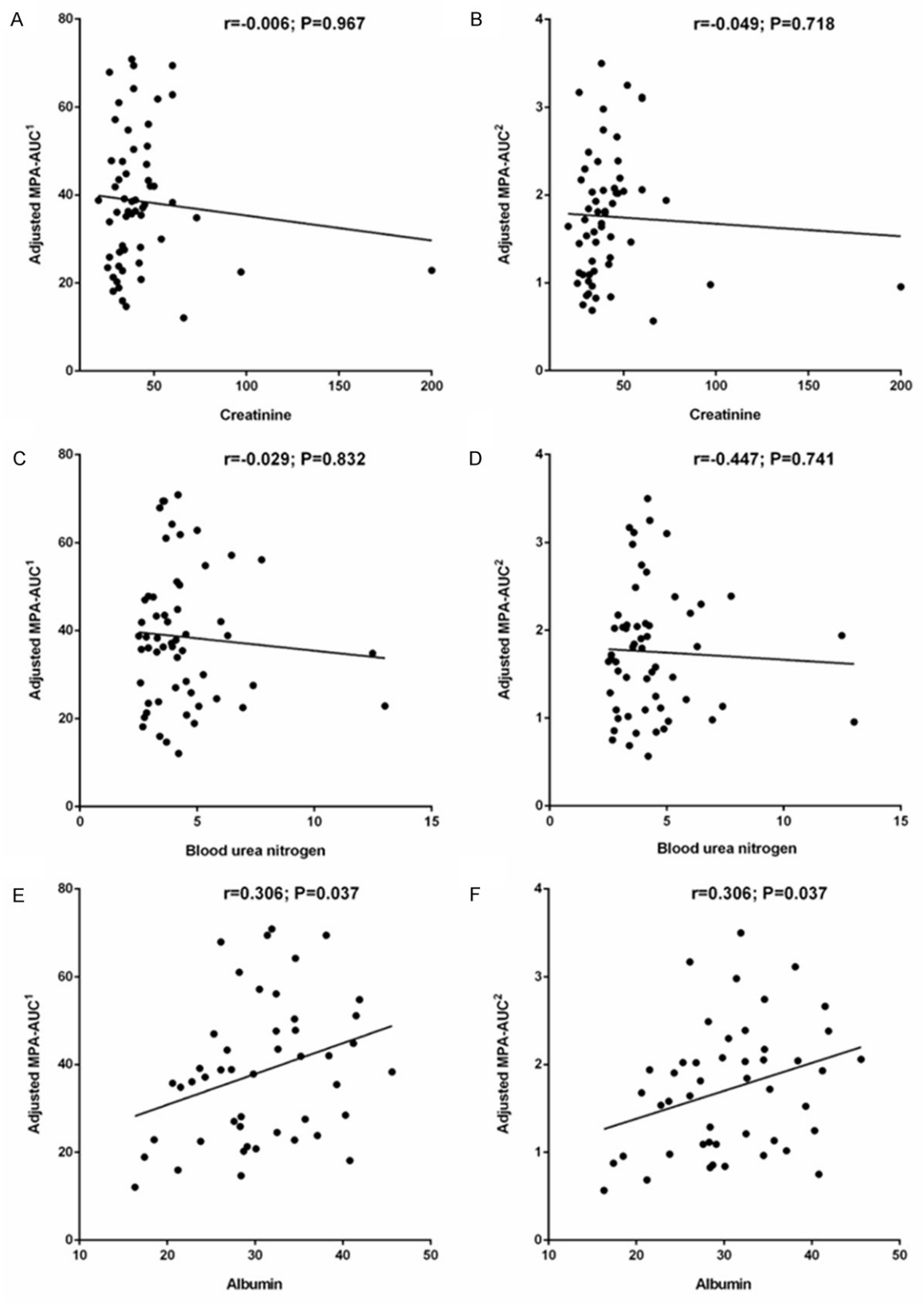

Figure 1. The correlation of dose-normalized MPA-AUC$_{0\text{-}12\ h}$ with serum albumin. MPA-AUC$_{0\text{-}12\ h}$ was normalized by dose/body surface area (adjusted MPA-AUC^1) or dose/total body weight (adjusted MPA-AUC^2). The correlation of creatinine (A, B), blood urea nitrogen (C, D), and serum albumin levels (E, F) with dose-normalized MPA-AUC$_{0\text{-}12\ h}$ were evaluated by Pearson's correlation analyses.

Table 3. The Pearson correlation analysis between dose-normalized MPA-$AUC_{0\text{-}12\ h}$ exposure and serum biochemical characteristics

Serum biochemical parameters	Mean ± SD	Adjusted MPA-AUC[1]		Adjusted MPA-AUC[2]	
		r1	*P1*	r1	*P1*
Creatinine (μmol/L)	43 ± 25	-0.006	0.967	-0.049	0.718
Blood urea nitrogen (mmol/L)	4.4 ± 2.0	-0.029	0.832	-0.447	0.741
Albumin (g/L)	30.6 ± 7.0	0.306	0.037*	0.306	0.037*
GPT (u/L)	17.3 ± 11.3	-0.133	0.324	-0.095	0.482
Leukocytes (10^9/L)	10.2 ± 3.9	-0.08	0.559	-0.027	0.845
Hemoglobin (g/L)	133 ± 13	0.017	0.9	0.034	0.800
Potassium (mmol/L)	3.5 ± 0.4	-0.191	0.531	-0.224	0.461
Sodium (mmol/L)	139 ± 3.8	0.451	0.122	0.470	0.105
Calcium (mmol/L)	1.1 ± 0.08	0.271	0.42	0.164	0.630
pH	7.39 ± 0.07	-0.075	0.807	0.026	0.933

PA-AUC, mycophenolic acid area under curve exposure; Adjusted MPA-AUC[1], MPA-$AUC_{0\text{-}12\ h}$ normalized by dose/body surface area; Adjusted MPA-AUC[2], MPA-$AUC_{0\text{-}12\ h}$ normalized by dose/total body weight; *A *P* value < 0.05 was considered statistically significant.

Table 4. The dose-normalized MPA-$AUC_{0\text{-}12\ h}$ in 57 patients grouped by gender, disease, and age

Parameters	N	Adjusted MPA-AUC[1] (μg•h/mL/600 mg/m^2)	Adjusted MPA-AUC[2] (μg•h/mL/mg/kg)
Gender			
Male	30	40.24 ± 16.65	1.84 ± 0.78
Female	27	36.74 ± 14.10	1.66 ± 0.66
Clinical diagnosis			
PNS	23	34.5 ± 16.8	1.53 ± 0.78
HSPN	20	43.1 ± 13.9	1.96 ± 0.68
IgAN	7	40.9 ± 17.0	1.91 ± 0.78
LN	7	36.7± 12.4	1.71 ± 0.50
Age			
3.0-5.9 years	8	38.94 ± 16.38	1.59 ± 0.66
6.0-11.9 years	39	39.64 ± 15.7	1.80 ± 0.75
12.0-16 years	10	34.16 ± 14.52	1.69 ± 0.75

Abbreviations: PNS, primary nephrotic syndrome; HSPN, Henoch schönlein purpura nephritis; IgAN, primary IgA nephropathy; LN, lupus nephritis; MPA-AUC, mycophenolic acid area under curve exposure. Adjusted MPA-AUC[1], MPA-$AUC_{0\text{-}12\ h}$ normalized by dose/body surface area; Adjusted MPA-AUC[2], MPA-$AUC_{0\text{-}12\ h}$ normalized by dose/total body weight.

As MMF exhibits wide inter- and intra-patient pharmacokinetic variability and the measurement of MPA-$AUC_{0\text{-}12\ h}$ is complex and difficult, researchers have tried to determine alternative strategies, such as trough concentration and single concentration time points for dosage prediction and monitoring [28]. A study on acute vs. chronic graft versus host disease found a significant correlation between MPA trough levels and clinical response [29]. Another study performed on Indian renal transplant patients revealed that the trough concentration of MPA significantly correlated with $AUC_{0\text{-}12\ h}$ (r = 0.69). However, if dosing in routine clinical practice was adjusted based on trough concentration alone, 41% of patients [25] would require a different dose compared with monitoring using $AUC_{0\text{-}12}$.

Since the MPA-$AUC_{0\text{-}12\ h}$ varies with the individual, as confirmed in the present study, we tried to identify factors which may influence the MPA-$AUC_{0\text{-}12\ h}$ in children. Previous studies have considered that the individual's metabolism of MPA may vary according to race, gender, drug compatibility, plasma albumin concentration, and renal function. In the present study, we found that MPA-$AUC_{0\text{-}12\ h}$ had no significant relationship to age, gender, weight, or GPT in Chinese children.

Additionally, the pharmacokinetics of MMF metabolites in children may be affected by various factors (treatment duration, therapeutic indication, co-administered drugs, genetics, physiological factors, and environmental factors, as well as kidney or liver dysfunction) [11, 30].

According to the literature, mycophenolate is the substrate of UGT [13], while prednisone, methylprednisolone, or prednisolone have no interaction with UGT [31]. Meanwhile, predni-

Table 5. Estimation of optimal minimum MMF dosage (dose/total body surface) achieving the target value of MPA-$AUC_{0\text{-}12\ h}$ (30 μg·h/mL) in children with various glomerular diseases with ROC analysis

Groups	N	AUC	Sensitivity	Specificity	MMF cutoff (mg/m^2)	PP	PN
Total	57	0.630	51.3%	77.8%	614.8	20/25	5/22
Clinical Diagnosis							
PNS	23	0.560	28.6%	100.0%	819.4	4/4	10/19
LN	7	1.000	100.0%	100.0%	594.1	3/3	0/4
HSPN	20	0.797	62.5%	100.0%	563.4	10/10	6/10
IgAN	7	0.750	66.7%	100.0%	554.0	5/5	1/2
LN+HSPN+IgAN	34	0.829	64.0%	100.0%	588.2	18/18	7/16
Serum albumin							
ALB < 30 g/L	23	0.616	30.8%	90.0%	717.7	4/5	9/18
ALB ≥ 30 g/L	24	0.684	36.8%	100.0%	667.9	6/6	12/18

Abbreviations: PNS, primary nephrotic syndrome; HSPN, Henoch schönlein purpura nephritis; IgAN, primary IgA nephropathy; LN, lupus nephritis; AUC, area under receiver operating characteristics curve; ALB, albumin; PP: patient proportion with MPA-$AUC_{0\text{-}12\ h}$ > 30 μg·h/mL in patients treated with MMF dose more than cutoff value; PP: patient proportion with MPA-$AUC_{0\text{-}12\ h}$ > 30 μg·h/mL in patients treated with MMF dose less than cutoff value.

Table 6. Estimation of optimal minimum MMF dosage (dose/total body weight) achieving the target value of MPA-$AUC_{0\text{-}12\ h}$ (30 μg·h/mL) in children with various glomerular diseases with ROC analysis

Groups	N	AUC	Sensitivity	Specificity	MMF cutoff (mg/kg)	PP	PN
Total	57	0.566	17.90%	100.00%	29.08	7/7	32/50
Clinical Diagnosis							
PNS	23	0.556	77.80%	57.10%	29.71	5/5	9/18
LN	7	0.917	66.70%	100.00%	20.15	2/2	1/5
HSPN	20	0.711	43.80%	100.00%	22.47	7/7	9/13
IgAN	7	0.583	33.30%	100.00%	23.37	2/2	4/5
LN+HSPN+IgAN	34	0.749	40.00%	100.0%	22.47	10/10	15/24
Serum albumin							
ALB < 30 g/L	23	0.562	30.80%	100.00%	29.71	4/4	9/19
ALB ≥ 30 g/L	24	0.602	33.30%	94.40%	14.3	17/21	1/3

Abbreviations: PNS, primary nephrotic syndrome; HSPN, Henoch schönlein purpura nephritis; IgAN, primary IgA nephropathy; LN, lupus nephritis; AUC, area under receiver operating characteristics curve; ALB, albumin; PP: patient proportion with MPA-$AUC_{0\text{-}12\ h}$ > 30 μg·h/mL in patients treated with MMF dose more than cutoff value; PP: patient proportion with MPA-$AUC_{0\text{-}12\ h}$ > 30 μg·h/mL in patients treated with MMF dose less than cutoff value.

sone, methylprednisolone, and prednisolone are substrates of CYP3A4 [32], and no effect of mycophenolate on CYP3A4 has been reported. Furthermore, no effect of prednisone on mycophenolic acid (MPA) trough concentration has been reported in the literature [33], and no impact of methylprednisolone on MPA pharmacokinetic parameters has been reported in the literature [21].

We also compared the MPA-$AUC_{0\text{-}12\ h}$ in children with different glomerular diseases and found no differences among the four types of disease studied other than the influence of albumin on MPA-$AUC_{0\text{-}12\ h}$ in children.

Honarbakhsh et al. [34] studied 31 Iranian patients who received kidney transplants and also received fixed doses of MMF for six months. They found no significant correlation between MPA-$AUC_{0\text{-}12\ h}$ and age, weight, or MMF daily dosage. We compared the MPA-AUC with different ages and genders in our study, but found no differences. However, a significant correlation was noted between the dose-normalized exposure and the age when studying 28 children who received a combination therapy of MMF and tacrolimus [30].

Another study was conducted to characterize the pharmacokinetic parameters of MPA in

Korean kidney transplant recipients. Patients' age, weight, body surface area, and renal function did not influence the MPA-$AUC_{0\text{-}12\ h}$. But the MPA-$AUC_{0\text{-}12\ h}$ was significantly different between men and women in spite of the small number of cases studied. They concluded that the free fraction of MPA (fMPA) was not affected by renal function when the creatinine clearance was above 40 mL/min [35].

In the current study, we found a correlation between MPA-AUC and albumin but not between MPA-AUC and creatinine. Many studies in the past have indicated that MPA-AUC was related to albumin in organ transplant patients. Recent research on patients after liver transplantation found an inverse relationship between serum albumin concentration and MPA clearance ($r^2 = 0.12$, $P < 0.05$). They also found a significant relationship between creatinine clearance and MPA clearance [27]. Another study in children with PNS found a significant positive correlation between MPA clearance and serum albumin levels less than 30 g/L, but not for serum albumin levels greater than 30 g/L [26]. On the other hand, Zhao et al. [36] studied twenty-three children with PNS and found body weight and serum albumin had a significant impact on MPA clearance. As MPA is bound extensively to serum albumin, the loss of binding protein might lead to greater elimination of MPA by the kidney which might then influence the MPA concentration in the blood and the AUC. The reason that we did not find a correlation between creatinine or blood ureanitrogenand MPA-AUC may be due the fact that the eGFR in all patients recruited was more than 90 ml/min.

No relationship between MPA-$AUC_{0\text{-}12\ h}$ and serum white blood cell count or hemoglobin level was found. We also did not find any correlation between serum pH values, or sodium, potassium, or calcium concentrations and MPA-$AUC_{0\text{-}12\ h}$, although Kamińska et al. [37] reported that MMF treatment decreased the plasma sodium concentration and concluded that MMF may affect sodium balance.

In addition, the previous reports suggested that MPA-AUC ≥ 30 μg·h/mL can be used to predicted favorable remission in steroid-dependent/frequent relapsing nephrotic syndrome [15, 16] and was used as a target value. Given that MPA-$AUC_{0\text{-}12\ h}$ was correlated with MMF dose per body surface area, we investigated optimal minimum MMF dose achieving the target value of MPA-AUC ≥ 30 μg·h/mL. In total patients and subgroups divided by albumin levels, the factor associated with dose-normalized MPA-$AUC_{0\text{-}12\ h}$, AUC and the sensitivity were relative small. After stratification by disease type, the AUC in PNS was only 0.560, however, in the patients with LN, HSPN, or IgAN, the AUC reached up to and anoptimal cutoff of MMF dosage of 588.2 mg/m^2 was obtained. The patients with MMF dosage more than 588.2 mg/m^2 all achieved the target value of 30 μg·h/mL (18 cases) and seven patients of the left 16 patients whose MMF dosage less than 30 μg·h/mL achieved the target value. The results suggested that MMF efficacy in PNS varied drastically and MPA-$AUC_{0\text{-}12\ h}$ should be monitored in timely, but in patient with LN, HSPN, or IgAN, 588.2 mg/m^2 MMF might be used as an initial dosage and detection of MPA-$AUC_{0\text{-}12\ h}$ could be avoid. The conclusions should be verified in more prospective independent cohorts with large scale samples.

In summary, MPA-AUC exposure in children with glomerular disease displayed individual differences. Plasma albumin levels were found to be associated with MPA-AUC. 588.2 mg/m^2 MMF could be used an initial dosage to ensure the MPA-$AUC_{0\text{-}12\ h}$ in the patients with LN, HSPN, or IgAN achieving the target value of 30 μg·h/mL while MPA-AUC varied largely in PNS and should be monitored in time to adjust MMF dosage.

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Disclosure of conflict of interest

None.

Abbreviations

AUC, area under curve; HSPN, Henoch Schönlein purpura nephritis; IgAN, primary IgA

nephropathy; LN, lupus nephritis; MMF, mycophenolate mofetil; MPA, mycophenolic acid; MPAG, MPA glucuronide; PNS, primary nephrotic syndrome; UGT, uridine diphosphate glucuronosyltramsferase.

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