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PHARMACOKINETICS AND DISPOSITION

Limited sampling strategy to predict mycophenolic acid area under the curve in pediatric patients with nephrotic syndrome: a retrospective cohort study

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Abstract

Purpose Limited sampling strategy (LSS) is a precise and relatively convenient therapeutic drug monitoring method. We evaluated LSSs for mycophenolic acid (MPA) in children with nephrotic syndrome treated with mycophenolic mofetil (MMF) and validated the LSSs using two different approaches.

Methods We measured MPA plasma concentrations in 31 children using HPLC-UV method and received 37 MPA pharmacokinetic profiles (0–12 h). For six children, MPA profiles were estimated twice after two MMF doses. LSSs were developed using multilinear regression with STATISTICA and R software and validated using validation group and bootstrap method, respectively.

Results The best three time point equations included C_1, C_3, C_6 (good guess 83%, bias − 2.78%; 95% confidence interval (CI) − 9.85–0.46); C_1, C_2, C_6 (good guess 72%, bias 0.72%; 95% CI − 5.33–7.69); and C_1, C_2, C_4 (good guess 72%, bias 2.05%; 95% CI − 4.92–13.01) for STATISTICA software. For R software, the best equations consisted of C_1, C_3, C_6 (good guess 92%, bias − 2.69%; 95% CI − 27.18–33.75); C_0, C_1, C_3 (good guess 84%, bias − 2.11%; 95% CI − 24.19–22.29); and C_0, C_1, C_2 (good guess 84%, bias − 0.48%; 95% CI − 30.77–54.07). During validation, better results were obtained for R evaluations, i.e., bootstrap method.

Conclusions The most useful equations included C_0, C_1, C_3 and C_0, C_1, C_2 time points; however, the most precise included C_1, C_3, C_6 time points because of MPA enterohepatic recirculation. Better results were obtained for bootstrap validation due to greater number of patients. Validated LSS should be used only in the population for which it was developed. As there is growing evidence that underexposure of MPA is associated with insufficient treatment response, we recommend the introduction of therapeutic drug monitoring for MPA in children with nephrotic syndrome.

Keywords Mycophenolic acid · Limited sampling strategy · Nephrotic syndrome · Pharmacokinetics · Therapeutic drug monitoring · Pediatric patients

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Introduction

Nephrotic syndrome, more frequently diagnosed in children, forms a group of clinical symptoms with the proteinuria [1, 2]. Children with nephrotic syndrome are vulnerable to suffer from many ailments, which may lead to cardiovascular disease, chronic kidney insufficiency, or even to the necessity of renal transplantation [1, 3]. The treatment of nephrotic syndrome is based on steroids and immunosuppressants [1, 4]; however, long-term steroid therapy unfavorably influences children's development and their further lives [2, 5]. The immunosuppressants most frequently administered are cyclosporine (CsA), chlorambucil, and cyclophosphamide, which

cause severe side effects, e.g., hematological, gonadal, and nephrological toxicities [4]. The aim of the treatment is to minimalize the incidence of proteinuria recurrence; however, in some patients, trace proteinuria may be observed, defined as protein concentration < 10 mg/kg body weight/day, which is rather related to the chronic kidney disease than nephrotic syndrome [6].

Mycophenolate mofetil (MMF), an immunosuppressive drug, is efficient in nephrotic syndrome and does not cause nephrotoxicity and additionally is well tolerated by patients [1, 4]. MMF is a pro-drug with an active moiety—mycophenolic acid (MPA). MPA is safe and effective when plasma concentrations are above the target minimum exposure. However, the necessity of monitoring MPA concentration is still a matter of debate. MPA pharmacokinetics is complex and differs between groups of patients what causes difficulties in reaching the target concentrations values. Monitoring MPA C_0 would be easy and convenient; however, numerous studies showed that MPA C_0 poorly correlated with the relapse rate [7]. MPA area under the time–concentration curve from 0 to 12 h ($AUC_{0–12}$) tends to be a more useful tool in therapeutic drug monitoring (TDM). Still measuring total $AUC_{0–12}$ is expensive, time-consuming, and inconvenient for patients, especially for children. Moreover, no specific MPA $AUC_{0–12}$ target was established for children with nephrotic syndrome [8].

Limited sampling strategy (LSS) is a useful approach to assess drug pharmacokinetics and safety. Collecting only few blood samples to establish $AUC_{0–12}$ is an easier than determining full pharmacokinetic profile. It is more convenient for patients and facilitates the work of nursing staff. There are studies concerning MPA LSS; however, most of them included renal transplant recipients, whereas each LSS should be evaluated and used in the same patient's group [9, 10]. Separate LSS should be evaluated for renal or heart transplant recipients and patients suffering from lupus nephritis or nephrotic syndrome. While there are two main possible calculation methods for evaluating LSS, multiple linear regression (MLR) [11–17] and Bayesian approach [18, 19], there are many software solutions [18, 20, 21]. In this study, we evaluated LSS for children with nephrotic syndrome using two methods of calculation, STATISTICA and R software, and two different methods of validation, validation group and bootstrap method, respectively. We hypothesized that the results obtained using two approaches should be comparable.

Material and methods

Study population

The retrospective study included 31 children (13 males and 18 females) with nephrotic syndrome, aged 3–18 years (mean age, 11 years). MMF was administered orally twice a day at the same dose (250–1000 mg) for at least 1 month prior to the pharmacokinetic study. Patients were hospitalized between 2012 and 2015 in Department of Pediatric Cardiology, Nephrology, and Hypertension, University of Medical Sciences in Poznan, Poland. The flow diagram of the patient selection is presented in Fig. 1. For children, demographical data as well as biochemical parameters were recorded.

The blood samples were collected into EDTA tubes before MMF administration (C_0) and subsequently 1 h (C_1), 2 h (C_2), 3 h (C_3), 4 h (C_4), 6 h (C_6), 9 h (C_9) and 12 h (C_{12}) after its administration. MMF was administrated in 12-h intervals at least for a month before blood collection for pharmacokinetic analysis; therefore, we assumed that all children were in steady state. In 12 children, blood samples were collected up to 6 h; therefore, as they were in steady state, it was assumed that C_{12} was equal to C_0 [11]. Additionally, we observed that MPA C_0 and C_{12} were comparable (2.78 ± 1.81 μg/mL and 2.07 ± 0.95 μg/mL for C_0 and C_{12}, respectively, $p = 0.055$, $n = 25$) for those children who had both samples collected. In six children, MPA pharmacokinetic profiles were determined twice after two MMF doses.

Children receiving CsA or MMF at not equal morning and evening doses or shorter than 1 month as well as children with too low number of blood samples collected were excluded from the study.

Four children included in the study received concomitantly steroids and seven children had trace proteinuria (< 10 mg/body weight/day) observed during blood sample collection. These two factors may be the source of potential bias.

Almost all children ($n = 35$) received enalapril and were supplemented with vitamins A and E ($n = 30$) as well as alfacalcidol ($n = 18$). Few children received additional antihypertensive drugs (losartanum, amlodipinum; $n = 7$).

All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethical Committee at Poznan University of Medical Sciences. Informed consent was obtained from the parents or guardians prior to initiating the study.

Analytical methods

MPA plasma concentrations were determined using HPLC-UV method. The analytical method for MPA determination was described elsewhere [22, 23]. MPA $AUC_{0–12}$ was calculated using linear trapezoidal rule and the maximal concentration (C_{max}) was extracted from the determined concentrations. To compare the data, we normalized MPA $AUC_{0–12}$ to the most frequently administered dose which was 500 mg b.i.d.

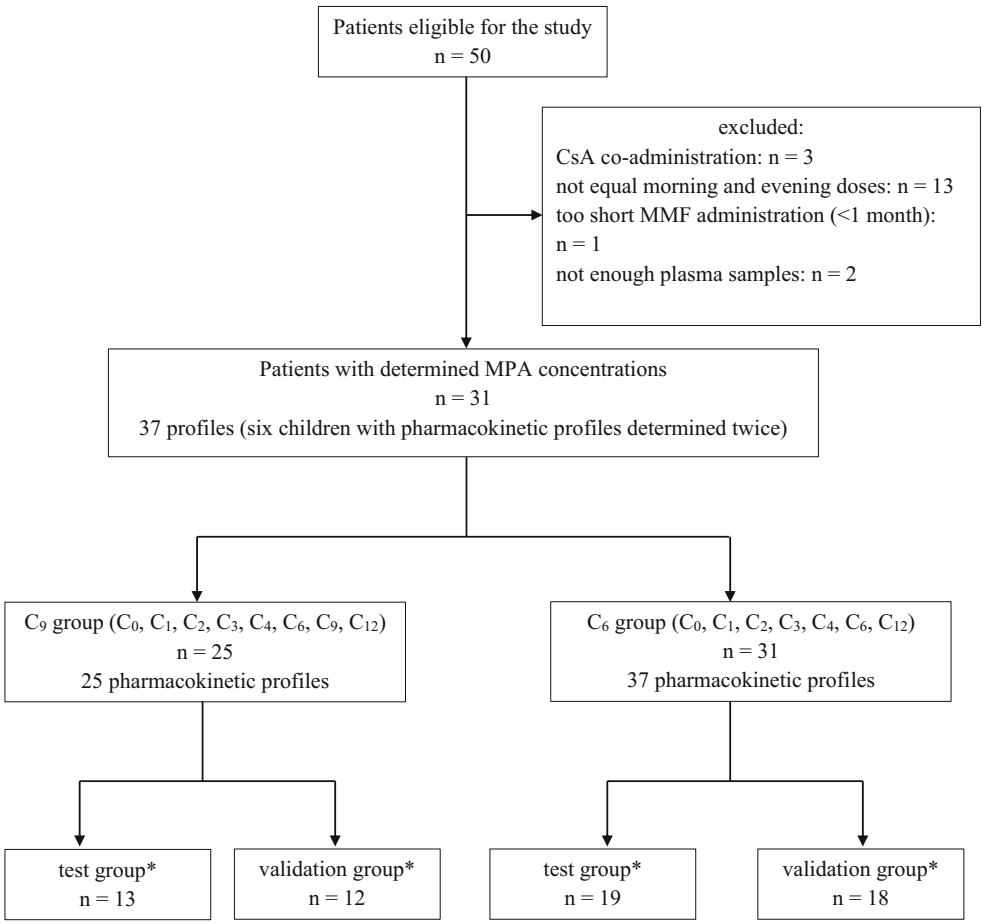

Fig. 1 The flow diagram of patient selection. The division into test and validation groups concerns only STATISTICA evaluations. For R evaluations, bootstrap procedure was used. The profiles were randomly divided into test group and validation group, and the procedure was performed 100 times. *Only for STATISTICA evaluations

All calculations were performed using MS Excel 2010 (Microsoft Corporation, Redmond, WA, USA).

LSSs evaluation

For evaluations, two groups with different blood sampling were considered separately. In the first group of children (C_9 group), 25 profiles with the following blood samples C_0, C_1, C_2, C_3, C_4, C_6, C_9, and C_{12} were included, and in the second group of children (C_6 group), 37 profiles without C_9 were included. The six children with MPA profiles determined twice were included in the C_6 group, and the second profile for each child comprised the validation group. The demographic data were not compared statistically as group C_6 comprises all patients from group C_6, and both groups seemed to be very similar. The characteristics are presented in Table 1.

LSSs were evaluated using STATISTICA 13.0 software (StatSoft, Inc., Tulsa, OK, USA) and R software (R Core Team, 2013). For each measured concentration, calculated $AUC_{0–12}$ and C_{max} normal distributions were confirmed using Shapiro–Wilk test (STATISTICA) and Kolmogorov–Smirnov test (R). For both evaluations method, MLR was applied to develop LSSs. The MPA $AUC_{0–12}$ was considered as the dependent variable, while MPA plasma concentrations at each sampling time point were the independent variables. All concentration–time profiles were completed; therefore, no estimation was done. There were no missing data to handle except for missing C_{12} in 12 children, which was previously described (C_{12} was assumed to equal C_0). The equations were evaluated as follows: $AUC_{pred} = A + B \times C_x + C \times C_y + D \times C_z$, where AUC_{pred} indicated predicted AUC; A indicated the intercept; C_x, C_y, C_z indicated the concentration for three different time points; and B, C, D indicated the coefficients for C_x, C_y, C_z, respectively.

For STATISTICA evaluations, children were divided into two groups, test group (13 and 19 MPA profiles for C_9 and C_6 group, respectively) and validation group (12 and 18 MPA profiles for C_9 and C_6 group, respectively). For test group, the correlations between MPA plasma concentrations at single time points and $AUC_{0–12}$ were firstly verified. Secondly, the equations were developed using stepwise regression with backward elimination. Finally, the number of possible equations based on samples collected during the first 3 h after drug administration was calculated according to the formula:

$$\binom{n}{k} = \frac{n!}{k!(n-k)1}$$

Table 1 The characteristics of children included in C_9 and C_6 groups (results as means)

	C_9 group	C_6 group
No. of patients (male/female)	25 (11/14)	31 (13/18)
Race (no. of Caucasian/other)	25/0	31/0
Age, years (range)	10 (3–18)	11 (3–18)
Body weight, kg (range)	36 (16–67)	38 (15–70)
Body surface, m^2 (range)	1.2 (0.7–1.9)	1.2 (0.6–1.9)
C_0 MPA, μg/mL (range)	2.78 (0.22–7.20)	2.46 (0.19–7.20)
C_1 MPA, μg/mL (range)	16.68 (2.11–44.22)	15.48 (2.11–44.22)
C_2 MPA, μg/mL (range)	7.14 (1.73–17.05)	6.64 (0.79–17.05)
C_3 MPA, μg/mL (range)	4.04 (0.53–12.57)	4.09 (0.37–12.57)
C_4 MPA, μg/mL (range)	3.12 (0.37–6.92)	3.06 (0.30–6.92)
C_6 MPA, μg/mL (range)	2.81 (0.42–7.88)	2.77 (0.25–7.88)
C_9 MPA, μg/mL (range)	2.47 (0.38–6.79)	
C_{12} MPA, μg/mL (range)	2.07 (0.53–3.84)	1.98 (0.19–3.84)
$AUC_{0–12}$ MPA, μg h/mL (range)	54.43 (9.31–95.88)	49.07 (7.19–94.54)
MPA t_{max}, h	1 (1–3)[a]	1 (1–3)[a]
No. of valid profiles	25	37[b]
MMF dose (0.25/0.3/0.35/0.4/0.5/0.6/0.75/1 g twice a day)	3/1/1/1/10/1/7/1 profiles	5/1/1/1/18/1/9/1 profiles
MMF usage period, months (range)	11 (2–29)	9 (1–29)

[a] Median (range)

[b] Six children (three boys, three girls) had MPA profiles determined twice

where k is the number of k-combinations (for equations with up to three time points) and n is the number of elements in the set (C_0, C_1, C_2, C_3; 4 elements). Subsequently, each of the possible 14 combinations was introduced manually into the software. Each developed equation was used for calculating AUC_{pred} for children in the validation group.

For R evaluations, it was assumed that the model should include up to three time points. For C_6 and C_9 groups, the profiles were randomly divided into two groups (test group and validation group). This procedure was performed 100 times to obtain the most varied and random test and validation groups (bootstrap procedure). Based on the results from test groups, all possible models were assessed. For each model, AUC_{pred} were calculated using coefficients which were medians from the previously calculated 100 coefficients. AUC_{pred} were calculated for all profiles because all profiles were previously used for building models.

For both evaluation methods, we calculated the values of r^2, adjusted r^2, good guess, and root square mean error (RMSE) for each equation to analyze the agreement between AUC_{pred} and AUC_{calc}. Good guess was determined by the number of profiles for AUC_{pred} within $AUC_{0–12} \pm 15\%$. The bias and precision for predicting $AUC_{0–12}$ was assessed based on mean prediction error (%MPE) and mean absolute error (%MAE), respectively. The accepted values for %MPE and %MAE were ± 15% and ± 10%, respectively [24]. The best model was chosen on the base of good guess, r^2, and adjusted r^2.

Results

Pharmacokinetic parameters

For all children included in the study, MPA concentrations from 0 to 12 h and $AUC_{0–12}$ are presented in Table 1. MPA $AUC_{0–12}$ was within 9.31 μg h/mL and 95.88 μg h/mL for C_9 group and within 7.19 μg h/mL and 94.54 μg h/mL for C_6 group. MPA $AUC_{0–12}$ below 10 μg h/mL was observed in three children receiving MMF dose of 250 mg b.i.d. The dose-normalized MPA $AUC_{0–12}$ was within 18.62 μg h/mL and 92.95 μg h/mL for C_9 group and within 12.65 μg h/mL and 131.56 μg h/mL for C_6 group. Mean MPA $AUC_{0–12}$ was lower in children with proteinuria (43.80 ± 33.26 μg h/mL vs. 50.30 ± 18.53 μg h/mL for children with and without proteinuria, respectively); however, the difference was not significant. Only one child with proteinuria had MPA $AUC_{0–12}$ below 10 μg h/mL. This child may have received too low MMF dose or may have not response to MMF. For six children with MPA $AUC_{0–12}$ above 10 μg h/mL, proteinuria may have been related to the chronic kidney disease with tissue proteinuria without the nephrotic syndrome and concomitant edema and coagulant disorders.

Table 2 The best LSSs in C9 group

	r^2	r^2 adjusted	%ME	%MAE	%RMSE	Good guess (%)	Median error	95% CI
STATISTICA equations								
$12.34 + 1.54 \times C_1 + 1.21 \times C_2 + 2.47 \times C_3$	0.6640	0.6304	4.51	8.76	10.63	67	10.96	− 0.98–21.42
$3.82 + 4.51 \times C_0 + 1.12 \times C_1 + 1.05 \times C_3 + 2.53 \times C_4 + 4.61 \times C_{12}$	0.4656	0.4121	5.00	9.45	13.26	67	0.69	− 4.44–29.57
$4.57 + 4.58 \times C_0 + 1.00 \times C_1 + 3.76 \times C_4 + 5.67 \times C_{12}$	0.3621	0.2983	6.21	11.05	15.55	67	− 0.65	− 4.57–34.83
$9.34 + 1.49 \times C_1 + 3.51 \times C_3 + 2.74 \times C_6$	0.7417	0.7159	3.50	7.08	8.97	58	9.19	− 0.57–15.84
$3.70 + 4.78 \times C_0 + 1.19 \times C_1 + 1.49 \times C_3 + 2.39 \times C_4 - 1.47 \times C_9 + 5.01 \times C_{12}$	0.3821	0.3203	5.33	10.53	14.52	58	0.59	− 4.85–31.85
$9.24 + 4.03 \times C_0 + 1.21 \times C_1 + 5.71 \times C_4$	0.3361	0.2697	8.46	12.61	16.88	58	8.62	1.01–36.53
$37.28 + 6.32 \times C_6$	0.3228	0.2550	1.31	9.81	13.54	58	− 0.17	− 9.42–23.72
$25.79 + 1.41 \times C_1 + 2.45 \times C_6$	0.5146	0.4661	3.74	9.68	17.79	58	11.08	− 2.86–23.07
$19.23 + 3.85 \times C_2 + 4.13 \times C_6$	0.5730	0.5303	8.13	11.32	14.96	58	12.59	1.46–32.03
$23.03 + 3.17 \times C_3 + 6.79 \times C_6$	0.3999	0.3399	0.97	10.77	13.53	58	− 3.34	− 11.10–20.63
$19.03 + 3.68 \times C_2 + 0.23 \times C_3 + 4.26 \times C_6$	0.5753	0.5328	7.80	11.16	14.66	58	11.45	1.02–31.27
R equations								
$7.10 + 1.21 \times C_1 + 3.75 \times C_3 + 3.08 \times C_6$	0.8388	0.8157	− 2.69	12.92	8.47	92	− 6.92	− 27.18–33.75
$3.49 + 3.66 \times C_0 + 1.39 \times C_1 + 3.82 \times C_3$	0.8516	0.8304	− 2.11	10.89	7.78	84	− 3.90	− 24.19–22.29
$10.96 + 2.25 \times C_0 + 1.11 \times C_1 + 2.18 \times C_2$	0.7903	0.7603	− 0.48	16.28	9.40	84	− 1.16	− 30.77–54.07
$11.66 + 0.96 \times C_1 + 2.10 \times C_2 + 4.83 \times C_{12}$	0.7869	0.7564	3.37	19.34	9.10	84	− 3.88	− 26.68–74.99
$20.06 + 0.72 \times C_3 + 3.94 \times C_6 + 6.04 \times C_9$	0.5698	0.5083	− 0.62	23.91	13.82	84	− 8.24	− 38.27–85.39
$13.17 + 1.32 \times C_1 + 1.21 \times C_2 + 2.47 \times C_3$	0.7859	0.7553	5.26	17.33	8.97	80	2.35	− 28.07–67.34
$12.42 + 1.37 \times C_1 + 4.15 \times C_3$	0.7372	0.7133	1.82	17.60	10.19	80	− 1.62	− 28.30–60.32
$11.43 + 1.35 \times C_1 + 3.37 \times C_3 + 1.24 \times C_4$	0.7516	0.7161	0.07	17.67	10.11	80	− 3.22	− 27.16–53.30
$14.85 + 1.11 \times C_1 + 3.04 \times C_4 + 3.42 \times C_6$	0.7237	0.6842	3.30	19.55	10.32	80	− 1.20	− 31.97–72.74
$18.96 + 2.12 \times C_2 + 2.06 \times C_4 + 3.54 \times C_6$	0.5637	0.5014	2.13	22.03	13.37	80	− 4.72	− 37.35–89.10
$22.85 + 1.92 \times C_2 + 0.79 \times C_3 + 3.75 \times C_6$	0.5294	0.4622	3.77	24.78	13.95	80	− 4.14	− 38.95–101.28
$23.34 + 2.45 \times C_0 + 2.42 \times C_2 + 0.43 \times C_3$	0.4524	0.3742	2.27	26.64	15.29	80	− 6.08	− 37.57–111.34
$7.56 + 0.73 \times C_1 + 4.63 \times C_6 + 10.45 \times C_{12}$	0.8147	0.7882	5.45	17.71	8.32	80	− 2.94	− 22.36–79.14
$14.03 + 1.02 \times C_1 + 1.46 \times C_2 + 4.37 \times C_9$	0.8154	0.7891	2.49	18.04	8.59	80	− 2.77	− 27.68–65.48
$11.14 + 1.21 \times C_1 + 3.49 \times C_3 + 4.96 \times C_{12}$	0.7781	0.7464	9.04	19.40	9.18	80	6.32	− 24.28–77.15
$11.26 + 5.03 \times C_6 + 3.46 \times C_9 + 9.38 \times C_{12}$	0.7141	0.6732	5.35	19.95	10.38	80	− 3.19	− 29.29–86.02
$18.10 + 2.81 \times C_4 + 3.25 \times C_6 + 6.54 \times C_9$	0.6372	0.5854	4.47	20.79	11.84	80	1.12	− 37.01–79.75
$11.41 + 3.05 \times C_0 + 1.01 \times C_1 + 7.99 \times C_{12}$	0.6904	0.6462	5.05	20.97	10.82	80	− 6.30	− 31.35–82.50
$22.53 + 2.08 \times C_2 – 0.52 \times C_3 + 7.66 \times C_{12}$	0.5600	0.4971	7.11	28.67	13.39	80	− 5.80	− 30.08–134.03

Table 3 The best LSSs in C_6 group

	r^2	r^2 adjusted	%ME	%MAE	%RMSE	Good guess (%)	Median error	95% CI
STATISTICA equations								
$1.62 + 2.22 \times C_0 + 1.27 \times C_1 + 2.32 \times C_3 + 1.32 \times C_4 + 3.07 \times C_6$	0.9477	0.9444	− 0.39	2.87	3.92	94	− 0.33	− 3.09–3.86
$2.08 + 1.85 \times C_0 + 1.28 \times C_1 + 2.88 \times C_3 + 3.67 \times C_6$	0.9289	0.9244	− 1.43	3.57	4.70	89	− 0.78	− 5.65–2.79
$2.55 + 1.28 \times C_1 + 2.98 \times C_3 + 4.76 \times C_6$	0.8838	0.8765	− 2.78	4.66	6.20	83	− 1.19	− 9.85–0.46
$2.87 + 0.81 \times C_1 + 2.74 \times C_2 + 5.69 \times C_6$	0.8542	0.8451	0.72	5.76	7.28	72	2.27	− 5.33–7.69
$12.67 + 1.19 \times C_1 + 6.38 \times C_6$	0.7504	0.7348	− 0.57	6.79	8.31	72	− 1.71	− 8.15–10.27
$2.71 + 1.36 \times C_1 + 1.39 \times C_2 + 5.60 \times C_4$	0.7246	0.7074	2.05	7.56	10.08	72	1.60	− 4.92–13.01
$1.37 + 4.66 \times C_0 + 1.39 \times C_1 - 0.14 \times C_2 + 1.64 \times C3 + 3.30 \times C_4$	0.7101	0.6920	2.40	7.79	9.28	72	7.14	− 1.87–15.17
$1.27 + 4.59 \times C_0 + 1.37 \times C_1 + 1.46 \times C_3 + 3.46 \times C_4$	0.7197	0.7022	2.60	7.68	9.21	72	7.44	− 1.47–15.31
R equations								
$3.59 + 1.25 \times C_1 + 3.06 \times C_3 + 4.65 \times C_6$	0.9522	0.9479	1.20	9.19	4.72	89	1.56	− 19.70–33.78
$5.81 + 0.97 \times C_1 + 2.20 \times C_2 + 4.69 \times C_6$	0.9353	0.9295	3.14	12.12	5.47	84	1.23	− 18.04–51.55
$2.39 + 3.97 \times C_0 + 1.44 \times C_1 + 3.38 \times C_3$	0.9017	0.8928	0.57	11.93	6.75	84	− 2.34	− 19.38–31.92
$6.32 + 1.18 \times C_1 + 3.87 \times C_4 + 4.50 \times C_6$	0.9111	0.9030	4.79	14.48	6.36	81	2.39	− 21.28–53.71
$6.09 + 1.48 \times C_1 + 2.46 \times C_3 + 2.86 \times C_4$	0.8438	0.8296	3.05	15.44	8.52	81	1.21	− 31.76–50.82
$9.78 + 2.45 \times C_2 + 2.00 \times C_4 + 5.85 \times C_6$	0.7864	0.7670	6.66	20.12	9.88	81	2.34	− 35.15–90.49
$5.43 + 0.90 \times C_1 + 5.11 \times C_6 + 7.33 \times C_{12}$	0.9097	0.9015	2.93	14.87	6.51	81	− 0.48	− 26.75–46.08
$5.98 + 3.67 \times C_0 + 1.20 \times C_1 + 2.25 \times C_2$	0.8473	0.8334	4.26	15.63	8.35	78	1.47	− 31.66–42.90
$5.12 + 2.00 \times C_2 + 5.77 \times C_6 + 7.19 \times C_{12}$	0.8504	0.8369	4.07	14.78	8.26	78	2.44	− 30.44–54.95

LSSs equations

The best equations for both evaluations methods are presented in Tables 2 and 3. Some of the other LSSs are included in Tables A.1 and B.1 (Appendices A and B).

For evaluations with STATISTICA software 53 and 48 equations in total, consisting of one up to seven concentration time points were obtained for C_9 and C_6 groups, respectively. For evaluations with R software, each model composed of one, two, or three time points and 92 models and 63 models were obtained in total for C_9 group and C_6 group, respectively.

For STATISTICA calculations, firstly, the equations included more than three time points. In total for both groups (C_6 and C_9), there were one equation with six time points, four with five time points, and four with four time points (Tables 2 and 3; Table A.1). The best r^2, adjusted r^2, and good guess (94%) were for the equation including C_0, C_1, C_3, C_4, C_6 for C_6 group. The appropriate good guess (89%), r^2, and adjusted r^2 (> 0.900) were obtained for four time point equation (C_0, C_1, C_3, C_6) for C_6 group. The equation with six time points (C_0, C_1, C_3, C_4, C_9, C_{12}) should be precise; however, its good guess amounted to only 58%.

For the C_9 group and equations with three time points, three best equations included C_1, C_2, C_3; C_1, C_3, C_6; and C_0, C_1, C_4 for STATISTICA software; however, the good guess values were rather poor for these equations (67%, 58%, 50%, respectively; Table 2). For R software, three best equations consisted of C_1, C_3, C_6; C_0, C_1, C_3; and C_0, C_1, C_2. Good guess was > 80% for five best equations evaluated with R, whereas for STATISTICA evaluations, the highest good guest amounted to 67%. The values of r^2 and adjusted r^2 for R evaluations were > 0.800 for two best equations and for one equation (C_1, C_4, C_9) with good guess 72%. The values of r^2

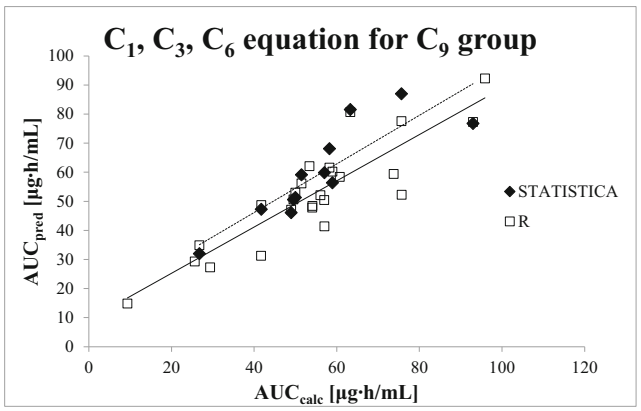

Fig. 2 The correlations between AUC_{pred} derived from C_1, C_3, C_6, and AUC_{calc} from 0 to 12 h MPA pharmacokinetic profile in C_9 group. The equations including C_1, C_3, and C_6 were evaluated with STATISTICA (closed diamonds; dotted line) and R (open squares; solid line). The equations were $9.34 + 1.49 \cdot C_1 + 3.51 \cdot C_3 + 2.74 \cdot C_6$ for STATISTICA and $7.10 + 1.21 \cdot C_1 + 3.75 \cdot C_3 + 3.08 \cdot C_6$ for R

and good guess were better for the equations evaluated with R software, whereas %MAE was lower for STATISTICA evaluations (Table 2; Table A.1). In Fig. 2, we compared the correlations between AUC_{pred} and AUC_{calc} for the best equation (C_1, C_3, C_6) estimated with STATISTICA and R in C_9 group.

For C_6 group and STATISTICA, the best three time point equations included C_1, C_3, C_6 (with the best good guess, i.e., 83%); C_1, C_2, C_6; and C_1, C_2, C_4. For R software, three best equations included C_1, C_3, C_6 (with the best good guess, i.e., 89%); C_1, C_2, C_6; and C_0, C_1, C_3. For STATISTICA evaluations, two equations with three time-points were characterized by r^2 and adjusted $r^2 > 0.800$ with good guess 83% and 72%. For R evaluations, good guess for seven equations was > 80%. The values of r^2 and adjusted r^2 were > 0.900 for four equations. For the C_6 group, the values of r^2, adjusted r^2, and good guess were also better for the evaluations with R software; however, the differences were smaller than within C_9 group for some equations as well as %MPE, %MAE, and median error were lower for STATISTICA evaluations (Table 3). In Fig. 3, we compared the correlations between AUC_{pred} and AUC_{calc} for the best equation (C_1, C_3, C_6) estimated with STATISTICA and R in C_6 group.

The best equations with two time points included C_1, C_6 for both groups and STATISTICA calculations, whereas for R calculations, C_1, C_3 and C_1, C_6 were the best for C_9 and C_6 groups, respectively. The good guess values were better for the R results (Tables 2 and 3).

For one time point equation, the highest good guess was 72% (C_1) for R evaluations and 58% (C_6) for STATISTICA for C_9 group (Table 2; Table A.1). For C_6 group, the best good guess was 68% (C_1) and 50% (C_1) for R and STATISTICA, respectively (data not shown). In C_9 group, the one time point equation with C_0 was characterized by the good guess of 72% for R calculations but only of 17% for STATISTICA (data not shown). For C_6 group, the good guess for C_0 equations amounted to 62% for R and 28% for STATISTICA (data not shown).

Among the best 47 equations presented in Tables 2 and 3, about 41% (14/47) included C_0.

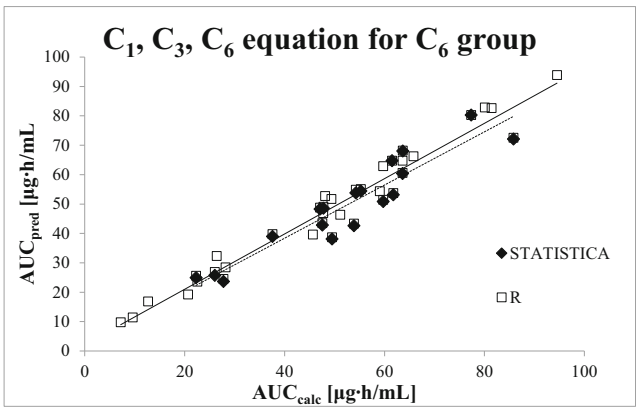

Fig. 3 The correlations between AUC_{pred} derived from C_1, C_3, C_6, and AUC_{calc} from 0 to 12 h MPA pharmacokinetic profile in C_6 group. The equations including C_1, C_3, and C_6 were evaluated with STATISTICA (closed diamonds; dotted line) and R (open squares; solid line). The equations were $2.55 + 1.28 \cdot C_1 + 2.98 \cdot C_3 + 4.76 \cdot C_6$ for STATISTICA and $3.59 + 1.25 \cdot C_1 + 3.06 \cdot C_3 + 4.65 \cdot C_6$ for R

Discussion

The MPA pharmacokinetic parameters are highly variable, and there are numerous factors which may contribute to this variability, e.g., treatment duration, therapeutic indication, drugs co-administered, genetic, physiologic, and environmental factors, as well as kidney or liver dysfunction [8]. For TDM, MPA $AUC_{0–12}$ is the most useful parameter; however, obtaining full pharmacokinetic profile is time-consuming and inconvenient for patients, especially for children [25], and there is still a need to establish target values for children with nephrotic syndrome [8]. In our previous study [22], we suggested that for those children, MPA $AUC_{0–12} > 60$ μg h/mL may be considered efficient to avoid proteinuria recurrence and ensure the safe and effective treatment. Moreover, we did not observe any toxicity in those children. Other studies showed that MPA $AUC_{0–12}$ above 30 μg h/mL is recommended for children with nephrotic syndrome [26] or described fewer relapses in children with MPA $AUC_{0–12}$ above 45 μg h/mL [8]. However, there are some cases where MMF is ineffective when standard doses are administered. The recent study of Kirpalani et al. [27] described the increase in MPA apparent clearance which may indicate that the unresponsiveness of MMF may be due to MPA underexposure. Therefore, it seems important to find if the reason why children do not respond to MMF is dose-related (infra or over dosing) and if so correct the dose before changing the treatment to other drugs with severe adverse effects (such as CsA or cyclophosphamide) in these children. The evaluation of MPA LSS should facilitate assessing treatment efficacy in pediatric patients.

There are two main approaches to evaluate LSS, Bayesian estimation and MLR, which can be performed using several software programs. NONMEM, a nonlinear mixed-effect modeling tool, is the gold standard for population pharmacokinetic analysis. However, working with this program is difficult because NONMEM is written in Fortran language [20, 21]. Therefore, we decided to evaluate and compare the LSSs using other software, STATISTICA and R. Both programs rely on MLR as computation methods. R software comprises powerful statistical techniques, supports object-oriented programming, and its accessible free of charge [20]. The STATISTICA line of software consists of a fully integrated line of analytic solutions which are easy to handle and offer wide options of algorithms, functions, and tests as well as effective graphic visualization.

Table 4 Multilinear regression equations found in the literature for predicting mycophenolic acid AUC_{0-12} for patients treated with mycophenolate mofetil (MMF)

Study group	MMF indication	Drugs co-administered	Equation	r^2	%MPE	Reference
Adults	Lung transplantation	Tacrolimus or cyclosporine	$\log AUC_{0-12} = 1.14 + 0.241 \times \log C_0 + 0.406 \times \log C_2$ $\log AUC_{0-12} = 1.09 + 0.202 \times \log C_0 + 0.411 \times \log C_{1.5}$	0.828 0.791	−5.82 −5.71	[30]
Adults	Heart transplantation	Cyclosporine	$AUC_{0-12} = 9.69 + 0.63 \times C_{0.5} + 0.61 \times C_1 + 2.20 \times C_2$	0.841	3.2	[9]
Adults	Kidney transplantation	Tacrolimus	$AUC_{0-12} = 4.24 + 2.05 \times C_2 + 8.51 \times C_7 + 2.29 \times C_{12}$	0.940	1.15	[13]
Adults	Kidney transplantation	Tacrolimus	$AUC_{0-12} = 7.951 + 1.893 \times C_2 + 4.04 \times C_6 + 4.542 \times C_{10}$ $AUC_{0-12} = 4.272 + 0.859 \times C_{0.5} + 1.896 \times C_2 + 4.074 \times C_6 + 4.680 \times C_{10}$	0.863 0.918	−0.3 −0.2	[14]
Adults	Kidney transplantation	Tacrolimus	$AUC_{0-12} = 3.542 + 3.332 \times C_{0.5} + 1.117 \times C_{1.5} + 3.946 \times C_4$ $AUC_{0-12} = 8.149 + 1.442 \times C_2 + 1.056 \times C_4 + 7.133 \times C_6$	0.900 0.88	1.7 −0.2	[16]
Adults	Heart transplantation	Cyclosporine	$AUC_{0-12} = 5.568 + 0.902 \times C_{1.25} + 2.022 \times C_2 + 4.594 \times C_6$ $AUC_{0-12} = 3.800 + 1.015 \times C_{1.25} + 1.819 \times C_2 + 1.566 \times C_4 + 3.479 \times C_6$	0.926 0.948	No data No data	[31]
Adults	Liver transplantation	Tacrolimus	$AUC_{0-12} = 6.03 + 0.89 \times C1 + 1.94 \times C2 + 2.24 \times C6 + 4.64 \times C8$	0.911	1.18	[15]
Children	Liver transplantation	Tacrolimus or cyclosporine	$AUC_{0-12} = 9.1 + 5.7 \times C_0 + 1.1 \times C_{40\ min} + 2.1 \times C_2$ $AUC_{0-12} = 5.2 + 7.1 \times C_0 + 1.0 \times C_{75\ min} + 5.4 \times C_6$	0.740 0.880	No data	[29]
Children	Kidney transplantation	Tacrolimus or cyclosporine	$AUC_{0-12} = 12.62 + 7.78 \times C_0 + 0.90 \times C_1 + 1.30 \times C_2$	0.750	No data	[11]
Children	Kidney transplantation	Cyclosporine	$AUC_{0-12} = 9.55 + 4.50 \times C_0 + 0.88 \times C_{0.5} + 2.67 \times C_2$ $AUC_{0-12} = 9.87 + 0.90 \times C_1 + 1.73 \times C_2 + 6.86 \times C_8$	0.770 0.910	6.48 3.56	[10]
Children	Kidney transplantation	Tacrolimus	$AUC_{0-12} = 10.01391 + 3.94791 \times C_0 + 3.24253 \times C_{0.5} + 1.0108 \times C_2$ $AUC_{0-12} = 8.217 + 3.163 \times C_0 + 0.994 \times C_1 + 1.334 \times C_2 + 4.183 \times C_4$	0.900 0.946	No data	[12]
Children	Kidney transplantation	Tacrolimus or cyclosporine	$AUC_{0-12} = 7.73 + 0.94 \times C_1 + 2.55 \times C_2 + 5.48 \times C_6$	0.845	No data	[28]

AUC_{0-12} area under the time-concentration curve from 0 to 12 h, *%MPE* mean prediction error

To our knowledge, in the literature, there are only a few studies concerning LSS as the approach for monitoring MPA therapy in children and adults with nephrotic syndrome. In none of the studies, MLR method was applied for evaluations. Saint-Marcoux et al. [18] and Zhao et al. [19] evaluated LSS for children with nephrotic syndrome basing on Bayesian estimator method. Some LSS for MMF treated patients evaluated using MLR concerned pediatric patients after renal [10–12, 28] or liver [15, 29] transplantation as well as adults patients after renal [13, 14, 16], lung [30], heart [9, 31], and liver [15] transplantation. The equations from these studies are shown in Table 4.

We assumed that it would be the most convenient procedure for patients if the LSS evaluated includes up to three time points. Therefore, the equations with four to six time points, generated with STATISTICA, although characterized by the best good guess (up to 94%) will not be useful in clinical practice. The most convenient approach for patients would be collecting only one or two blood samples; however, in our study, the results obtained for these equations were not satisfactory comparing with the three time point equations.

The best three time point equations in our study included C_1, C_3, C_6; C_1, C_2, C_6; and C_1, C_2, C_4 for evaluations with STATISTICA software and C_1, C_3, C_6; C_1, C_2, C_6; C_0, C_1, C_2; and C_0, C_1, C_3 for evaluations with R software. The same time points were included in the best equation for R calculations for both (C_6 and C_9) groups. The literature data with LSS for pediatric patients included $C_{20\ min}$, $C_{60\ min}$, $C_{180\ min}$ [18], or C_0, C_1, C_4 [19]; however, these LSS were evaluated based on Bayesian estimator. Among MLR equations found in the literature, not only none of them included C_1, C_2, C_3 and C_1, C_3, C_6 time points, but also none of them considered children with nephrotic syndrome. Therefore, we suggest that LSS should be validated and used only in the population for which it was developed. It is in accordance with our previous data [22] as we concluded that MPA pharmacokinetic parameters must be calculated and applied separately for patients after renal transplantation and with nephrotic syndrome. The differences may derive from the MPA pharmacokinetic intra- and intervariability.

Apart from including three time points, the most useful equations should also contain time points collected up to 3 h after drug administration because most of the children are not hospitalized but treated in clinics. Therefore, although C_1, C_3, C_6 comprised the best equation, collecting C_0, C_1, C_3 or C_0, C_1, C_2 should be more practical. However, this approach in the case of MPA may lead to misprediction of AUC_{0-12} due to MPA enterohepatic recirculation and the occurrence of MPA second C_{max}. According to the literature, the most accurate LSS should probably include at least one blood sample collected at time point close to second C_{max} of MPA due to MPA enterohepatic recirculation [32].

In our study, only a few equations included C_0 which is in accordance with the literature data as MPA C_0 did not predict the response to the drug sufficiently [9].

In general, the results obtained using STATISTICA and R were different and better for R. Apart from two best three time point equations for C_6 group which are the same, the subsequent equations differ. The best equation for C_9 group for STATISTICA (C_1, C_2, C_3) is the sixth for R calculations. The second best equation for STATISTICA (C_1, C_3, C_6) is the best for R. Better values of r^2, adjusted r^2, and good guess were obtained for LSS generated with R program. The difference may be explained by different validations for both computing methods. At the same time, %MPE and %MAE were lower for STATISTICA results. The difference is smaller for C_6 group as this group was more numerous. The greater number of patients contributed to the better results for STATISTICA in C_6 group than C_9 group. We assumed that test group and validation group should be more numerous to achieve better results with STATISTICA.

The limitation of our study is the fact that we were unable to collect the samples more frequently, especially within the first 2 h after MMF administration. More studies on the larger number of pediatric patients are needed to confirm our observations. Other limitations are the inclusion of only Caucasian children, children receiving steroids, and children with trace proteinuria during the day of blood collection. These factors may influence MPA exposure and limit the generalizability of the study.

Conclusion

The best equations in our study included C_1, C_3, C_6; C_0, C_1, C_3; and C_0, C_1, C_2. The most useful in everyday practice equations include C_0, C_1, C_3 and C_0, C_1, C_2; however, the most precise is the equation including C_1, C_3, C_6 time points. Better results were obtained with R software.

For validation method, the number of patients is essential; therefore, better results were obtained with R calculations and the bootstrap validation as validation groups for STATISTICA calculations were smaller.

The proposed equations may be an useful implement for MPA monitoring in children with nephrotic syndrome treated with MMF as there is growing evidence that underexposure of MPA is associated with insufficient treatment response. Still, it is important to remember that properly validated LSS should be used only in the population for which it was developed.

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Authors' individual contributions JS, TP, DO-N, and MC designed the study; JS, MR, DO-N, and JZ collected the data; JS and MR carried out the quantifications of analytes in blood; JS, TP, and PŻ carried out the

statistical analyses; JS, TP, and MC analyzed data; and JS and TP wrote the first draft of the manuscript. All authors contributed to, and have approved, the final manuscript.

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Compliance with ethical standards

The study was approved by the Bioethical Committee at Poznan University of Medical Sciences.

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent to participate was obtained from all parents or guardians of individual participants included in the study.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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