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Pharmacokinetic Study of Mycophenolate Mofetil in Patients with Systemic Lupus Erythematosus and Design of Bayesian Estimator Using Limited Sampling Strategies

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Abstract

Background: Monitoring of the area under the plasma concentration-time curve (AUC) of mycophenolic acid (MPA) has been developed for individual dose adjustment of mycophenolate mofetil (MMF) in renal allograft recipients. MMF is currently used as an off-label drug in the treatment of systemic lupus erythematosus (SLE), but factors of its exposition may be different in these patients and need to be determined for therapeutic drug monitoring (TDM) purposes.

Objective: The aim of the study was to develop a maximum *a posteriori* probability (MAP) Bayesian estimator of MPA exposition in patients with SLE, with the objective of TDM based on a limited sample strategy.

Methods: Twenty adult patients with SLE given a stable 1 g/day, 2 g/day or 3 g/day dose of MMF orally for at least 10 weeks were included in the study. MPA was measured by high-performance liquid chromatography (HPLC) coupled to a photodiode array detector (11 plasma measurements over 12 hours post-dose per patient). Free MPA concentrations were measured by HPLC with fluorescence detection. Two different one-compartment models with first-order elimination were tested to fit the data: one convoluted with a double γ distribution to describe secondary concentrations peaks, and one convoluted with a triple γ distribution to model a third, later peak.

Results: A large interindividual variability in MPA concentration-time profiles was observed. The mean maximum plasma concentration, trough plasma concentration, time to reach the maximum plasma concentration and AUC from 0 to 12 hours (AUC_{12}) were 13.6 ± 8.4 μg/mL, 1.4 ± 1.2 μg/mL, 1.1 ± 1.2 hours and 32.2 ± 17.1 μg • h/mL, respectively. The mean free fraction of MPA was 1.7%. The one-compartment model with first-order elimination convoluted with a triple γ distribution best fitted the data. Accurate Bayesian estimates of the AUC_{12} were obtained using three blood samples collected at 40 minutes, 2 hours and 3 hours, with a coefficient of correlation (R) = 0.95 between the observed and predicted AUC_{12} and with a difference of <20% in 16 of the 20 patients.

Conclusion: A specific pharmacokinetic model was built to accurately fit MPA blood concentration-time profiles after MMF oral dosing in SLE patients, which allowed development of an accurate Bayesian estimator of MPA exposure that should allow MMF monitoring based on the AUC_{12} in these patients. The predictive value of targeting one specific or different AUC values on patients' outcome using this estimator in SLE will need to be evaluated.

Background

Mycophenolate mofetil (MMF) is an immunosuppressant agent that is extensively used for prevention of allograft rejection in combination with ciclosporin or tacrolimus after organ transplantation.[1] Recently, the predictive value of MMF exposition for efficacy against allograft rejection has been established using the area under the plasma concentration-time curve (AUC) from 0 to 12 hours (AUC_{12}) of mycophenolic acid (MPA, the active metabolite of MMF) between two dose intakes at steady state. A randomized concentration-controlled trial has suggested that the incidence of acute rejection is minimized when the values of the AUC_{12} of MPA lie between 30 and 60 μg • h/mL.[2] However, determination of the individual AUC_{12} of any drug using the standard pharmacokinetic approach is difficult in routine clinical practice since it requires multiple (10–12) blood samples over a 12-hour period. Previous studies have proposed an abbreviated MPA AUC to substitute for the full MPA AUC.[3-11] We have developed a Bayesian estimation of the concentration-time area at steady state between two drug intakes, which allows AUC_{12} estimation using only three points of plasma MPA concentrations and has been used successfully for monitoring of MMF in renal transplantation.[12]

Because MPA downregulates adhesion molecule expression and reduces lymphocyte and monocyte adhesion,[13] it has recently been used in the treatment of autoimmune diseases, especially in systemic lupus erythematosus (SLE).[14] Indeed, several randomized trials have demonstrated the efficacy of MMF in lupus nephritis in both the maintenance[15,16] and induction phases.[17] In contrast to patients after organ transplant, lupus patients do not receive calcineurin inhibitors, which are known to affect the pharmacokinetic parameters of MPA.[18,19] Ciclosporin has been shown to reduce plasma concentrations of MPA by inhibiting the biliary excretion of MPA 7-*O*-glucuronide (MPAG, the inactive main MPA metabolite), leading to reduction of the enterohepatic cycle of MPA.[20,21] Indeed, Neumann et al.[22] have demonstrated that MPA pharmacokinetic parameters such as the maximum plasma concentration (C_{max}) and the concentration at 12 hours (C_{12}) are different in renal transplant recipients compared with autoimmune disease patients. Whether the Bayesian estimation model developed for renal transplantation can be used for SLE patients must therefore be questioned. We therefore conducted a study with the objective of setting up the parameters of the pharmacokinetic model of MPA in patients with SLE who do not receive calcineurin inhibitors in order to develop and validate a maximum *a posteriori* probability (MAP) Bayesian estimator of MPA exposition in these patients using a limited sampling strategy (LSS).

Patients and Methods

Patients

Twenty consecutive adults with a diagnosis of SLE fulfilling the American College of Rheumatology criteria[23] were included in the study. Written informed consent was obtained from all patients. The study protocol was approved by the local ethics committee.

MMF was administrated at a stable dosage (0.5 g, 1 g or 1.5 g twice daily) for at least 10 weeks, in addition to prednisone. These doses were given according to the clinical stage of SLE and not because of adverse effects with higher doses. Pregnant and breastfeeding females were excluded.

None of the patients enrolled was taking drugs known to interact with MMF (aciclovir, antacids, cholestyramine, ganciclovir, metronidazole, iron, tacrolimus or ciclosporin).

Pharmacokinetic Study Protocol

MMF was administrated orally twice daily before the pharmacokinetic investigations. The patients were required to fast overnight before dosing. Blood samples were drawn immediately before administration of MMF and 20 minutes, 40 minutes and 1, 1.5, 2, 3, 4, 6, 8 and 12 hours after administration. All blood samples were collected in tubes containing EDTA as an anticoagulant, and the plasma was separated and stored at −20°C until analysis. Three standard meals were served 1, 5 and 10 hours, respectively, after the first blood sample was drawn in all patients.

Pharmacokinetic Analyses

Samples were analyzed for MPA and MPAG by high-performance liquid chromatography (HPLC) coupled to a photodiode array detector at a 250 nm wavelength, using a method developed by Westley et al.[24] with some modifications. The method proved to be accurate and precise in the range of 0.1–20 μg/mL, and the within-day precision (coefficient of variation [CV]) was <10%. The lower limits of quantification (LLQs) for MPA and MPAG were 0.1 and 0.5 μg/mL, respectively.

The free MPA concentration in plasma was determined 20 minutes, 40 minutes and 1 hour post-dose by ultrafiltration followed by fluorescence detection, modified from the method described by Shen et al.[25] The LLQ with such detection was 5 ng/mL (accuracy 4%, CV 3.2%).

The prednisolone plasma concentration was determined for all samples by HPLC coupled to UV detection at a 242-nm wavelength, using a modification of the method of Alvinerie et al.,[26] and the LLQ was 20 ng/mL (CV 4.4%).

Pharmacokinetic Modelling

Two pharmacokinetics models were tested:

- The one-compartment model with first-order elimination convoluted with a double γ absorption phase, describing an early peak and a possible secondary peak, which has been previously described by Premaud et al.[12] This model is currently used for AUC_{12} estimation in renal transplantation.
- Another γ model of absorption with three parallel absorption routes. The absorption rate at time t was described by a sum of three γ distributions (equation 1):

$$V_{abs(t)} = F \bullet D\sum_{i=1}^{3} r_i \bullet f_i(t), \text{ with:}$$

$$f_i(t) = \frac{b_i^{a_i} \bullet t^{a_i - 1} \exp(-b_i \bullet t)}{\Gamma(a_i)}$$

(Eq. 1)

where F is the bioavailability coefficient, D is the administered dose, Γ is the Gamma function, a_i and b_i are the parameters of the Gamma distributions, r_i is the fraction of the drug absorbed through the ith route, and f_i is the probability density function for the Gamma distribution. The disposition kinetics were described by a one-compartment model, according to equation 2:

$$I(t) = A_{IV} \exp(-\alpha \bullet t)$$

(Eq. 2)

where I(t) is the drug concentration at time t following an intravenous (IV) bolus of a unit dose D_0, A_{IV} is the disposition coefficient and α is the disposition rate constant.

The convolution product of the absorption rate and disposition function was computed analytically, as described previously[12,27] (equation 3):

$$C(t) = C_0 + F \bullet D\, A_{IV} \exp(-\alpha \bullet t)\sum_{i=1}^{3} r_i\, [b_i/(b_i - \alpha)]^{a_i} \bullet P[a_i,(b_i - \alpha)t]$$

(Eq. 3)

where C(t) is the concentration at time t and C_0 is the trough concentration. P, the incomplete Gamma function, is represented by equation 4:

$$P(n, x) = [1/\Gamma(n)]\int_0^x z^{n-1} \bullet \exp(-z) \bullet dz$$

(Eq. 4)

where n is the exponent (analogous to a_i), x is the argument of the function (analogous to t), z is the integration variable and dz is its differential.

The population pharmacokinetic parameters were determined by the iterative two-stage method of Steimer et al.[28]

The apparent volume of distribution (V_d/F) and apparent clearance (CL/F) after oral administration were computed from (equation 5):

$$V_d/F = \frac{D_0}{A_{IV}}$$

$$CL/F = \frac{D}{AUC}$$

(Eq. 5)

where AUC is the area under the plasma concentration-time curve during the time between two drug administrations.

Design of Maximum *A Posteriori* Bayesian Estimators

The best LSS was selected on the basis of combination of a maximum of three sampling times within 3 hours post-dose, as it seemed to be a good compromise between the precision of parameters estimated and possible implementation in clinical practice.

The LSS was tested with respect to its predictive performance for estimation of the AUC_{12} by studying the correlation between the estimated and trapezoidal AUC_{12}, the mean relative bias and the percentage of patients with acceptable estimation (within ±20% of the trapezoidal AUC_{12}).[29]

Validation of Bayesian Estimators

Due to the small number of patients, it was not possible to divide them into two groups. The model was validated using circular permutation (the so-called 'jack-knife' method)[28] excluding the three time-concentration profiles of each patient in turn, building a reference population and Bayesian estimator with the 57 concentration-time profiles of the 19 remaining patients in the group, estimating the pharmacokinetic parameters and exposure indices of the excluded patient and comparing them with the measured values or the parameters computed using the full concentration-time profiles.

Results

Patient Characteristics

The patients were 15 women and 5 men with a mean age at sampling of 35 years and a diagnosis of SLE. All were treated by MMF for at least 10 weeks at a dosage of 1 g/day (n = 7), 2 g/day (n = 11) or 3 g/day (n = 2). For all patients, a physical examination and standard laboratory analyses, including haematological, biochemical and immunological tests, were performed. The main patient characteristics are shown in table I.

Table I. Characteristics of the 20 patients with systemic lupus erythematosus[a]

Parameters	Value
Male (%)	25
Age (y)	35 ± 11.6; 35 (69–18)
Bodyweight (kg)	63.3 ± 10.1; 66 (82–45)
Serum creatinine (μmol/L)	86.1 ± 56.4; 76 (303–43)
Serum albumin (g/L)	38.8 ± 6.9; 37 (50–27)
Proteinuria (g/L)	1.1 ± 1.8; 0.3 (6.9–0.1)
Haemoglobin (g/dL)	11.9 ± 1.8; 11.7 (15.2–8.9)
Leukocytes ($\times 10^{-3}$/mm^3)	6.9 ± 2.8; 6.42 (12.9–2.9)
Platelets ($\times 10^{-3}$/mm^3)	279 ± 104; 273 (514–127)
Anti-double-stranded DNA antibodies (IU/L)	60.5 ± 87.1; 40 (374–2.3)
Complement C3 (g/L)	0.9 ± 0.4; 0.89 (1.71–0.32)

a The values are expressed as the mean ± SD; median (range) unless specified otherwise.

Mycophenolic Acid (MPA) Pharmacokinetics

Table II summarizes the pharmacokinetics parameters of MPA and the metabolic AUC ratio (MPAG **:** MPA) obtained with the standard noncompartmental analysis. The pharmacokinetic profiles of MPA in patients treated with 1 g twice daily are presented in figure 1. The individual plasma MPA concentration-time curves showed important between-subject variability, with one, two or three concentration peaks. The first and second peaks occurred during the first 2 hours of the absorption phase. A third peak occurred 6–12 hours following administration and corresponded to the enterohepatic cycle. The amplitude of the three peaks varied widely between subjects (see figures 1 and 2).

Models

With the double γ distribution model – the one used for renal transplant recipients – the mean ± SD delta between the observed and estimated AUC_{12} was −8.6 ± 11.5%. A model with triple γ distribution provided a lower bias between the estimated and observed data: mean ± SD 1.6 ± 13% ($p = 0.008$ by the Mann-Whitney U test). With this model, the AUC_{12} estimation error was lower than ±10% in 18 individuals. Two profiles exhibited an estimation error of >20% (34.8%, 46.7%). Overall, the correlation between the estimated and observed concentrations was very good with this model (coefficient of correlation [R] = 0.9858; see figure 3a).

Bayesian Estimation of MPA Exposition

With the double γ distribution model, all possible limited sampling strategies failed to provide an acceptable difference between the observed and estimated AUC_{12}. Indeed, all possible strategies gave a difference of >20% in at least five patients.

Table II. Pharmacokinetic parameters and exposure indices of mycophenolic acid (MPA) and MPA 7-*O*-glucuronide (MPAG) calculated using nonlinear regression in the study participants[a]

Parameter	Value			
	n = 7	n = 11	n = 2	All (n = 20)
MMF daily dosage (mg)	1000	2000	3000	1750 ± 639
MPA AUC_{12} (μg • h/mL)	23.3 ± 8.8	33.6 ± 16	55.2 ± 27.7	32.2 ± 17.1
DN MPA AUC_{12} (μg • h/mL/g)	50.8 ± 21.7	33.6 ± 16	35.1 ± 19	45.1 ± 16.7
C_{max} (μg/mL)	8.8 ± 5.4	13.5 ± 6	31 ± 5.7	13.6 ± 8.4
t_{max} (h)	0.7 ± 0.4	1.5 ± 1.6	0.6	1.1 ± 1.2
C_0 (μg/mL)	0.9 ± 0.7	1.3 ± 1.3	2.6 ± 2	1.4 ± 1.2
V_d/F (L)	27 ± 14.9	38.4 ± 21	21.6 ± 4	32.7 ± 18.6
CL/F (L/h)	23.7 ± 10.5	52 ± 66.6	33.4 ± 18.1	40.3 ± 50.7
MPAG AUC (μg • h/mL)	350.5 ± 158.5	629.3 ± 370.4	601 ± 243.4	522.7 ± 324.4
AUC ratio (MPAG **:** MPA)	14.5 ± 6.8	17 ± 6.9	10.8 ± 1.4	15.6 ± 6.6
MPA free fraction (%)	1.8 ± 0.9	2.1 ± 1.8	2 ± 0.3	1.7 ± 0.9
MPA free fraction (ng/mL)	82 ± 70.5	95.3 ± 74	235.4 ± 206.2	103.5 ± 94.1

a The values are expressed as the mean ± SD.

AUC = area under the plasma concentration-time curve; **AUC_{12}** = AUC from 0 to 12 h; **C_0** = trough plasma concentration; **CL/F** = apparent oral clearance; **C_{max}** = peak plasma concentration; **DN MPA AUC_{12}** = dose-normalized MPA AUC_{12}; **MMF** = mycophenolate mofetil; **t_{max}** = time to reach the C_{max}; **V_d/F** = apparent volume of distribution after oral administration.

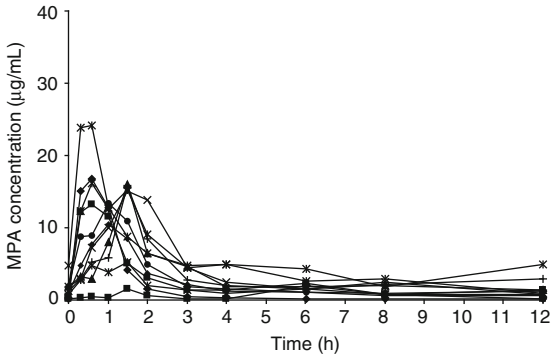

Fig. 1. Mycophenolic acid (MPA) concentration-time profiles obtained in 11 systemic lupus erythematosus patients treated with mycophenolate mofetil 1 g twice daily.

With the triple γ distribution model, the best sampling strategy with respect to AUC_{12} estimation and the D-optimality criterion for Bayesian estimation was 40 minutes, 2 hours and 3 hours. With this sampling strategy, the observed and estimated AUC_{12} correlation was good (R = 0.9506) [figure 3b]. The difference between the observed and estimated AUC_{12} was <20% in 16 of 20 patients (mean difference 6.42%) [table III] and in 14 patients, the bias was within ±12%. Four patients had a difference of slightly over 20% (24%, −21.7%, 27.4% and 30.2%).

To assess the agreement between the AUC calculated from all 11 sampling points and the abbreviated AUC, Bland and Altman analysis was performed. This analysis indicated good agreement without values beyond the mean ± 2 SD and an average error of 2.11 μg • h/mL (figure 4).

Typical examples of computed concentration-time curves are shown in figure 2 with the position of the three points at 40 minutes, 2 hours and 3 hours.

Major Factors Possibly Influencing the MPA Area under the Plasma Concentration-Time Curve in Systemic Lupus Erythematosus Patients

Effect of Serum Albumin Levels

Seven of 20 patients had low albumin levels (<37 g/L). Serum albumin levels and MPA clearance were negatively correlated (R = −0.6, p = 0.008). A significant positive correlation was found between the MPA AUC_{12} and serum albumin levels (R = 0.55, p = 0.016). The mean prediction error between the observed and estimated MPA AUC was similar in the group of SLE patients with low albumin levels compared with the group with normal serum albumin levels (p = 0.36 by the Mann-Whitney U test).

Effect of Prednisolone Pharmacokinetics

All SLE patients were treated with prednisone (mean daily dose 16.3 ± 6.7 mg). The mean C_{max}, C_{12} and AUC_{12} of prednisolone (active metabolite) were 167 ± 80 ng/mL, 29 ± 17 ng/mL and 881 ± 376 ng • h/mL, respectively. We did not find a significant correlation between the MPA AUC_{12} and the prednisolone AUC_{12} (R = 0.138; p = 0.5 by the Spearman rank correlation test), the MPAG AUC_{12} and the prednisolone AUC_{12}, the prednisolone AUC_{12} and the MPGA **:** MPA ratio, and daily doses of prednisone and the MPA AUC_{12} (data not shown).

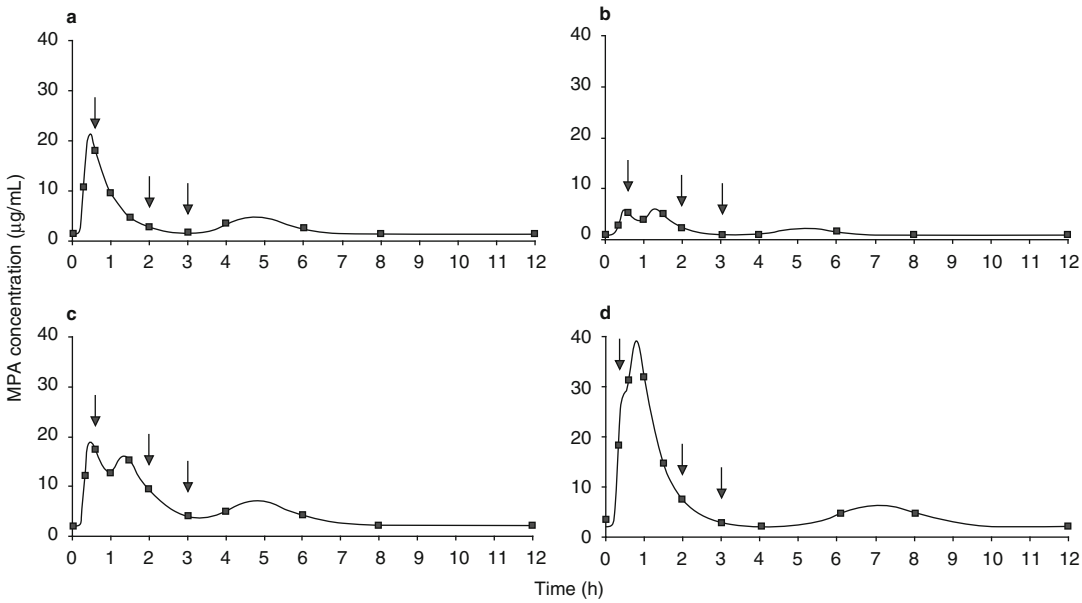

Fig. 2. Typical examples of Bayesian estimates of mycophenolic acid (MPA) pharmacokinetics in patients (**a**–**d**) with systemic lupus erythematosus. The arrows indicate the data points included in the limited sampling strategies.

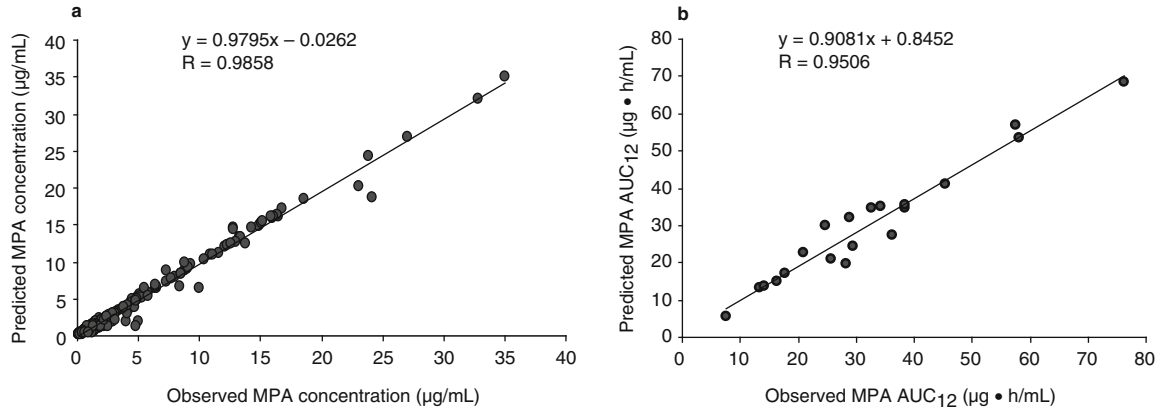

Fig. 3. (**a**) Plots of observed versus maximum *a posteriori* probability (MAP)-Bayesian estimated concentrations of mycophenolic acid (MPA). (**b**) Plots of observed versus MAP-Bayesian estimated area under the plasma concentration-time curve from 0 to 12 hours (AUC_{12}) of MPA using a 40-minute, 2-hour and 3-hour limited sampling strategy. **R** = coefficient of correlation.

Discussion

In a previous study, Premaud et al.[12] showed that the MPA AUC_{12} could be accurately estimated in renal transplant patients by an abbreviated sampling strategy based on a double γ absorption model. We first tried to develop an LSS to assess the MPA AUC in SLE patients, which was initially based on this double γ distribution model. We found that the Bayesian estimator developed for transplant patients could not be used satisfactorily in SLE patients, mainly because of its lack of estimation of the third peak. This later peak, which is due to enterohepatic recycling of MPAG to MPA, represented a significant proportion of the AUC in some SLE patients. The mean AUC_{12} of MPAG of patients treated with 2 g/day of MMF was lower in our study than the value reported previously for patients receiving a similar daily dose of MMF and ciclosporin concomitantly (629 ± 370 µg • h/mL vs 1230 ± 250 µg • h/mL).[30] This low level of MPAG concentration is likely due to the absence in SLE patients of drugs that may interact with enterohepatic recycling of MPAG to MPA.[11,12] Indeed, ciclosporin has been shown to inhibit MPA enterohepatic recirculation[31] by inhibiting the biliary excretion of MPAG.[9,10]

For SLE patients, a triple γ distribution model that provided the best fit to the third peak in addition to the first and second absorption peaks could lead to the development of a good Bayesian estimator. An LSS based on this Bayesian estimator was

Table III. Determination of the best limited sampling strategy using mycophenolic acid (MPA) area under the plasma concentration-time curve (AUC) Bayesian estimation

Sampling strategy	Triple γ distribution			Double γ distribution		
	R	MRE ± SD (%) [extremes]	Number of patients with unsatisfactory estimation (beyond ±20% of the trapezoidal AUC_{12})	R	MRE ± SD (%) [extremes]	Number of patients with unsatisfactory estimation (beyond ±20% of the trapezoidal AUC_{12})
40 min, 90 min, 3 h	0.90	8.58 ± 20.2 [−25.89 to 53.93]	5	0.91	10.02 ± 17.88 [−52.53 to 28.86]	6
40 min, 2 h, 3 h[a]	0.95	6.42 ± 13.2 [−21.72 to 30.22]	4	0.92	19.89 ± 16.04 [−4.64 to 59.69]	9
20 min, 2 h, 3 h	0.88	−4.14 ± 23 [−69.33 to 31.77]	5	0.67	8.58 ± 24.07 [−50.88 to 41.27]	8
20 min, 40 min, 3 h	0.92	6.67 ± 19.9 [−23.17 to 52.62]	4	0.73	3.22 ± 38.05 [−75.9 to 36.41]	9
20 min, 1 h, 3 h	0.83	2.75 ± 24 [−46.52 to 58.59]	7	0.87	10.33 ± 17.79 [−15.66 to 53.18]	5

a The sampling strategy that gave the best correlation between the AUC_{12} calculated using the trapezoidal rule and the AUC triple γ Bayesian estimation. None of the sampling strategies developed using the double γ Bayesian estimation gave satisfactory results.

AUC_{12} = AUC from 0 to 12 hours; **MRE** = mean relative error; **R** = coefficient of correlation.

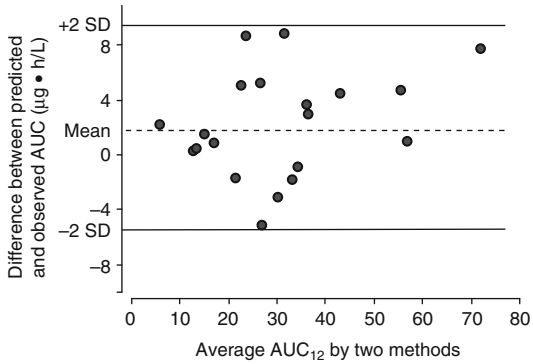

Fig. 4. Bland and Altman analysis between the areas under the plasma concentration-time curve (AUC) derived from full pharmacokinetic profiles and the estimate derived from concentrations at 40 minutes, 2 hours and 3 hours. **AUC_{12}** = AUC from 0 to 12 hours; **MPA** = mycophenolic acid.

developed and was able to accurately estimate the MPA AUC in SLE patients. The best sampling strategy obtained with the Bayesian estimator of MPA exposition was 40 minutes, 2 hours and 3 hours. As expected, this blood sampling schedule is different from the one previously devised for renal transplantation patients (20 minutes, 1 hour and 3 hours) in the post-transplantation period.[12]

Our study confirmed the wide between-subject variability of MPA pharmacokinetics in SLE patients, as has been previously observed in transplant patients. Factors affecting the interindividual variability of the MPA AUC have been extensively investigated in transplant patients[32-34] and are likely the same for SLE patients because they are drug related. As for transplant patients,[35] low serum albumin levels were associated with faster clearance of MPA in SLE patients. Despite this variability in serum albumin levels, our Bayesian estimator was able to provide accurate estimations in all patients.

An interference between glucocorticoids and MPA metabolism was found in the study by Cattaneo et al.[36] Since corticosteroids are often used in addition to MMF in SLE, we studied the possible interactions between these two drugs. Neither the prednisolone AUC_{12} nor daily doses of prednisone were found to be correlated with MPA pharmacokinetic parameters. However, since we could not withdraw corticosteroids in patients with SLE, as Cattaneo et al.[36] did, we cannot completely rule out a possible interaction between corticosteroids and MMF.

Conclusion

This study provides the first simple sampling strategy that can easily be integrated into clinical practice for monitoring MMF in SLE patients. Therapeutic drug monitoring based on the MPA AUC_{12} has been proposed in transplant patients and could reduce the risk of acute rejection and occurrence of adverse effects. It is proposed that the sampling strategy presented here could be used for therapeutic drug monitoring purposes not only in SLE but also in other autoimmune diseases when patients are treated with MMF not associated with calcineurin inhibitors.

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