The utility of trough mycophenolic acid levels for the management of lupus nephritis

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ABSTRACT

Background. Monitoring of mycophenolic acid (MPA) levels may be useful for effective mycophenolate mofetil (MMF) dosing. However, whether commonly obtained trough levels are an acceptable method of surveillance remains debatable. We hypothesized that trough levels of MPA would be a poor predictor of area under the curve (AUC) for MPA.

Methods. A total of 51 patients with lupus nephritis who were on MMF 1500 mg twice a day and had a 4-h AUC done were included in this study. MPA levels were measured prior to (C0) and at 1 (C1), 2 (C2) and 4 (C4) h, followed by 1500 mg of MMF. The MPA AUC values were calculated using the linear trapezoidal rule. Regression analysis was used to examine the relationship between the MPA trough and AUC. Differences in the MPA trough and AUC between different clinical and demographic categories were compared using *t*-tests.

Results. When grouped by tertiles there was significant overlap in MPA, AUC 0–4 and MPA trough in all tertiles. Although there was a statistically significant correlation between MPA trough levels and AUC, this association was weak and accounted for only 30% of the variability in MPA trough levels. This relationship might be even more unreliable in men than women. The use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers was associated with increased MPA trough levels and AUC at 0–4 h (AUC_{0–4}).

Conclusion. Trough levels of MPA do not show a strong correlation with AUC. In clinical situations where MPA levels are essential to guide therapy, an AUC_{0–4} would be a better indicator of the adequacy of treatment.

Keywords: area under the curve (AUC), lupus nephritis, mycophenolic acid levels, mycophenolate mofetil dosing, renal function

INTRODUCTION

Lupus nephritis is one of the most serious manifestations of systemic lupus erythematosus (SLE), affecting >50% of patients with SLE [1]. While mycophenolate mofetil (MMF) therapy is commonly used to attain clinical remission [2, 3], relapses following initial remission are prevalent in patients with proliferative lupus nephritis [4–8], often requiring maintenance therapy with MMF. Although well tolerated and relatively safe, long-term use of MMF is associated with gastrointestinal side effects as well as risks of immunosuppression [9]. Therefore, establishing and targeting well-defined therapeutic levels is important to optimize clinical outcomes while limiting exposure to MMF and minimizing side effects.

Previous studies have shown that the area under the curve for plasma concentration–time of mycophenolic acid (MPA), the active moiety of MMF from 0 to 12 h (AUC_{0–12}), is associated with favorable clinical outcomes [10]. Recent small, uncontrolled trials have suggested that a patient-tailored therapy based on therapeutic drug monitoring of MPA can improve clinical outcomes in patients with lupus nephritis [11–13]. Based on the available literature, an MPA AUC_{0–12} of 30–90 mg·h/L [11, 13–17] and/or a therapeutic trough level between 3 and 4.5 mg/L [18, 19] is recommended to decrease the risk of treatment failure in patients with lupus nephritis treated with MMF.

However, MPA AUC_{0–12} is cumbersome and expensive to perform. An abbreviated MPA AUC over 0–4 h (AUC_{0–4}) correlates reasonably with the full AUC ($r = 0.87$, $P < 0.001$) [20] and predicts total MPA exposure [21, 22] in transplant patients. Nevertheless, most clinicians routinely only obtain trough levels of MPA, which, at least in transplant recipients, does not adequately reflect exposure to MPA [23, 24], due to the wide interindividual variability. Variability may even be more of a challenge in patients

with lupus, who often have increased inflammatory markers, hypoalbuminemia and renal dysfunction [25], all of which can affect MMF levels. However, the relationship between trough levels and MPA AUC has not been studied. We hypothesized that trough levels of MPA would be a poor predictor of MPA AUC in patients with lupus nephritis. The purpose of our study was to examine the correlation of MPA AUC₀₋₄ and trough levels of MMF in patients with lupus nephritis.

MATERIALS AND METHODS

Data source and participants

The study was approved by the Institutional Review Board at the University of Florida. A retrospective analysis was performed on a cohort of 51 patients diagnosed with biopsy-proven lupus nephritis who received their care at the University of Florida between 25 July 2008 and 16 August 2016. All patients were receiving a fixed dose of MMF 1500 mg twice a day, as induction/maintenance therapy of their underlying disease, and had MPA AUC₀₋₄ and trough levels determined as a part of their routine clinical care. For assaying, MPA AUC₀₋₄ blood samples were collected from subjects at four time points [0 (predose, C₀) and 1 (C₁), 2 (C₂) and 4 (C₄) h postdose] and MPA plasma concentrations were measured using high-performance liquid chromatography. The MPA AUC₀₋₄ was calculated using the linear trapezoidal rule [26]. Medical records were reviewed to abstract demographic and clinical characteristics, including the medical treatment received and renal function. We used standardized reference serum creatinine, gender, race and age to calculate a reference estimated glomerular filtration rate (eGFR) based on the Modification of Diet in Renal Disease equation [27]. The degree of proteinuria was determined by the random urine albumin:creatinine ratio (in µg/mg). All serum and urine chemistries and immunoassays were performed in the central hospital clinical laboratory or Quest referral laboratories.

Statistical analysis

Continuous variables, if normally distributed, were reported as mean ± standard deviation and compared using independent *t*-test and if normalcy assumptions were not met, presented as median ± interquartile range and compared by Mann–Whitney U-test. Categorical variables were presented as frequency and percentage and compared using chi-square or Fisher's exact test. Spearman's rank correlation analyses were performed to examine the unadjusted association between MPA AUC and trough levels. Multivariate regression analyses were employed to control for other potential confounding factors. Normality was examined for linear regression analysis. Significance tests were two-sided, with a *P*-value <0.05 considered statistically significant. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

The study included a total of 51 patients ranging from 15 to 62 years of age, with a mean age of 33 years. They were

Table 1. Baseline characteristics (N = 51)

Characteristic/parameter	Value
Age (years)	33.0 ± 13.0
Gender	39 female, 12 males
Race	35 white, 16 black
Creatinine clearance (mL/min)	83.8 ± 38.9 (GFR)
BMI (kg/m ²)	26.6 ± 6.2
Weight (lbs)	149.0 ± 38.0
Albumin/Cr	1141.56 ± 1173.24
Smoking status	4 smokers and 47 nonsmokers

Data are presented as mean ± SD unless otherwise noted.

predominantly of Caucasian descent [35 of 51 patients (68.6%)] and female (with a male:female ratio of 3:10). All patients had World Health Organization (WHO) Class III or higher lupus nephritis at the time of presentation. At the time of analysis, everyone was being treated with MMF 1500 mg twice a day for management of lupus nephritis. Along with MMF, patients were on steroids and/or Plaquenil. Among all the patients, 45% were on Plaquenil only, 22% were on only steroids and 34% were on both Plaquenil and steroids. Of the cohort, 90% of patients were on one or more antihypertensive drugs at the time of treatment for lupus nephritis: 86% were on either angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) and 53% were prescribed diuretics. At the time of the MPA AUC measurement, only three patients were determined to have nephrotic syndrome and only two had an eGFR level <15 mL/min/1.73 m². Renal function was preserved in most patients, with a mean eGFR of 83.8 mL/min/1.73 m². The albuminuria ranged from 4 to 3974 mg/24 h, with a mean of 1141 mg/24 h. The baseline characteristics of the patients are shown in Table 1.

MPA AUC₀₋₄ displayed a mean of 68.08 mg*h/L with a standard deviation of 35.33 mg*h/L. The mean trough level was 4.24 mg/L with a standard deviation of 3.56 mg/L. There was a modest correlation of MPA trough levels with MPA AUC₀₋₄ (*R* = 0.55, *P* = 0.02) (Figure 1A). However, when the range of troughs was divided into tertiles (0–4, 5–9 and 10–12) there was significant overlap in MPA AUC₀₋₄ values in the different MPA trough level ranges (Figure 1B). Taking the therapeutic MPA AUC for transplant patients as 30–90 mg*h/L [28], therapeutic levels can be seen at every trough level. In addition, values of MPA AUC₀₋₄ <30 mg*h/L can be found both in the group with trough levels 0–4 mg/L as well as the group with trough levels 10–12 mg/L. While there are a lot more MPA AUC₀₋₄ levels >100 mg*h/L in the group with trough levels 10–12 mg/L, there was one patient with MPA AUC₀₋₄ levels >100 mg*h/L in the group with trough levels 0–4 mg/L. To further examine the ability of the MPA trough to predict MPA AUC₀₋₄, the trough MPA values were graphed versus the difference between measured and predicted MPA AUC₀₋₄ levels from the regression equation (Figure 1C). The measured MPA AUC₀₋₄ and the predicted MPA AUC₀₋₄ demonstrate significant disagreement through the range of trough values.

The relationship between the MPA trough and AUC₀₋₄ levels with clinical variables is shown in Figure 2. The MPA trough and AUC₀₋₄ levels did not differ between races. The mean MPA trough level was 3.3 ± 3.1 mg/L for African Americans and

FIGURE 1: Relationship between MPA trough plasma concentration and MPA AUC₀₋₄. All subjects on oral MMF 1500 mg twice a day. (A) Plot of trough plasma concentrations verse MPA AUC₀₋₄ with a regression line. (B) Box plot of tertiles of trough plasma concentrations and MPA AUC₀₋₄. (C) Plot of trough MPA levels and the difference between measured MPA AUC₀₋₄ and the MPA AUC₀₋₄ predicted from the regression equation. The shaded area depicts 95% CI for the differences between predicted and measured AUC₀₋₄.

4.0 ± 3.4 mg/L for Caucasians ($P=0.48$), whereas the mean MPA AUC₀₋₄ level was 53.0 ± 23.7 mg·h/L for African Americans and 66.6 ± 36.4 mg·h/L for Caucasians ($P=0.19$). Also, there was no significant difference in MPA trough levels or MPA AUC₀₋₄ levels between males and females. The mean MPA trough level was 5.2 ± 3.8 mg/L in males and 3.4 ± 3.1 mg/L in females ($P=0.11$), whereas the mean MPA AUC₀₋₄ level was 54.8 ± 22.4 mg·h/L in males and 64.4 ± 35.6 mg·h/L in females ($P=0.42$). However, while the trough levels were lower in females than males, the AUC levels were higher in women than in men. An interaction analysis showed that there was a strong interaction between gender and trough levels in determining AUC₀₋₄ levels ($P=0.009$). When comparing smokers with nonsmokers, trough levels and MPA AUC₀₋₄ levels were not different between the two groups ($P=0.75$ and $P=0.76$, respectively). Neither was a significant difference noted between MPA AUC₀₋₄ and trough levels in patients with hypertension (HTN), diabetes mellitus (DM) or hyperlipidemia compared with those patients who did not have these comorbidities ($P=0.36$, 0.99 and 0.92 for trough levels and $P=0.73$, 0.60 and 0.19 for MPA AUC, respectively).

We also compared the relationship of MPA trough and MPA AUC₀₋₄ levels with concurrent medication use, including

Plaquenil, steroids, ACE inhibitors and diuretics, as shown in Figure 3. Our study revealed that there were no statistically significant differences in MPA AUC₀₋₄ levels with the concurrent use of steroids, Plaquenil or diuretics ($P=0.12$, 0.46 and 0.93, respectively). Moreover, concomitant use of steroids, Plaquenil and diuretics did not cause a significant change in MPA trough levels ($P=0.78$, 0.53 and 0.20, respectively). However, concurrent ACE inhibitor/ARB use resulted in increased MPA AUC₀₋₄ and trough levels ($P=0.04$ and 0.01, respectively).

DISCUSSION

Our results demonstrate that although there was a statistically significant correlation between the trough levels of MPA and MPA AUC₀₋₄ ($r=0.55$, $P<0.001$), trough levels only explained 30% of the variance in AUC₀₋₄ and all tertiles of trough levels showed significant overlap of MPA AUC₀₋₄ levels.

There have been several small studies on MPA pharmacokinetics in patients with SLE [19]. Our findings are at odds with those of Lertdumrongluk *et al.* [11] showing that, the plasma trough concentration of MPA₀₋₁₂ significantly correlated with the AUC₀₋₁₂ ($r=0.90$, $P<0.05$) and was associated with response to MMF therapy. However, their study was small, with

FIGURE 2: Relationship of (A) MPA trough levels and (B) MPA AUC₀₋₄ levels and clinical variables. 1 = male, 2 = female; 1 = White, 2 = African American; 1 = smoker, 2 = nonsmoker; 1 = DM, 2 = no DM; 1 = HTN, 2 = no HTN; 1 = hyperlipidemia, 2 = no hyperlipidemia. Error bars depict the standard deviation.

FIGURE 3: The relationship between (A) MPA AUC₀₋₄ levels and (B) MPA trough levels with concurrent medication use. 1 = steroid use, 2 = no steroid use; 1 = Plaquenil use, 2 = no Plaquenil use; 1 = ACE inhibitor use, 2 = no ACE inhibitor use; 1 = diuretic use, 2 = no diuretic use. Error bars depict the standard deviation.

only 18 subjects, all of whom were Asian. Our results are consistent with Alexander *et al.* [17] who studied a small cohort of patients from India with proliferative lupus nephritis, and found only a weak correlation between trough levels and MPA AUC₀₋₁₂, with only 31% of the variance in AUC explained by the trough levels. Similarly, Neumann *et al.* [18] showed a weak, but significant, correlation between 12-h trough MPA concentrations and MPA AUC₀₋₁₂ ($r = 0.578$; $P < 0.05$) in patients with autoimmune diseases including lupus nephritis. However, only 12 of these patients had lupus. A detailed review of published studies over the past 15 years that have addressed the relationship between blood levels, the method of measuring MMF and clinical outcomes is shown in Table 2. To the best of our knowledge, our study is the largest study in lupus nephritis and the only one to include patients whose demographic makeup is characteristic of the USA and all of whom had biopsy-confirmed lupus nephritis.

We found a significant interaction between gender, AUC₀₋₄ and trough levels. In women, MPA trough levels were lower but, surprisingly, AUC levels were higher. Although a few small

studies have looked at the effect of gender on MPA pharmacokinetics, they have found conflicting results [33]. A recent study by Tornatore *et al.* [34] looked at the influence of sex and race on MPA pharmacokinetics in renal transplant patients. The authors found no significant difference in 12-h MPA AUC or 12-h trough levels. However, they did find that females had slower MPA clearance and more severe gastrointestinal side effects. Pescovitz *et al.* [35] showed that in renal transplant patients, the mean concentration time profiles of MPA in the male versus female subjects were very similar when dosed by race and gender. However, they did not study the correlation between trough and AUC. Our study suggests that using trough levels to predict AUC might be even more unreliable in men than women. However, we only had 12 male subjects. Thus further studies with more male patients are needed before making definite conclusions about how gender influences the ability of trough levels to predict MPA.

We did not find any significant correlation between MPA trough and AUC₀₋₄ concentrations in smoking, diabetes, hyperlipidemia or HTN (Figure 2). Previous studies have also

Table 2. A review of published studies over the past 15 years showing the relationship between MMF blood levels, the method of measuring MMF and clinical outcomes

Year	Chan <i>et al.</i> [4] 2005	Zahr <i>et al.</i> [29] 2008	Neumann <i>et al.</i> [18] 2008	Zahr [13] 2010	Lertdumrongluk <i>et al.</i> [11] 2010	Sagat-Gironella <i>et al.</i> [30] 2011	Kittanamongkolkhai <i>et al.</i> [12] 2013	Daleboudt <i>et al.</i> [14] 2013	Weillard <i>et al.</i> [31] 2014	Alexander <i>et al.</i> [17] 2014	Zabotti <i>et al.</i> [15] 2015	Hui-Yuen <i>et al.</i> [32] 2016
Study population/design	64 adults with lupus nephritis, prospective interventional study	71 adults with SLE, prospective interventional study	13 adults with SLE, prospective interventional study	71 adults with SLE, prospective interventional study	18 adults with lupus nephritis, prospective interventional study	19 children with SLE, prospective interventional study	19 adults with lupus nephritis, prospective interventional study	16 adults with lupus nephritis, retrospective non-interventional study	36 children with lupus nephritis, retrospective non-interventional study	34 adults with lupus nephritis, retrospective non-interventional study	5 adults with lupus nephritis, prospective interventional study	17 children with lupus nephritis, retrospective non-interventional study
Study objective	MMF versus cyclophosphamide-azathioprine for induction maintenance	Develop maximum a posteriori probability Bayesian estimator of MPA	Therapeutic drug monitoring of MPA based on troughs for effective MMF dosing	Assess relationships between SLE activity and MPA AUC ₀₋₁₂	Pharmacokinetic monitoring (MPA AUC) to optimize dosing of MPA	Characterize pharmacokinetics and pharmacodynamics of MPA and SLE disease activity	Monitoring MPA AUC in the treatment of severe, active lupus nephritis	Concentration-controlled treatment (MPA AUC) on renal outcomes in patients with lupus nephritis	MPA pharmacokinetics to develop a Bayesian estimator of AUC, relationship of MPA and clinical status	Pharmacokinetic and clinical correlations	Pharmacokinetics of MPA	Use of MPA and MPA-G levels in routine care of pediatric lupus nephritis and correlation with clinical response
Lupus nephritis class	Class IV	Class III 2 Class IV 2 Class V 1	Class III 2 Class IV 2 Class V 1	NA	Class III 4 Class IV 9	NA	Class III 1 Class IV 13 Class III/IV 1 Class IV/IV 4	Class III 5 Class IV 11	NA	Class III 4 Class IV 30	Class III 2 Class IV 3	Class III 4 III/V 6 IV 1 IV/V 3 V 3
Dose	2000 mg/day	1000-3000 mg/day	1000 mg/day	1846 ± 612 mg/day in active SLE and 1877 ± 490 mg/day in inactive SLE	1000-1500 mg/day	1000-3000 mg/day	1000-2000 mg/day	1000-4000 mg/day	728 ± 255 (300, 1250) mg/day	1000-2000 mg/day	2800 ± 400 mg/day	1200 mg/m ² /day
Drug, no. of patients	MMF, 33	MMF, 71	MMF, 13	MMF, 71	MMF, 12	MMF, 19	MMF, 19	MMF, 16	MMF, 36	MMF, 34	MMF, 5	MMF, 17
Sampling times (h)	NA	0.67, 2, 3	0, 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 12, 14 and 24	0.67, 2 and 3	EC-MPS, 6 0, 0.5, 1, 2, 3, 4, 8 and 12	0, 0.33, 0.67, 1, 1.5, 2, 3, 4, 6 and 9	0, 0.5, 1, 2, 3, 4, 8 and 12	0, 1, 2 and 3 h	0, 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8 and 12	0.5, 1, 1.5, 2, 2.5, 3, 4, 5 and 6	0.05, 1.25, 2, 4, 6, 8 and 12	6
Method of MPA, AUC ₀₋₁₂ calculation	Not mentioned	Bayesian estimation	Linear trapezoidal rule	Bayesian estimation	Linear trapezoidal rule	Extrapolation	Linear trapezoidal rule	LSS, Bayesian estimation	Bayesian estimation	Extrapolation LSS	Linear trapezoidal rule	NA
Single-time plasma MPA correlation with AUC ₀₋₁₂	NA	NA	NA	NA	C0, C1, C4, C8	NA	C1 (13 mg/L)	C0, C1, C2, C3	NA	C0.5, C0, C1, C1.5, C2, C2.5, C3, C4, C5, C6	NA	NA
Analysis method	Not described	HPLC	HPLC	HPLC	Roche, MPA assay	HPLC	Roche, MPA assay	HPLC	HPLC	HPLC	HPLC	NA
Baseline renal function	SCr (μmol/L): 112.29 ± 65.43 in one group and 113.18 ± 46.86 in the other group	Mean SCr (μmol/L): 86.1 ± 56.4	Mean SCr (μmol/L): 63.2 ± 27.8	Creatinine clearance (MDRD): mL/min/1.73 m ² : 113.0 ± 44.9 in active SLE and 98.4 ± 33.5 in inactive SLE	SCr (μmol/L): 111.41 ± 49.52 and eGFR (MDRD): 69.94 ± 42.09 mL/min/1.73 m ²	NA	Roche (MDRD): 84.4 ± 32.7 mL/min/1.73m ²	SCr (μmol/L): 98.6 ± 55.0	SLIDEAI score: 6 ± 6 (0, 20)	Mean SCr (μmol/L): 78.7 ± 30.1 in one group and 91.15 ± 31.8 in another group	eGFR mL/min: 87.6 (± 34.4)	SCr (μmol/L): 53.04 (44.2-79.56) in clinical response group, 56, 58 (32.71-73.37) in patients not in clinical response
Baseline urine protein	6.21 ± 4.11 g/24 h in one group and 4.44 ± 3.62 g/24 h in the other group	Mean ± SD: 1.1 ± 1.8 g/L Median: 0.3 g/L	NA	NA	6.3 ± 4.42 g/24 h	NA	2.92 ± 1.60 g/24 h	1.3 ± 1.3 g/24 h	NA	3.8±2.9 g/24 h in one group and 3.4±2.8 g/24 h in another group	2.6 ± 2.3 g/24 h	NA
Urine analysis	NA	NA	NA	NA	Bland	NA	Inactive	NA	Inactive	NA	NA	NA
Correlation of MPA level and outcome	NA	NA	Lower MPA trough levels associated with disease recurrence, 28/68 samples (41%) with an MPA <2 mg/L had recurrence.	The MPA AUC ₀₋₁₂ threshold value of 35 mg•h/L was associated with the lowest risk of active SLE	Successful treatment was seen in patients with MPA AUC >45 mg•h/L. The dosage of the drug was not related to MPA pharmacokinetics	Patients with MPA AUC ₀₋₁₂ of at least 30 mg•h/L (n = 5) had a significantly greater percent-reduction in BILAG scores	MPA C1 >13 mg/L resulted in response to therapy in 17 (89%) patients at Week 24 with 4 (21%) patients having complete response	Targeting MPA-AUC ₀₋₁₂ of 60-90 mg•h/L at 12 months of follow-up, 87.5% of patients had achieved either partial (18.7%) or complete (68.8%) remission	AUC <44 mg•h/L and AUC/dose <0.06 were associated with an increased risk of active disease. This was not the case for trough concentration values	Patients with an AUC ≥30 mg•h/L had greater renal response at 1 year	Targeting of MPA-AUC ₀₋₁₂ of 45-60 mg•h/L resulted in a complete renal response in three of five patients	Calculated steady-state levels do not reflect midinterval plasma levels of MPA. Midinterval plasma levels of MPA do not appear to correlate with clinical response
Correlation of MMF dose and outcome	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

BILAG, British Isles Lupus Assessment Group; HPLC, high-performance liquid chromatography; LSS, limited sampling strategy; MDRD, Modification of Diet in Renal Disease; NA, not mentioned in the reference papers; SCr, serum creatinine;

suggested that the MPA AUC and trough levels are not influenced by patient-specific factors [36–38]. Monitoring of MMF exposure in lupus nephritis is made more challenging because of the use of diverse concomitant medications [18, 39]. We found no significant difference in AUC_{0-4} or trough levels between patients on steroids or Plaquenil. It should be noted, however, that the available literature on the effect of corticosteroids on MPA AUC is controversial, with one study showing no effect and another demonstrating lower MPA exposure [33, 40–42].

An incidental finding in our study was that among all the medications, concurrent use of ACE inhibitors/ARBs was noted to have a correlation with increased MPA trough and AUC_{0-4} levels. A thorough search of the literature did not reveal any evidence of an interaction between ACE inhibitors/ARBs and MMF, that explains the increase in MMF exposure. However, there is some evidence that a change in renal function and albumin (both of which may be affected by ACE inhibitors/ARBs) may affect MMF exposure [33]. van Hest *et al.* [33] performed a population pharmacokinetic meta-analysis of MPA in renal transplant recipients to explore potential determinants of total MPA exposure. Among other things, they found that exposure to total MPA significantly increased with decreasing renal function, especially when GFR was <25 mL/min. Changes in renal function above the 25 mL/min threshold for GFR had a minimal effect on MPA clearance. We did not find a significant association between renal function and MPA clearance, possibly because most patients in our cohort had an eGFR >25 mL/min. In addition, an increase in albumin level was significantly associated with increasing MPA exposure. In healthy patients, MPA binds extensively to albumin (97–99%) [19]. A decrease in albumin level can cause an increase in the MPA free fraction and clearance. Acidosis, uremia and accumulation of MPA-7-O-glucuronide (an inactive glucuronide metabolite), all of which are associated with impaired renal function, can also decrease MPA binding to albumin [43] and result in increased clearance. ACE inhibitors and ARBs have been shown to decrease urinary excretion of protein (albumin) and slow the progression of kidney disease [44–46]. Therefore, in patients receiving MMF, concomitant use of an ACE inhibitor or ARB may be associated with a higher serum albumin level than in those who do not take ACE inhibitors/ARBs, which may decrease the clearance of MPA and thereby increase MPA exposure. In our study, there were no significant differences in serum albumin levels between those patients who received ACE inhibitors or ARBs and those who did not (3.2 versus 3.7; $P = 0.2$). Also, in reviewing the pharmacokinetics of MPA, one possible mechanism is that a minor MPA metabolite, acyl mycophenolic acid glucuronide (Ac-MPAG) is partially eliminated in the urine. One of the commercially available assays (Thermo Fisher Scientific, Waltham, MA, USA) for detecting MPA in human plasma also demonstrates cross-reactivity with the minor Ac-MPAG metabolite. It is possible that small changes in GFR, seen with ACE inhibitors and ARBs, could result in a small decrease in renal elimination of the Ac-MPAG metabolite. If this particular assay is used to measure the drug level, this could result in a minor increase in the reported blood concentration of MPA. However, the University of Florida and Quest Clinical laboratories use the

Roche Diagnostics (Basel, Switzerland) assay, which does not demonstrate significant cross-reactivity with the Ac-MPAG metabolite. Therefore, this explanation is not applicable.

The limitations of this study are the retrospective nature of the study and the relatively small sample size. Also, all of our patients were on a fixed oral dose of 1500 mg twice a day of MMF and the majority were on Plaquenil and/or corticosteroids. In order to assess the pharmacokinetics of MMF, different dosing regimens without the confounding effect of concomitant medications would be ideal. Moreover, the pharmacokinetic profiles of MPA were assessed only once during the 3- to 6-month period and do not assess within-patient variability. Multiple blood samplings for MPA AUC may give us an idea of within-patient variability, but frequent measurements of MPA levels in clinical practice is not cost effective or feasible. However, our pragmatic study is the largest thus far of the pharmacokinetics of MMF in patients with biopsy-confirmed lupus nephritis and reflects real-world conditions under which MMF is likely to be used in lupus nephritis.

CONCLUSION

Studies suggest that clinical outcomes might be optimized by targeting MMF to defined targets rather than using fixed-dose regimens. Clinicians routinely obtain MMF trough levels to guide therapeutic choices. Our study suggests that trough levels do not correlate with the AUC and an abbreviated AUC_{0-4} can and must be obtained in all patients to guide MMF dosing. Larger randomized clinical trials are required to evaluate the effect of using MPA AUC_{0-4} to impact clinical outcomes in patients with lupus nephritis.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Feldman CH, Hiraki LT, Liu J *et al.* Epidemiology and sociodemographics of systemic lupus erythematosus and lupus nephritis among US adults with Medicaid coverage, 2000–2004. *Arthritis Rheum* 2013; 65: 753–763
2. Houssiau FA, Vasconcelos C, D'Cruz D *et al.* Immunosuppressive therapy in lupus nephritis: the Euro-Lupus Nephritis Trial, a randomized trial of low-dose versus high-dose intravenous cyclophosphamide. *Arthritis Rheum* 2002; 46: 2121–2131
3. Appel GB, Contreras G, Dooley MA *et al.* Mycophenolate mofetil versus cyclophosphamide for induction treatment of lupus nephritis. *J Am Soc Nephrol* 2009; 20: 1103–1112
4. Chan TM, Tse KC, Tang CS *et al.* Long-term study of mycophenolate mofetil as continuous induction and maintenance treatment for diffuse proliferative lupus nephritis. *J Am Soc Nephrol* 2005; 16: 1076–1084
5. Swaak AJ, van den Brink HG, Smeenk RJ *et al.* Systemic lupus erythematosus. Disease outcome in patients with a disease duration of at least 10 years: second evaluation. *Lupus* 2001; 10: 51–58

6. Ioannidis JP, Boki KA, Katsorida ME *et al.* Remission, relapse, and remission of proliferative lupus nephritis treated with cyclophosphamide. *Kidney Int* 2000; 57: 258–264
7. Grootsholten C, Berden JH. Discontinuation of immunosuppression in proliferative lupus nephritis: is it possible? *Nephrol Dial Transplant* 2006; 21: 1465–1469
8. Moroni G, Gallelli B, Quaglini S *et al.* Withdrawal of therapy in patients with proliferative lupus nephritis: long-term follow-up. *Nephrol Dial Transplant* 2006; 21: 1541–1548
9. Davies NM, Grinyo J, Heading R *et al.* Gastrointestinal side effects of mycophenolic acid in renal transplant patients: a reappraisal. *Nephrol Dial Transplant* 2007; 22: 2440–2448
10. Arns W, Cibrik DM, Walker RG *et al.* Therapeutic drug monitoring of mycophenolic acid in solid organ transplant patients treated with mycophenolate mofetil: review of the literature. *Transplantation* 2006; 82: 1004–1012
11. Lertdumrongluk P, Somparn P, Kittanamongkolchai W *et al.* Pharmacokinetics of mycophenolic acid in severe lupus nephritis. *Kidney Int* 2010; 78: 389–395
12. Kittanamongkolchai W, Ruksung C, Supasiri T *et al.* Therapeutic drug monitoring of mycophenolate mofetil for the treatment of severely active lupus nephritis. *Lupus* 2013; 22: 727–732
13. Zahr N, Arnaud L, Marquet P *et al.* Mycophenolic acid area under the curve correlates with disease activity in lupus patients treated with mycophenolate mofetil. *Arthritis Rheum* 2010; 62: 2047–2054
14. Daleboudt GM, Reinders ME, den Hartigh J *et al.* Concentration-controlled treatment of lupus nephritis with mycophenolate mofetil. *Lupus* 2013; 22: 171–179
15. Zabotti A, Baraldo M, Quartuccio L *et al.* Optimizing the dose of mycophenolate mofetil for the maintenance treatment of lupus nephritis by therapeutic drug monitoring. *Clin Rheumatol* 2015; 34: 171–174
16. van Gelder T, Berden JH, Berger SP. To TDM or not to TDM in lupus nephritis patients treated with MMF? *Nephrol Dial Transplant* 2015; 30: 560–564
17. Alexander S, Fleming DH, Mathew BS *et al.* Pharmacokinetics of concentration-controlled mycophenolate mofetil in proliferative lupus nephritis: an observational cohort study. *Ther Drug Monit* 2014; 36: 423–432
18. Neumann I, Fuhrmann H, Fang IF *et al.* Association between mycophenolic acid 12-h trough levels and clinical endpoints in patients with autoimmune disease on mycophenolate mofetil. *Nephrol Dial Transplant* 2008; 23: 3514–3520
19. Łuszczynska P, Pawiński T. Therapeutic drug monitoring of mycophenolic acid in lupus nephritis: a review of current literature. *Ther Drug Monit* 2015; 37: 711–717
20. Ghio L, Ferrareso M, Viganò SM *et al.* Mycophenolate mofetil pharmacokinetic monitoring in pediatric kidney transplant recipients. *Transplant Proc* 2005; 37: 856–858
21. Weber LT, Shipkova M, Armstrong VW *et al.* The pharmacokinetic-pharmacodynamic relationship for total and free mycophenolic acid in pediatric renal transplant recipients: a report of the german study group on mycophenolate mofetil therapy. *J Am Soc Nephrol* 2002; 13: 759–768
22. van Gelder T, Le Meur Y, Shaw LM *et al.* Therapeutic drug monitoring of mycophenolate mofetil in transplantation. *Ther Drug Monit* 2006; 28: 145–154
23. Kaplan B. Mycophenolic acid trough level monitoring in solid organ transplant recipients treated with mycophenolate mofetil: association with clinical outcome. *Curr Med Res Opin* 2006; 22: 2355–2364
24. Cox VC, Ensom MH. Mycophenolate mofetil for solid organ transplantation: does the evidence support the need for clinical pharmacokinetic monitoring? *Ther Drug Monit* 2003; 25: 137–157
25. Joy MS, Hilliard T, Hu Y *et al.* Influence of clinical and demographic variables on mycophenolic acid pharmacokinetics in antineutrophil cytoplasmic antibody-associated vasculitis. *Ann Pharmacother* 2009; 43: 1020–1027
26. Filler G, Feber J, Lepage N *et al.* Universal approach to pharmacokinetic monitoring of immunosuppressive agents in children. *Pediatr Transplant* 2002; 6: 411–418
27. Levey AS, Bosch JP, Lewis JB *et al.* A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med* 1999; 130: 461–470
28. Jeong H, Kaplan B. Therapeutic monitoring of mycophenolate mofetil. *Clin J Am Soc Nephrol* 2007; 2: 184–191
29. Zahr N, Amoura Z, Debord J *et al.* Pharmacokinetic study of mycophenolate mofetil in patients with systemic lupus erythematosus and design of Bayesian estimator using limited sampling strategies. *Clin Pharmacokinet* 2008; 47: 277–284
30. Sagcal-Gironella AC, Fukuda T, Wiers K *et al.* Pharmacokinetics and pharmacodynamics of mycophenolic acid and their relation to response to therapy of childhood-onset systemic lupus erythematosus. *Semin Arthritis Rheum* 2011; 40: 307–313
31. Woillard JB, Bader-Meunier B, Salomon R *et al.* Pharmacokinetics of mycophenolate mofetil in children with lupus and clinical findings in favour of therapeutic drug monitoring. *Br J Clin Pharmacol* 2014; 78: 867–876
32. Hui-Yuen JS, Tran T, Taylor J *et al.* Use of glucuronidated mycophenolic acid levels for therapeutic monitoring in pediatric lupus nephritis patients. *J Clin Rheumatol* 2016; 22: 75–79
33. van Hest RM, Mathot RA, Pescovitz MD *et al.* Explaining variability in mycophenolic acid exposure to optimize mycophenolate mofetil dosing: a population pharmacokinetic meta-analysis of mycophenolic acid in renal transplant recipients. *J Am Soc Nephrol* 2006; 17: 871–880
34. Tornatore KM, Meaney CJ, Wilding GE *et al.* Influence of sex and race on mycophenolic acid pharmacokinetics in stable African American and Caucasian renal transplant recipients. *Clin Pharmacokinet* 2015; 54: 423–434
35. Pescovitz MD, Guasch A, Gaston R *et al.* Equivalent pharmacokinetics of mycophenolate mofetil in African-American and Caucasian male and female stable renal allograft recipients. *Am J Transplant* 2003; 3: 1581–1586
36. Budde K, Bauer S, Hambach P *et al.* Pharmacokinetic and pharmacodynamic comparison of enteric-coated mycophenolate sodium and mycophenolate mofetil in maintenance renal transplant patients. *Am J Transplant* 2007; 7: 888–898
37. Budde K, Glander P, Kramer BK *et al.* Conversion from mycophenolate mofetil to enteric-coated mycophenolate sodium in maintenance renal transplant recipients receiving tacrolimus: clinical, pharmacokinetic, and pharmacodynamic outcomes. *Transplantation* 2007; 83: 417–424
38. Johnston A, He X, Holt DW. Bioequivalence of enteric-coated mycophenolate sodium and mycophenolate mofetil: a meta-analysis of three studies in stable renal transplant recipients. *Transplantation* 2006; 82: 1413–1418
39. Neumann I, Haidinger M, Jäger H *et al.* Pharmacokinetics of mycophenolate mofetil in patients with autoimmune diseases compared renal transplant recipients. *J Am Soc Nephrol* 2003; 14: 721–727
40. Jaklic A, Collins CJ, Mrhar A *et al.* High prevalence of potential drug interactions affecting mycophenolic acid pharmacokinetics in nonmyeloablative hematopoietic stem cell transplant recipients. *Int J Clin Pharmacol Ther* 2013; 51: 711–717
41. Le Guellec C, Bourgoin H, Buchler M *et al.* Population pharmacokinetics and Bayesian estimation of mycophenolic acid concentrations in stable renal transplant patients. *Clin Pharmacokinet* 2004; 43: 253–266
42. Cattaneo D, Perico N, Gaspari F *et al.* Glucocorticoids interfere with mycophenolate mofetil bioavailability in kidney transplantation. *Kidney Int* 2002; 62: 1060–1067
43. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of mycophenolate in solid organ transplant recipients. *Clin Pharmacokinet* 2007; 46: 13–58
44. Lebovitz HE, Wiegmann TB, Nnaan A *et al.* Renal protective effects of enalapril in hypertensive NIDDM: role of baseline albuminuria. *Kidney Int Suppl* 1994; 45: S150–S155
45. Ravid M, Lang R, Rachmani R *et al.* Long-term renoprotective effect of angiotensin-converting enzyme inhibition in non-insulin-dependent diabetes mellitus. A 7-year follow-up study. *Arch Intern Med* 1996; 156: 286–289
46. Xu R, Sun S, Huo Y *et al.* Effects of ACEIs versus ARBs on proteinuria or albuminuria in primary hypertension: a meta-analysis of randomized trials. *Medicine (Baltimore)* 2015; 94: e1560

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