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Mycophenolic Acid Area Under the Curve Correlates With Disease Activity in Lupus Patients Treated With Mycophenolate Mofetil

Noël Zahr,[1] Laurent Arnaud,[1] Pierre Marquet,[2] Julien Haroche,[1] Nathalie Costedoat-Chalumeau,[1] Jean-Sébastien Hulot,[1] Christian Funck-Brentano,[1] Jean-Charles Piette,[1] and Zahir Amoura[1]

Objective. **Mycophenolic acid (MPA) is the active metabolite of mycophenolate mofetil (MMF), which is widely used to treat systemic lupus erythematosus (SLE). In transplantation, MPA area under the plasma concentration–time curve from 0 to 12 hours (MPA $AUC_{0–12}$) is correlated with clinical outcome. We undertook the present study to assess possible relationships between SLE activity and MPA $AUC_{0–12}$.**

Methods. **Using a Bayesian estimator, MPA $AUC_{0–12}$ was determined in 71 consecutive SLE patients (61 women and 10 men; mean ± SD age 34 ± 10 years) receiving a stable MMF dose. On the same day, SLE activity was assessed using the SLE Disease Activity Index (SLEDAI; active disease defined as a SLEDAI score ≥6) and the British Isles Lupus Assessment Group (BILAG) index (active disease defined as BILAG A or B).**

Results. **Two groups were studied: patients with active SLE (mean ± SD SLEDAI score 11.6 ± 4.4; n = 26) and patients with inactive SLE (mean ± SD SLEDAI score 1.9 ± 1.6; n = 45). MPA $AUC_{0–12}$ correlated weakly with the dose of MMF (r = 0.33, $P = 0.005$). Mean ± SD MPA $AUC_{0–12}$ in the group with active SLE was significantly lower than that in the group with inactive SLE (26.8 ± 13.6 μg.hour/ml versus 46.5 ± 16.3 μg.hour/ml; $P < 0.0001$). MPA $AUC_{0–12}$ was negatively correlated with the SLEDAI (r = –0.64, $P < 0.0001$). In multivariate analysis, MPA $AUC_{0–12}$ was the sole parameter associated with SLE activity (odds ratio 0.89 [95% confidence interval 0.83–0.96], $P = 0.002$). The MPA $AUC_{0–12}$ threshold value of 35 μg.hour/ml was associated with the lowest risk of active SLE.**

Conclusion. **Our data show that SLE activity is strongly correlated with MPA $AUC_{0–12}$. An individualized dosing regimen of MMF, with a target $AUC_{0–12}$ of 35 μg.hour/ml, should be considered for SLE patients.**

Mycophenolate mofetil (MMF) is an inactive prodrug that is converted to its active metabolite (mycophenolic acid [MPA]) by intestinal, liver, and plasma esterases. MMF is now widely used for the treatment of systemic lupus erythematosus (SLE) (1–5). In clinical practice, the prescribed daily dose of MMF is based on data from clinical trials in transplantation. A fixed dose of 2 or 3 gm/day of MMF, given in 2 divided doses in combination with steroids, is prescribed for adults with SLE (1–4). Doses are further reduced in case of side effects of MMF, such as leukopenia, thrombocytopenia, infections, or gastrointestinal side effects.

As with many immunosuppressants (e.g., cyclosporin A, tacrolimus, sirolimus, everolimus) (6), therapeutic drug monitoring of MMF leading to individualized doses has been developed in transplantation (7). MPA area under the plasma concentration–time curve from 0 to 12 hours (MPA $AUC_{0–12}$) is the MPA pharmacokinetic parameter that has the best relationship with clinical outcome in solid organ transplant

[1]Noël Zahr, DPharm, PhD, Laurent Arnaud, MD, Julien Haroche, MD, PhD, Nathalie Costedoat-Chalumeau, MD, PhD, Jean-Sébastien Hulot, MD, PhD, Christian Funck-Brentano, MD, PhD, Jean-Charles Piette, MD, Zahir Amoura, MD: Pitié-Salpêtrière Hospital, Paris, France; [2]Pierre Marquet, MD, PhD: Centre Hospitalier Universitaire, INSERM U850, and Université de Limoges, Limoges, France.

Drs. Zahr and Arnaud contributed equally to this work.

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Address correspondence and reprint requests to Zahir Amoura, MD, Service de Médecine Interne 2, Centre National de Référence Lupus, Groupe Hospitalier Pitié-Salpêtrière, 47-83 bd de l'Hôpital, 75013 Paris, France. E-mail: zahir.amoura@psl.aphp.fr.

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patients (7). Therapeutic MPA $AUC_{0–12}$ monitoring has been shown to significantly reduce the risk of treatment failure and acute rejection in renal allograft recipients in randomized concentration-controlled trials (8,9).

MMF exposure monitoring is challenging in SLE because the MPA pharmacokinetic profile in lupus is different from that in transplantation (10,11). MPA maximum concentration and MPA concentration after 12 hours are different in renal transplant recipients than in autoimmune disease patients who are not receiving cyclosporin A (10,12). In contrast with transplant recipients, SLE patients often have a third peak of MPA (10,12). This last peak is due to the lack of use of calcineurin inhibitors, which affect MPA pharmacokinetic parameters, in SLE (13,14). Plasma concentrations of MPA are reduced by cyclosporine, which inhibits the biliary excretion of MPA 7-O-glucuronide (the inactive main MPA metabolite), leading to reduction of the enterohepatic cycle of MPA (15,16). Evaluation of MPA $AUC_{0–12}$ using tools developed in transplantation cannot be utilized in SLE (11). Bayesian estimators developed for assessing MPA $AUC_{0–12}$ in transplantation do not correctly estimate MPA exposure in SLE (11). We have recently developed a new Bayesian estimator for determining MPA exposure in SLE between two drug intakes (11). This model accurately evaluates MPA $AUC_{0–12}$ in patients who are not receiving calcineurin inhibitors (11), and it can easily be applied to patients, since it employs a limited 3-point MPA concentration sampling strategy.

Using this estimator with a limited sampling strategy, we studied correlations between MPA $AUC_{0–12}$ and SLE activity in a prospective study of 71 SLE patients. We also determined the threshold of MPA $AUC_{0–12}$ that is best associated with inactive SLE status.

PATIENTS AND METHODS

Patients. Seventy-one patients followed up in the Internal Medicine Department of Pitié-Salpêtrière Hospital (the French National Reference Center for SLE) between April 2005 and May 2009 were included in the study. Patients were enrolled if they fulfilled all the following criteria: disease that met the American College of Rheumatology classification criteria for SLE (17,18); treatment with MMF at a dosage of 0.5 gm, 0.75 gm, 1 gm, or 1.5 gm twice daily; treatment with MMF at a dosage that was stable for at least 10 weeks; no intake of drugs known to interact with MMF, including acyclovir, antacids, cholestyramine, ganciclovir, metronidazole, iron, tacrolimus, or cyclosporine; and not pregnant or breastfeeding. The study protocol was approved by the local ethics committee, and informed consent was obtained from all patients before the study began.

Study design. On the day of sampling, MMF was administered to the patients orally in order to ensure that they actually received the prescribed dose. On the same day, the patients underwent a complete physical examination and complete laboratory testing (blood cell count, measurement of serum creatinine, serum albumin, and C3 levels, urinalysis, anti–double-stranded DNA [anti-dsDNA] antibody measurement by Farr assay, and pharmacokinetic analyses). The primary outcome measure was the existence of an active SLE status on the day of sampling. SLE status was assessed using the SLE Disease Activity Index (SLEDAI) (19), and active SLE was defined as a SLEDAI score of ≥ 6 (20). Disease activity was also evaluated using the British Isles Lupus Assessment Group (BILAG) index (21), and active disease was defined as BILAG A or B (21). All patients were assessed by the same physician (ZA), who was blinded with regard to the MPA $AUC_{0–12}$.

Pharmacokinetic analysis. *MPA $AUC_{0–12}$ determination.* Samples were analyzed for MPA by high-performance liquid chromatography (HPLC) coupled to a 250-nm wavelength photodiode array detector, using a method developed by Westley et al (22), with some modifications. This method proved to be accurate and precise in the range 0.1–20 μg/ml; the within-day precision (coefficient of variation %) was $<10\%$. The limit of quantification was 0.1 μg/ml for MPA (data not shown). MPA $AUC_{0–12}$ was determined using a Bayesian estimator developed by our group, from 3 samplings at 40 minutes, 2 hours, and 3 hours after dosing (11). We have previously demonstrated that this Bayesian estimator was able to predict MPA $AUC_{0–12}$ with a very good correlation with the one measured during the 12 hours following MMF intake ($R^2 = 0.90$) (11).

Blood hydroxychloroquine (HCQ) concentrations. HCQ concentrations were measured in whole blood using HPLC with fluorometric detection, as described previously (20). The lower limit of detection was 10 ng/ml.

Statistical analysis. Quantitative data were expressed as the mean ± SD, and qualitative data as numbers and percentages. Comparison of quantitative values was performed using Mann-Whitney U test. Qualitative values were compared using Fisher's exact test. Correlations were analyzed using the nonparametric Spearman's test. Parameters significantly associated with SLE activity were determined using a multivariate analysis (logistic regression). A receiver operating characteristic (ROC) curve (a plot of sensitivity versus 1 minus specificity) was constructed to determine the target MPA $AUC_{0–12}$.

RESULTS

Patient characteristics. Seventy-one consecutive SLE inpatients (61 women and 10 men; mean ± SD age at sampling 34 ± 10 years) were included in our study. All were treated with MMF as follows: 6 patients received 3 gm/day, 49 patients received 2 gm/day, 1 patient received 1.5 gm/day, and 15 patients received 1 gm/day. Indications for MMF treatment were lupus nephropathy (n = 61), refractory cutaneous involvement (n = 4), central nervous system involvement (n = 2),

Table 1. Characteristics of the patients with active SLE and with inactive SLE, as defined using the SLEDAI*

	Active SLE (SLEDAI score ≥6) (n = 26)	Inactive SLE (SLEDAI score <6) (n = 45)	*P*
Age, years	35.0 ± 9.3	33.6 ± 10.4	0.51
Women, no. (%)	22 (85)	39 (87)	0.99
Ethnicity, no. (%)			
White	15 (58)	29 (64)	
Black	10 (38)	9 (20)	0.12
Asian	1 (4)	7 (16)	
BMI, kg/m^2	24.3 ± 4.8	23.3 ± 5.4	0.33
Lupus nephropathy, no (%)	22 (84)	39 (87)	0.81
Creatinine clearance (MDRD), ml/minute/$1.73m^2$	113.0 ± 44.9	98.4 ± 33.5	0.23
Prednisone dosage, mg/day	18.8 ± 10.8	14.8 ± 8.5	0.10
MMF dosage, mg/day	1,846 ± 612	1,877 ± 490	0.79
HCQ, ng/ml†	616 ± 472	816 ± 687	0.42
Albuminemia, mg/liter	33.1 ± 7.4	39.5 ± 7.4	0.0008
C3 level, gm/liter	0.77 ± 0.34	1.00 ± 0.19	0.004
Anti-dsDNA, IU/ml	108 ± 158	28 ± 66	0.002
SLEDAI score	11.6 ± 4.4	1.9 ± 1.6	<0.0001

* Except where indicated otherwise, values are the mean ± SD. Statistical comparisons were made using the Mann-Whitney U test, except for sex and proportion of patients with lupus nephropathy, which were made using Fisher's exact test, and ethnicity, which was made using the Kruskal-Wallis test. The presence of anti–double-stranded DNA (anti-dsDNA) antibodies was assessed using the Farr assay. SLE = systemic lupus erythematosus; SLEDAI = SLE Disease Activity Index; BMI = body mass index; MDRD = Modification of Diet in Renal Disease study; MMF = mycophenolate mofetil; HCQ = hydroxychloroquine.
† Assessed in 68 patients, of whom 25 were in the active SLE group and 43 were in the inactive SLE group.

liver involvement (n = 1), bronchiolitis obliterans (n = 1), lupus vasculitis (n = 1), and pulmonary arterial hypertension (n = 1). All patients were also treated with oral prednisone (mean ± SD dosage 16.3 ± 9.6 mg/day). Fifty-seven patients received HCQ at a dosage of at least 200 mg/day (mean ± SD 743 ± 620 ng/ml).

Disease activity determined using the SLEDAI and BILAG. According to the SLEDAI, 26 patients (36.6%) had active SLE (SLEDAI score ≥6) and 45 patients (63.4%) had inactive SLE on the day of sampling. Among the patients with active SLE, the mean ± SD SLEDAI score was 11.6 ± 4.4, and among the patients with inactive SLE, the mean ± SD SLEDAI score was 1.9 ± 1.6 ($P < 0.0001$). The active and inactive SLE groups were similar in terms of sex ratio, mean age at sampling, mean weight, mean body mass index (BMI), mean daily dose of MMF, mean daily dose of steroids, and mean blood HCQ levels (Table 1).

According to the BILAG index, 28 patients (39.4%) had active SLE (BILAG A or B) and 43 (60.6%) had inactive SLE on the day of sampling. Mean anti-dsDNA antibody levels were significantly higher among patients with active SLE than among patients with inactive SLE (99.7 ± 154.4 IU/ml versus 30.3 ± 67.5 IU/ml; $P = 0.0154$). C3 levels were significantly lower among patients with active SLE than among patients with inactive SLE (0.81 ± 0.33 gm/liter versus 0.99 ± 0.22 gm/liter; $P = 0.0225$). The groups with active and inactive SLE were similar in terms of sex ratio (24 women in the active SLE group versus 37 women in the inactive SLE group; $P = 0.99$), mean age at sampling (36.3 ± 9.5 years versus 32.7 ± 10.1 years; $P = 0.12$), mean weight (67.4 ± 14.6 kg versus 63.7 ± 16.0 kg; $P = 0.23$), mean BMI (24.4 ± 4.7 kg/m^2 versus 23.2 ± 5.5 kg/m^2; $P = 0.18$), mean daily dose of MMF (1,893 ± 567 mg versus 1,848 ± 518 mg; $P = 0.72$), mean daily dose of steroids (18.4 ± 10.6 mg versus 14.9 ± 8.7 mg; $P = 0.14$), and mean blood HCQ level (603.1 ± 480.9 ng/ml versus 834.8 ± 687.8 ng/ml; $P = 0.29$).

Correlation of MPA AUC_{0-12} with SLE activity. MPA AUC_{0-12} displayed wide variability, with a median concentration of 36.7 μg.hour/ml and extremes of 7.5 and 83.7 μg.hour/ml. Median MPA dose-standardized AUC_{0-12} was 42.4 μg.hour/ml per gram of MMF, with extremes of 7.5 and 98.1 (Figure 1). MPA AUC_{0-12} correlated weakly with daily MMF dose ($r = 0.33$, $P = 0.005$). The mean ± SD MPA AUC_{0-12} of the group with active SLE was significantly lower than that of the group with inactive SLE (26.8 ± 13.6 μg.hour/ml versus 46.5 ± 16.3 μg.hour/ml, $P < 0.0001$) (Figure 2A). Similarly, the mean ± SD MPA AUC_{0-12} of the group with active SLE, as defined using the BILAG index, was significantly lower than that of the group with inactive SLE (29.4 ± 15.2 μg.hour/ml versus 45.7 ± 16.8

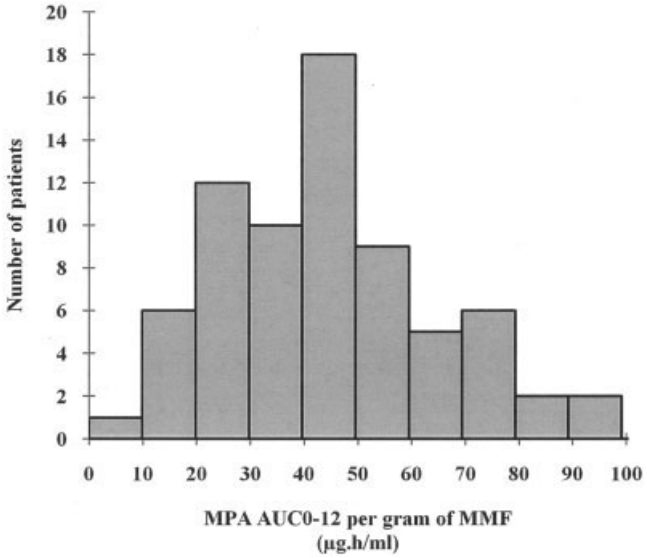

Figure 1. Between-patient variations of mycophenolic acid area under the plasma concentration–time curve from 0 to 12 hours (MPA AUC_{0-12})/gm of mycophenolate mofetil (MMF) received, in patients with systemic lupus erythematosus (SLE). MPA AUC_{0-12} was determined using a Bayesian estimator that was previously developed to assess data from SLE patients (11).

μg.hour/ml, $P < 0.0001$). MPA AUC_{0-12} was negatively correlated with the SLEDAI ($r = -0.64$, $P < 0.0001$) (Figure 2B) and with anti-dsDNA levels ($r = -0.25$, $P = 0.04$). All but 3 patients with a SLEDAI score of ≥6 had an MPA AUC below 35 μg.hour/ml. A positive correlation was found between MPA AUC_{0-12} and C3 levels ($r = 0.38$, $P = 0.001$) (Figure 2C).

MPA AUC_{0-12} is a major parameter influencing SLE activity. To assess parameters that may have influenced SLE activity on the day of sampling, we constructed a multivariate logistic regression model including sex, MPA AUC_{0-12}, daily dose of steroids, daily dose of MMF, blood HCQ concentrations, ethnicity, C3 levels, and anti-dsDNA levels. In multivariate analysis, MPA AUC_{0-12} was the sole independent parameter associated with SLE activity (Table 2). Interestingly, serum albumin level was not identified as an independent parameter influencing SLE activity when added to this model ($P = 0.44$).

Parameters influencing MPA AUC_{0-12}. Adherence to treatment regimen was assessed using blood HCQ concentrations, as previously described by our group (23). The mean blood HCQ concentration was not significantly different between patients with active SLE and patients with inactive SLE ($P = 0.42$) (Table 1). The proportion of patients with low (<400 ng/ml) or very low

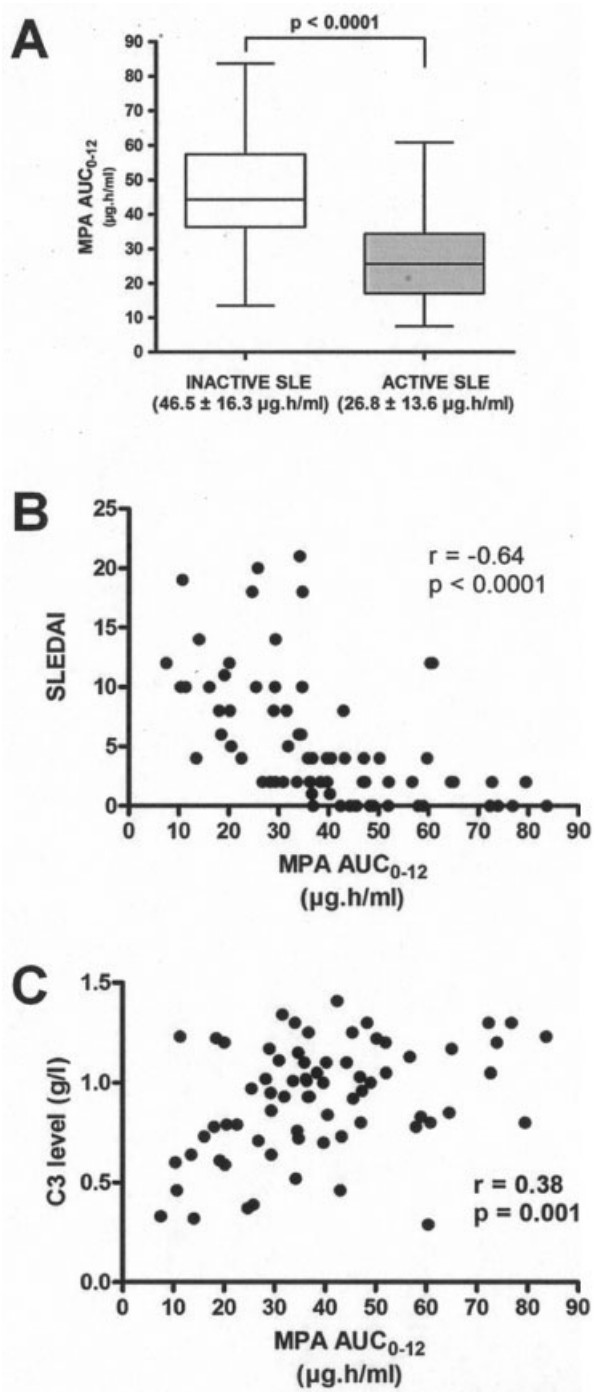

Figure 2. A, Significantly lower MPA AUC_{0-12} in patients with active SLE (SLE Disease Activity Index [SLEDAI] score ≥6; n = 26) than in patients with inactive SLE (n = 45). Data are shown as box plots. Each box represents the 25th to 75th percentiles. Lines outside the boxes represent the 10th and the 90th percentiles. Lines inside the boxes represent the median. **B** and **C,** Correlation of the MPA AUC_{0-12} with SLE activity, as shown by the SLEDAI score (**B**) and C3 levels (**C**). See Figure 1 for other definitions.

Table 2. Multivariate analysis of parameters potentially influencing SLE activity in patients treated with MMF*

Parameter	OR (95% CI)
Sex (male)	1.47 (0.20–11.12)
Ethnicity (black)	33.78 (0.59–1,940)
C3 level	0.04 (0.001–1.38)
Anti-dsDNA level	1.004 (0.996–1.011)
Blood HCQ levels	0.999 (0.998–1.000)
Daily dose of prednisone	1.07 (0.99–1.15)
Daily dose of MMF	1.001 (0.999–1.002)
MPA AUC_{0-12}	0.89 (0.83–0.96)†

* Multivariate analysis was performed by logistic regression. OR = odds ratio; 95% CI = 95% confidence interval; MPA AUC_{0-12} = mycophenolic acid area under the plasma concentration–time curve from 0 to 12 hours (see Table 1 for other definitions).
† $P = 0.002$.

(<100 ng/ml) blood HCQ concentrations was also not significantly different between patients with active SLE and patients with inactive SLE ($P = 0.43$ and $P = 0.36$, respectively). To assess other parameters that may influence MPA AUC_{0-12}, we constructed a multivariate regression model including sex, ethnicity, MPA AUC_{0-12}, daily dose of steroids, daily dose of MMF, blood HCQ concentrations, BMI, albuminemia, and creatinine clearance as defined by the Modification of Diet in Renal Disease study (MDRD). In multivariate analysis, MDRD creatinine clearance ($P = 0.039$), daily dose of MMF ($P = 0.0095$), and serum albumin level ($P = 0.0036$) were the 3 parameters independently associated with MPA AUC_{0-12}.

Estimation of a target MPA AUC_{0-12} in SLE. We used ROC curve analysis to determine the MPA AUC_{0-12} associated with the lowest risk of active SLE, as defined by the SLEDAI or the BILAG index, on the day of sampling. For both the SLEDAI and the BILAG index, the threshold value of 35 μg.hour/ml provided the best tradeoff between sensitivity and specificity (Figures 3A and B). After 35 μg.hour/ml, the curve plateaued. When MPA AUC_{0-12} was between 35 and 45 μg.hour/ml, the SLEDAI dramatically decreased. Considering the threshold value of 35 μg.hour/ml, the positive predictive value (PPV) and the negative predictive value (NPV) of MPA AUC_{0-12} for active SLE as defined by the SLEDAI were 71.9% and 92.3%, respectively. The PPV and NPV for active SLE as defined by the BILAG index were 68.8% and 84.6%, respectively.

Lack of association between MPA AUC_{0-12} and hematologic toxicity. Six patients had neutropenia on the day of sampling. Of those, 2 had active SLE (SLEDAI scores of 8 and 11). Four had low MPA AUC_{0-12} (19.2, 20.2, 20.5, and 22.6 μg.hour/ml), and the other 2 had high MPA AUC_{0-12} (59.8 and 72.3 μg.hour/ml). Six patients had a clinically significant nonhemolytic anemia (hemoglobin level <10 gm/dl). Of those, 3 patients had both low MPA AUC_{0-12} (7.5, 24.7, and 29.2 μg.hour/ml) and active SLE. Among the 3 others, 2 had

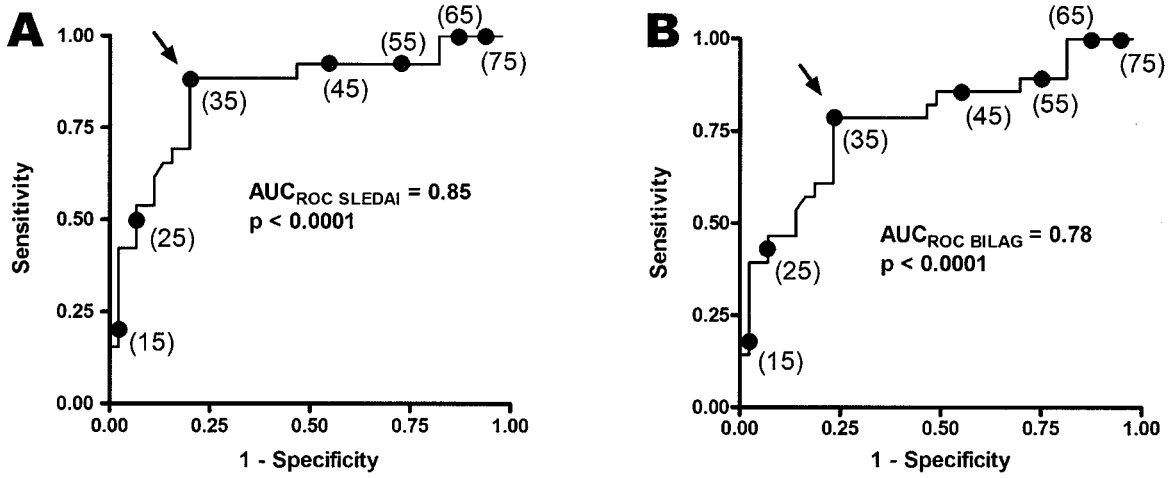

Figure 3. Receiver operating characteristic (ROC) curve estimates for the 71 SLE patients, obtained using the SLE Disease Activity Index (SLEDAI) (**A**) and the British Isles Lupus Assessment Group (BILAG) index (**B**). Sensitivity and 1 minus specificity for the risk of active SLE on the day of sampling are shown. Numbers in parentheses are the MPA AUC_{0-12} cutoff values (in μg.hour/ml). The number indicated by the arrow is the proposed threshold cutoff value (35 μg.hour/ml). $AUC_{ROC\ SLEDAI}$ and $AUC_{ROC\ BILAG}$ are the areas under the ROC curve. The reported *P* values were calculated to test the null hypothesis that the AUC = 0.50. See Figure 1 for other definitions.

inactive SLE with "normal" MPA $AUC_{0–12}$ (40.5 and 73.9 μg.hour/ml) and 1 had inactive SLE with low MPA $AUC_{0–12}$ (28.3 μg.hour/ml). Four patients had thrombocytopenia (platelet count $<120{,}000/mm^3$) and MPA AUCs of 14.0, 34.1, 36.6, and 40.5. Two had active disease. We found no correlation between MPA $AUC_{0–12}$ and white blood cell count ($r = 0.02$, $P = 0.90$), neutrophil count ($r = 0.01$, $P = 0.91$), hemoglobin level ($r = 0.24$, $P = 0.05$), or platelet count ($r = –0.04$, $P = 0.72$).

DISCUSSION

MMF is the morpholinoethyl ester of MPA, which is hydrolyzed to MPA in the stomach and in the proximal small bowel. It is an inactive prodrug that is converted into its active metabolite, MPA, by intestinal, liver, and plasma esterases (24). We have shown that fixed daily doses of MMF do not guarantee ideal exposure to MPA, as we observed a 10-fold variation of MPA $AUC_{0–12}$/gm of MMF received. This interindividual variation in the exposure to MMF is a critical issue, since fixed-dose and nonindividualized dosing regimens of MMF are currently in use in most randomized controlled trials.

In transplant patients, this wide between-subject variability has been previously reported (25–27) and has lent support to the development of strategies of MPA $AUC_{0–12}$ monitoring. These strategies were based on the findings that MPA pharmacokinetic parameters, especially MPA $AUC_{0–12}$, are associated with the outcome of transplantation (rate of acute rejection) in renal, heart, and liver transplantation (7).

Herein we report for the first time a strong association between MPA $AUC_{0–12}$ and SLE activity, as assessed by scoring the disease with both the SLEDAI and the BILAG index. In a recent study of 20 SLE patients, Rolland et al (5) observed only a trend toward a lower $AUC_{0–4}$ in patients who had low complement C3 concentrations, suggesting a possible relationship between AUC and SLE disease activity. However, unlike the current study, that study did not assess disease activity using validated indexes such as the SLEDAI and the BILAG index.

We found that the mean MPA $AUC_{0–12}$ of a group of patients with active SLE was significantly lower than that of a group of patients with inactive SLE, who were similar to the active SLE group in mean age, sex distribution, mean body weight, mean BMI, mean daily dose of steroids, mean daily dose of MMF, and mean blood HCQ concentration. We have demonstrated that the MPA $AUC_{0–12}$ is correlated with the SLEDAI and with C3 and anti-dsDNA levels, the 2 main biologic markers of SLE activity (19). In our multivariate analysis, MPA $AUC_{0–12}$ was the sole factor that was significantly associated with active SLE status on the day of sampling. It is very unlikely that other factors such as duration of treatment with MMF or prior treatment with other drugs may have influenced these results, since all patients were treated using MMF for at least 10 weeks and the MMF dosage was not modified during that period. Moreover, the daily dose of corticosteroids among patients with active SLE was similar to that among patients with inactive SLE.

A critical issue is irregular therapeutic compliance, since this may account for some variability in the AUC values and in disease activity (20,23). To address this, MMF was administered to the patients on the day of sampling, to ensure that they received the prescribed dose. Additionally, we used blood HCQ concentrations to assess therapeutic observance objectively, as previously described by our group (23). We found that the mean HCQ concentrations as well as the proportion of patients with low and very low HCQ concentrations were not significantly different between patients with active SLE and patients with inactive SLE, suggesting similar compliance in both groups.

Creatinine clearance, serum albumin level, and the daily dose of MMF were recognized as independent parameters influencing MPA $AUC_{0–12}$ (Table 3). Thus, it is legitimate to discuss whether differences in those 3 parameters may explain the difference of MPA $AUC_{0–12}$ we observed between patients with active disease and

Table 3 Univariate and multivariate analysis of parameters potentially influencing MPA AUC_{0-12} in patients treated with MMF

Parameter	*P*	
	Univariate analysis*	Multivariate analysis†
Sex (male)	0.87	0.49
Ethnicity (black)	0.43	0.58
BMI	0.28	0.49
Daily dose of steroids	0.83	0.83
Blood HCQ levels	0.49	0.18
Creatinine clearance (MDRD)	0.01	0.04
Daily dose of MMF	0.008	0.01
Albumin level	0.001	0.0036

* For univariate analysis, association between mycophenolic acid area under the plasma concentration–time curve from 0 to 12 hours (MPA AUC_{0-12}) and both sex and ethnicity was determined using Fisher's exact test. For all other parameters, association was determined using linear regression. See Table 1 for other definitions.
† Multivariate analysis was performed using the least squares method.

patients with inactive disease. First, both groups had similar creatinine clearance level and received similar daily doses of MMF. Thus, both creatinine clearance and the daily dose of MMF cannot be considered as significant confounding factors. Second, even though serum albumin level was found to be lower in patients with active SLE, multivariate analysis revealed that it was not an independent parameter influencing disease activity. This suggests that MPA AUC_{0-12} is not lower in the patients with active disease solely because these patients have lower albumin levels.

Using ROC curve analysis, we found that an MPA AUC_{0-12} above 35 μg.hour/ml was associated with the lowest risk of active SLE, as assessed by both the SLEDAI (score $\geq$6) and the BILAG index (BILAG A and B). We therefore propose 35 μg.hour/ml as the target MPA AUC_{0-12} threshold for SLE. The very high NPV of MPA AUC_{0-12} for active SLE, as defined by both the SLEDAI and the BILAG index, suggests that it is very unlikely that patients with MPA AUC_{0-12} above this threshold may have active SLE. Based on our data, we cannot recommend an upper MPA AUC_{0-12} limit. In renal transplantation, the recommended target MPA AUCs are between 30 and 60 μg.hour/ml (28,29). The upper limit is usually based on drug toxicity. In SLE, we did not find that high MPA AUC_{0-12} was associated with a higher occurrence of hematologic adverse events. This finding was not unexpected, since free MPA has been found to be better correlated with MMF toxicity (30), while total MPA exposure was better correlated with efficacy. At MPA AUC_{0-12} above 45 μg.hour/ml the ROC curve nearly plateaued (Figure 3), but still suggested a moderate gain of efficacy.

Among the important limitations of our study are its cross-sectional design. Definitive conclusion regarding the association between MPA AUC_{0-12} levels and SLE activity may only be established through prospective monitoring of MPA AUC_{0-12} levels. Such a prospective longitudinal study is currently under way. Yet, the results presented herein may be seen as a valuable addition to the current knowledge of MMF pharmacokinetics in SLE.

In conclusion, there is a strong association between disease activity and MPA AUC_{0-12} in SLE. This finding provides evidence for the benefit of individualized dosing regimens of MMF, with a recommended target AUC above 35 μg.hour/ml to improve the efficacy of MMF in SLE. Because there is a high interindividual variability of MMF pharmacokinetics, inclusion of MPA AUC_{0-12} as a parameter in future clinical trials evaluating MMF in SLE could be important for more relevant comparisons of MMF-treated patients and controls. A prospective longitudinal monitoring of MPA AUC_{0-12} levels to assess whether low MPA AUC_{0-12} levels precede SLE flares is currently in progress at our center.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Amoura had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Zahr, Arnaud, Hulot, Piette, Amoura.

Acquisition of data. Zahr, Arnaud, Marquet, Haroche, Costedoat-Chalumeau, Piette, Amoura.

Analysis and interpretation of data. Zahr, Arnaud, Hulot, Funck-Brentano, Amoura.

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