



## ORIGINAL ARTICLE

# Therapeutic drug monitoring of enteric-coated mycophenolate sodium by limited sampling strategies is associated with a high rate of failure

Jean-Michel Hougardy<sup>1,\*</sup>, Laurette Maufort<sup>1,\*</sup>, Frédéric Cotton<sup>2</sup>, Julien Coussement<sup>1</sup>, Dimitri Mikhalski<sup>1</sup>, Karl M. Wissing<sup>3</sup>, Alain Le Moine<sup>1</sup>, Nilufer Broeders<sup>1</sup> and Daniel Abramowicz<sup>4</sup>

<sup>1</sup>Department of Nephrology, ULB Hôpital Erasme, Brussels, Belgium, <sup>2</sup>Department of Clinical Chemistry, ULB Hôpital Erasme, Brussels, Belgium, <sup>3</sup>Department of Nephrology, Universitair Ziekenhuis Brussel, Brussels, Belgium and <sup>4</sup>Department of Nephrology, Universitair Ziekenhuis Antwerpen, Brussels, Belgium

Correspondence and offprint requests to: Daniel Abramowicz; E-mail: daniel.abramowicz@gmail.com or dabram@ulb.ac.be

\*These authors contributed equally to this work.

## Abstract

**Background:** Therapeutic drug monitoring of mycophenolic acid (MPA) is usually performed with a limited sampling strategy (LSS), which relies on a limited number of blood samples and subsequent extrapolation of the global exposure to MPA. LSS is usually performed successfully with mycophenolate mofetil (MMF), but data on enteric-coated mycophenolate sodium (EC-MPS) are scarce. Here, we evaluated the feasibility of 6-h LSS therapeutic drug monitoring with EC-MPS compared with MMF monitoring among kidney transplant recipients.

**Methods:** Sixty-two patients who received EC-MPS during the first 6 months of transplantation were compared with a matched group of 64 MMF-treated kidney transplant recipients. The area under the curve (AUC) was computed by LSS using multiple concentration time points (0, 1, 2, 3 and 6 h post-dose) and a trapezoidal rule. Patients had MPA therapeutic drug monitoring performed on two occasions, one within 2 weeks and the second after 3–4 months of transplantation.

**Results:** EC-MPS monitoring and MMF therapeutic drug monitoring were not interpretable in 34.5% ( $n = 40/116$ ) and 1.8% ( $n = 2/112$ ) of patients, respectively [relative risk [RR] 19.3 [95% confidence interval (CI) 4.8–78.0];  $P < 0.0001$ ]. The main cause of abnormal EC-MPS therapeutic drug monitoring was delayed absorption of both the previous evening and the morning dose, resulting in MPA plasma levels before the next morning dose being higher than MPA plasma levels measured at 1, 2 and 3 h after taking EC-MPS. Cyclosporin in association with MMF significantly increased the risk of low AUC values ( $<30$  mg h/L) in comparison with tacrolimus [55% ( $n = 11/20$ ) and 10% ( $n = 9/88$ ), respectively; RR 5.4 (95% CI 2.6–11.2);  $P < 0.0001$ ].

**Conclusions:** The risk of therapeutic drug monitoring failure with EC-MPS is  $>30\%$  during the first 6 months of renal transplantation. Delayed pharmacokinetics was the main reason. In contrast, the risk of therapeutic drug monitoring failure was substantially lower with MMF.

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**Key words:** suppression of area under curves, enteric coating, kidney transplantation, mycophenolate mofetil, pharmacokinetics

## Introduction

Mycophenolic acid (MPA), the active compound of mycophenolate mofetil (MMF) and enteric-coated mycophenolate sodium (EC-MPS), is the most widely used antiproliferative agent in kidney transplantation [1, 2]. The main side effects of MPA are gastrointestinal disturbances, haematologic disorders (e.g. anaemia and leucopenia) and infections [3–5]. These adverse events are reported more frequently with high MPA exposure (>60 mg h/L of plasma). In contrast, the relative risk (RR) of acute rejection significantly increases with low MPA exposure (<30 mg h/L) [3, 4, 6–8]. Accordingly, an MPA exposure between 30 and 60 mg h/L is considered optimal [9].

Like some other immunosuppressive drugs, MPA exhibits a narrow therapeutic index and large inter- and intra-individual variability [10, 11]. Therefore, an expert panel has recommended MPA therapeutic drug monitoring for kidney transplant recipients who receive dual immunosuppressive therapy, reduced-dosage calcineurin inhibitor (CNI) therapy, CNI switch or withdrawal and in recipients with high immunologic risk [9]. The full area under the plasma concentration–time curve (AUC 0–12 h) is the gold standard method to assess MPA exposure, but it is time-consuming and expensive [4–6, 9, 12]. In routine clinical practice, limited sampling strategies (LSSs) from multiple concentration time points are perhaps a more practical method to evaluate the MPA AUC in the hours following drug intake. LSSs are translated into AUCs by computing the results by trapezoidal rule, multiple linear regressions or Bayesian estimation [9]. While both MPA formulations, MMF and EC-MPS, have shown equivalent efficacy in kidney transplant recipients [13–15], EC-MPS exhibits delayed and prolonged intestinal absorption. Therefore, LSS may not be accurate to evaluate MPA AUCs. Indeed, LSS is frequently performed with MMF [9, 16] but remains rarely reported with EC-MPS [8, 17–19]. Here, we retrospectively reviewed the performance of a trapezoidal rule-based LSS in kidney transplant recipients receiving either EC-MPS or MMF in combination with CNI and steroids.

## Materials and methods

### Study design

We started performing MPA AUC in our centre in September 2005. We retrospectively reviewed the charts of all kidney transplant recipients (N = 434) who had transplants performed at our centre between September 2005 and May 2011. We excluded 15 patients who received a combined graft (kidney and pancreas or liver), 5 patients who were already transplanted with another solid organ transplant and 23 paediatric recipients. Among the remaining 391 patients, 72 received EC-MPS from the transplantation, but 2 patients had no MPA AUC during the first 6 months and 8 patients had incomplete blood sampling. A total of 62 evaluable patients were therefore included in analyses. For comparison, sex- and age-matched MMF-treated kidney transplant recipients with an MPA AUC performed during the first 6 months were selected as a comparison group (N = 64). By centre protocol, most MPA AUCs were performed during the first 10 days, with the objective of avoiding MPA underexposure (EC-MPS, N = 52/116; MMF, N = 57/112; ‘early therapeutic drug monitoring’), or after (almost always during months 2–3), mainly to avoid MPA

overexposure (EC-MPS, N = 64/116; MMF, N = 55/112; ‘late therapeutic drug monitoring’).

### Monitoring methods

The MPA AUC level was computed on the basis of five sampling time points (before and 1, 2, 3 and 6 h after medication intake). As previously reported, MPA plasma levels were measured at 1, 2 and 3 h (C<sub>1</sub>, C<sub>2</sub> and C<sub>3</sub>) to evaluate peak concentration (C<sub>max</sub>) [8, 10, 20]. A later time, 6 h (C<sub>6</sub>), was selected to assess enterohepatic MPA cycling. AUC values were computed by the trapezoidal rule developed in our centre in 2005 according to Hale et al.’s publication in kidney transplant recipients [21, 22]. Internal validation consistently demonstrated that trapezoidal rule was equivalent to several published equations derived from linear regression and was validated by full 12-h AUC measurements that were performed in kidney transplant recipients (n = 8, unpublished data). MPA plasma concentrations were measured by high-performance liquid chromatography–ultraviolet light as recommended [23]. According to their kinetic profile, curves were classified as interpretable or not. Curve qualification criteria required that peak MPA plasma concentrations (C<sub>max</sub>) be higher than those at both C<sub>6</sub> and C<sub>0</sub>. In other words, C<sub>max</sub> had to occur at C<sub>1</sub>, C<sub>2</sub> or C<sub>3</sub>. Indeed, the occurrence of C<sub>max</sub> at C<sub>0</sub> or C<sub>6</sub> strongly suggests that the 6-h sampling period does not capture adequate MPA exposure.

### Patient demographics

The analysis included 62 and 64 kidney transplant recipients who were treated with EC-MPS and MMF, respectively. Demographics are summarized in Table 1. Within the EC-MPS group, cyclosporin was the most frequently used CNI (n = 59/62), whereas it was tacrolimus (n = 50/64) among MMF patients. The systematic association of EC-MPS with cyclosporin in the context of a clinical trial explained this difference in CNI distribution between the two patient populations.

### Data collection

For each patient, the following data were collected: age, sex, body weight at transplant, graft characteristics (date of transplant, donor age, graft order, current panel reactive antibody), the date the AUC was calculated, characteristics of concomitant immunosuppressive drugs, classical biological parameters (e.g. creatinine) and comorbidities (e.g. diabetes, oesophagitis and digestive ulcer). For EC-MPS-treated kidney transplant recipients, we also focussed on factors that may influence MPA exposure, such as oesophagitis and/or gastritis (n = 23), chronic viral hepatitis (n = 4), anti-HIV therapy (n = 0) and other co-medications taken at the time of therapeutic drug monitoring and possibly interfering with MPA absorption/metabolism [i.e. prophylactic use of drugs against herpetic virus [i.e. aciclovir, valaciclovir, ganciclovir or valganciclovir; n = 46], proton pump inhibitor [PPI] use [n = 95] and antibiotics [i.e. penicillins (n = 6), quinolone (n = 2)]]. No patients were taking metronidazole or rifampicin.

### Statistical analyses

Continuous data are expressed as mean (SD) or median [interquartile range (IQR)] as appropriate. The D’Agostino and Pearson

Table 1. Patient characteristics

	MMF (N = 64)	EC-MPS (N = 62)	P-value
Recipient age (mean ± SD; years)	54 ± 13	52 ± 14	0.38
Donor age (mean ± SD; years)	45 ± 14	44 ± 14	0.77
BMI (mean ± SD; kg/m <sup>2</sup> )	26 ± 5	25 ± 5	0.55
Proportion of male (% , n)	69% (44)	73% (45)	0.70
Proportion of first graft (% , n)	94% (60)	100% (62)	0.12
PRA max >0%	11% (7)	5% (3)	0.32
AUCs, early period (% , n)	51% (57)	45% (52)	0.43
AUCs, late period (% , n)	49% (55)	55% (64)	
Treatment by cyclosporin (% , n)	19% (12)	95% (59)	<0.0001
MPA dose, early period (mean ± SD; mg/kg)	28 ± 6	20 ± 4	NA
MPA dose, late period (mean ± SD; mg/kg)	28 ± 7	20 ± 5	NA
Creatinine, early period (mean ± SD, mg/dL) <sup>a</sup>	3.3 ± 3.0	3.2 ± 2.2	0.35
Creatinine, late period (mean ± SD, mg/dL) <sup>b</sup>	1.4 ± 1.2	1.4 ± 0.7	0.08

Cyclosporin was more frequently prescribed in the EC-MPS group in comparison to the MMF group (mainly treated with tacrolimus) (see text).

BMI, body mass index; PRA, panel reactive antibody; NA, not applicable.

<sup>a</sup>Mean on day of MPA assay: 5.7 ± 1.8 (n = 57, MMF) and 4.8 ± 1.7 (n = 52, EC-MPS).

<sup>b</sup>Mean on day of MPA assay: 83.7 ± 42.9 (n = 55, MMF) and 74.4 ± 46.2 (n = 64, EC-MPS).

omnibus normality test was used to determine normality. Unpaired non-parametric data were compared by the Mann-Whitney test. For some data, contingency tables were established and  $\chi^2$  was determined. Correlations were obtained by linear regression analysis and the non-parametric Spearman rank correlation coefficient. P-values <0.05 were considered statistically significant. Statistical analyses were performed using Prism 5.0a (GraphPad Software, La Jolla, CA, USA).

## Results

### Adequacy of therapeutic drug monitoring

A mean of 1.87 and 1.75 AUC was obtained for EC-MPS- and MMF-treated kidney transplant recipients, respectively. EC-MPS monitoring and MMF therapeutic drug monitoring were not interpretable in 34.5% (n = 40/116) and 1.8% (n = 2/112) of assays, respectively [RR 19.3 [95% confidence interval (CI) 4.8–78.0]; P < 0.0001]. Within the MMF-treated group, the rate of therapeutic drug monitoring failure was low whether patients were taking cyclosporin A (n = 12) or tacrolimus (n = 42) (4.8 versus 1.1%, respectively; P = 0.34). For EC-MPS, the proportion of inadequate therapeutic drug monitoring was similar whether they were performed early or late [n = 18/52 (35%) versus 22/64 (34%); P = 1.00]. We failed to identify any risk factor for an abnormal kinetic profile among EC-MPS patients (i.e. oesophagitis and/or gastritis, diabetes, early versus late post-transplant period, PPI use, cyclosporin or antihyperlipidemic drugs and obesity) (Table 2).

### MPA pharmacokinetics

MPA plasma concentrations were plotted according to time for EC-MPS (n = 116) and MMF (n = 112) (Figure 1). In the case of a valid AUC, EC-MPS (n = 76) showed delayed peak MPA plasma concentrations compared with MMF (n = 110). Peak MPA plasma concentrations were shown after 1 h in 74% (81/110) of MMF and 32% (24/76) of EC-MPS assays (P < 0.0001). Peak MPA plasma concentrations were shown most often at 2 h with EC-MPS (41%, n = 31/76). C<sub>max</sub> values were similar (10.5 ± 4.8 and 12.5 ± 9.0 mg/L for MMF and EC-MPS, respectively; P = 0.23). The two abnormal MMF AUCs showed lower peak concentrations and higher C<sub>6</sub>, suggesting delayed absorption of the morning dose (Figure 1A). In

Table 2. Risk factors for therapeutic drug monitoring failure with EC-MPS

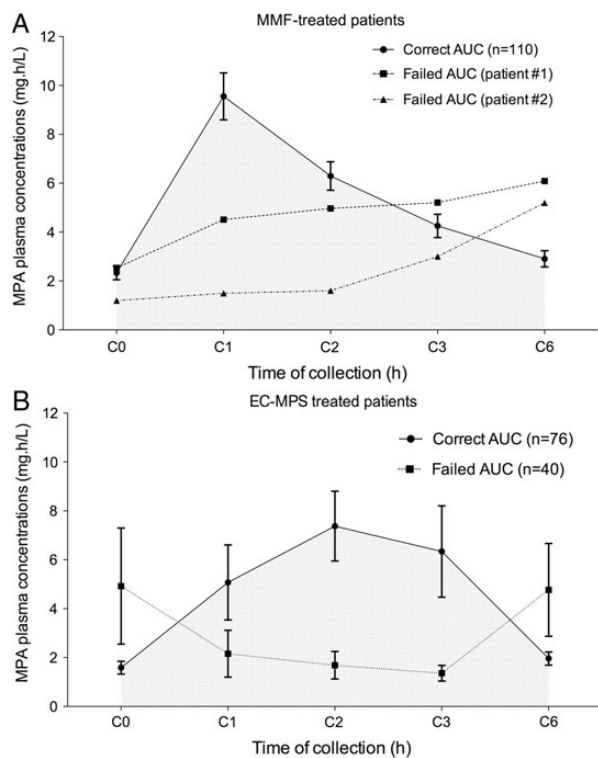
	Proportion of therapeutic drug monitoring failures, % (n/N)		P-value
	Absent	Present	
Oesophagitis/gastritis	31 (12/39)	39 (9/23)	0.58
Diabetes	35 (17/48)	29 (4/14)	0.76
Early period	33 (4/12)	34 (17/50)	1.00
PPI use	33 (4/12)	34 (17/50)	1.00
Antiviral drugs (herpes virus) intake	38 (6/16)	32 (15/46)	0.76
BMI ≥30 mg/kg	32 (17/53)	44 (4/9)	0.47

PPI, proton pump inhibitors.

comparison with valid EC-MPS AUCs (n = 74), abnormal EC-MPS AUCs (n = 40) exhibited higher C<sub>0</sub> (4.9 ± 7.4 versus 1.6 ± 1.1 mg/L; P = 0.04) and delayed peak MPA plasma levels (Figure 1B). As previously described, cyclosporin was associated with lower exposure to MPA when compared with tacrolimus [cyclosporin + MMF: 32.3 ± 13.8 mg/h/L (n = 20) versus Tac + MMF: 48.4 ± 18.8 mg/h/L (n = 88); P < 0.0001; cyclosporin + EC-MPS: 35.3 ± 15.2 mg/h/L (n = 73) versus Tac + EC-MPS: 45.9 ± 1.0 mg h/L (n = 3)]. Accordingly, cyclosporin in association with MMF increased the risk of low AUC values (<30 mg/h/L) by nearly 50% in comparison with tacrolimus [55% (n = 11/20) and 10% (n = 9/88), respectively; RR 5.4 (95% CI 2.6–11.2); P < 0.0001].

## Discussion

The main finding of our study is the poor performance of the 6-h LSS for therapeutic drug monitoring of EC-MPS. Indeed, about one-third of therapeutic drug monitoring failed with EC-MPS, whereas it was uncommon with MMF. It seems that by virtue of its pharmacokinetic properties, the extended-release EC-MPS formulation leads to an unusually delayed absorption in a significant subset of patients. As a result, absorption of the previous evening dose is still ongoing when the patient undergoes the MPA AUC, and the intake of the morning dose also results in a



**FIGURE 1:** (A) MPA AUC after one dose of MMF. Normal AUC values, solid line ( $n = 110$ ); abnormal AUC values, dashed line ( $n = 2$ ). Vertical lines represent 95% CI. (B) MPA AUC after one dose of EC-MPS. Normal AUC values, solid line ( $n = 76$ ); abnormal AUC values, dashed line ( $n = 40$ ). Vertical lines represent 95% CI. MPA plasma levels, hours after intake.

similar late absorption. Cattaneo et al. [10] reported that  $C_{max}$  could occur as late as 16 h after EC-MPS intake. In our study, the pharmacokinetic profile of MPA (i.e. plasma concentration–time curves) was similar to that reported in the literature for both formulations of MPA [20]. de Winter et al. [24] have also reported the weak performance of LSS with EC-MPS, but their pharmacokinetic analysis was limited to 3 h. We noted high pharmacokinetic variabilities of EC-MPS, reflected in wide CIs in plasma MPA concentrations, which could contribute to therapeutic drug monitoring failure [10, 20, 24].

We investigated whether factors known to influence the absorption of MPA could help to identify patients at risk of EC-MPS LSS failure. The presence of diabetes, obesity, PPI use, antihypertensive drug intake and the timing of LSS (i.e. early versus late) did not predict EC-MPS therapeutic drug monitoring failure (although this study is too small to rule out small effects of these factors on therapeutic drug monitoring failure). Other study limitations include the retrospective design, the low acute graft rejection rate and the fact that MMF or EC-MPS dose modification in response to therapeutic drug monitoring could have reduced the reporting of side effects. Most patients on MMF were taking tacrolimus. In contrast, most patients on EC-MPS were taking cyclosporin. This distribution bias could be a limitation because cyclosporin exhibits well-documented interactions with MPA, whereas tacrolimus does not. However, the virtual absence of MMF therapeutic drug monitoring failure among patients taking cyclosporin strongly suggests that EC-MPS therapeutic drug monitoring failure is an intrinsic characteristic of EC-MPS, irrespective of the associated CNI. Finally, an additional limitation

is the absence of full 12-h AUC values for MMF and EC-MPS in order to compare the ability of the LSS to predict exposure in the two groups. However, we believe that prolongation of the LSS is futile, as the need for a longer collection time could be impractical from a clinical point of view.

In conclusion, EC-MPS therapeutic drug monitoring by a 6-h LSS is associated with a high failure rate because of its delayed absorption. These patients may require the performance of a full 12-h AUC to capture MPA exposure efficiently [9]. For patients who require MPA therapeutic drug monitoring, MMF is currently the most practical therapeutic option and EC-MPS might be best reserved for use in those kidney transplant recipients who do not require therapeutic drug monitoring.

## Conflict of interest statement

We declare that the results presented in this paper have not been published previously in whole or part, except in abstract format.

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