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Drug Monitoring and Toxicology

Comparison of the Emit Immunoassay with HPLC for Therapeutic Drug Monitoring of Mycophenolic Acid in Pediatric Renal-Transplant Recipients on Mycophenolate Mofetil Therapy

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Background: **HPLC is currently the preferred method for accurate measurement of mycophenolic acid (MPA). This study was designed to validate the Emit compared with HPLC in relation to clinical outcome measurements.**

Methods: **Pediatric renal-transplant recipients (n = 50) on an immunosuppressive triple regimen consisting of cyclosporin A, prednisone, and mycophenolate mofetil (600 mg/m^2 twice per day) were investigated in an open-label prospective study. Pharmacokinetic profiles over 12 h were obtained at 1 week, 3 weeks, 3 months, and 6 months posttransplant. Plasma MPA was measured by both reversed-phase HPLC and the Emit immunoassay.**

Results: **There was an association between the risk of acute rejection episodes and low area under the curve values from t_0 to t_{12h} (AUC_{0-12}) for MPA (MPA-AUC_{0-12}) or predose concentrations of MPA derived from both HPLC and Emit measurements. According to ROC analysis, an AUC value of 33.8 mg · h/L for MPA from t_0 to t_{12h} (MPA-AUC_{0-12}) determined by HPLC had a diagnostic sensitivity of 80% and a diagnostic specificity of 57%. The corresponding value of the Emit was 36.1 mg · h/L. For the predose concentration (MPA-c_{12}), a concentration of 1.2 mg/L determined by HPLC and 1.4 mg/L determined by Emit gave a sensitivity of 80% and a specificity of 60%, respectively. There was no association of any pharmacokinetic variables derived from total MPA measurements with an increased risk of side effects related to mycophenolate mofetil.**

Conclusions: **The Emit assay appears to have a comparable diagnostic efficacy to HPLC for assessing the risk of acute rejection in pediatric renal-transplant recipients. However, because of the cross-reactivity of the antibody used in the Emit assay with the active MPA acyl glucuronide metabolite, the decision thresholds for the Emit were higher than those calculated from HPLC measurements.**

Mycophenolate mofetil (MMF)[3] is widely used for maintenance immunosuppressive therapy both in adult *(1)* and pediatric renal-transplant recipients *(2)*. MMF is rapidly metabolized in vivo to its active constituent, mycophenolic acid (MPA), a reversible, noncompetitive inhibitor of inosine monophosphate dehydrogenase. Inhibition of inosine monophosphate dehydrogenase II in activated lymphocytes causes a reduction in intracellular guanine nucleotide pools and leads to an arrest of lymphocyte proliferation. MPA is extensively bound to albumin, with a protein-binding range of 97–99% in patients with healthy renal and liver function *(3–5)*. The primary metabolite of MPA is the phenolic MPA glucuronide (MPAG) 7-O-MPAG. Two further metabolites have been identified in humans, namely the acyl glucuronide

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[3] Nonstandard abbreviations: MMF, mycophenolate mofetil; MPA, mycophenolic acid; PK, pharmacokinetic; PD, pharmacodynamic; AUC, area under the curve; MPAG, MPA glucuronide; AcMPAG, MPA acyl glucuronide; c_0, concentration immediately before dosing; c_{max}, maximum (peak) concentration; and c_{12}, concentration 12 h after dosing.

(AcMPAG) and the phenolic glucoside of MPA *(6)*. Of these three metabolites, only the acyl glucuronide is capable of inhibiting human inosine monophosphate dehydrogenase II *(7)* and proliferation of human mononuclear leukocytes *(8)* in vitro.

The pharmacokinetic (PK) profile of MPA shows large interindividual variability. PK monitoring of MPA with the aim of optimizing the dosage of this drug to achieve adequate immunosuppression with minimized risk of graft rejection or toxicity has therefore been under investigation *(9)*. In adult renal-transplant recipients on cyclosporin A, MMF, and steroids, PK/pharmacodynamic (PD) relationships between the area under the curve (AUC) of MPA or predose MPA concentrations and the risk of acute rejection have been established on the basis of measurements by reversed-phase HPLC with ultraviolet detection *(9–11)*. Low MPA-AUC values estimated with an abbreviated sampling schedule and a validated HPLC procedure were found to be associated with cardiac allograft rejection in heart transplant recipients *(12)*. A commercial, Emit-based immunoassay is available for the determination of MPA. In general, the Emit assay has the advantage of being less laborious and time-consuming and therefore better suited for routine drug monitoring. In a study involving heart-transplant recipients receiving a combination therapy of tacrolimus and MMF, low plasma predose MPA concentrations determined by the Emit procedure were found to be associated with an increased incidence of acute rejection *(13)*. Several studies revealed a systematic positive bias between the results obtained with the Emit assay and those found with HPLC *(14–16)*. This bias is primarily attributable to cross-reactivity of the immunosuppressive metabolite AcMPAG with the antibody used in the Emit MPA assay *(16)*. In contrast, 7-O-MPAG does not display any notable cross-reactivity in this assay.

The interpretation of the PK/PD relationship of MPA in different patient populations can be influenced by several factors, such as the nature of the organ transplanted, the age of the patient, use of concomitant immunosuppressive therapy, protein binding, the presence of active metabolites and the assay used. The purpose of this study, therefore, was to investigate the following: (*a*) the clinical utility of the Emit assay in comparison with HPLC for identifying patients at risk of acute graft rejection and MMF-related side effects in pediatric renal-transplant recipients on an immunosuppressive regimen with cyclosporin A, MMF, and corticosteroids; (*b*) the method-dependent therapeutic ranges for MPA-AUC_{0-12} values and predose MPA concentrations in this patient population; and (*c*) the clinical effectiveness of an abbreviated AUC estimation protocol in comparison to the full MPA-AUC and single time point MPA concentrations.

Materials and Methods

PATIENTS

This study was an open-label longitudinal evaluation of the PK/PD relationship of MPA in pediatric renal-transplant recipients. The inclusion and exclusion criteria have been described in previous reports *(5, 17)*. The study protocol was approved by the local ethics committee of each contributing center. The analyses of the clinical results of the study have been published elsewhere *(18)* as have the PK/PD results according to MPA measurements with a validated HPLC procedure *(19)*. The present comparison involved a subgroup of 50 patients [31 males, 19 females; mean age, 11.8 years (range, 3.2–16.0 years)], in whom both Emit and HPLC data were collected. All patients were Caucasian. Forty of the patients had primary transplant function; 10 had delayed graft function defined as the requirement for dialysis in the first 3 weeks posttransplant. No graft loss occurred in the delayed graft function group. Immunosuppressive therapy consisted of 300 mg/m^2 methylprednisolone on the day of transplant surgery, which was then tapered to 60 mg/m^2 for the first, 30 mg/m^2 for the second, 15 mg/m^2 for the third, 12 mg/m^2 for the fourth, 9 mg/m^2 for the fifth, and 6 mg/m^2 for the sixth week after transplantation and 4 mg/m^2 thereafter. Cyclosporin A (microemulsion formulation) was administered in a dose of 500 mg/m^2 per day given in two divided doses for 24 h starting 6 h after surgery. Thereafter, doses (~300 mg/m^2 per day) were adjusted to achieve 12-h trough concentrations of 150–250 μg/L, as measured by whole-blood monoclonal fluorescence polarization immunoassay on a TDx analyzer (TDx mFPIA; Abbott), in the first 3 months posttransplant; thereafter, 12-h trough concentrations of 100–200 μg/L were targeted. The mean cyclosporin A dose and the respective 12-h predose concentrations at the four PK sampling time points are listed in Table 1. Serum creatinine (intra- and interassay CVs, 3% and 4.5%, respectively) was measured by a CX7 (Beckman Instruments). The glomerular filtration rate was estimated with the formula of Schwartz et al. *(20)*, stratified according to the age and gender of the patients.

Table 1. Cyclosporin A dose and whole-blood predose concentrations in 50 pediatric renal-transplant recipients at the four PK sampling time points posttransplant.[a]

	PK sampling time			
Cyclosporin A	1 week	3 weeks	3 months	6 months
Dose, mg · kg^{-1} · day^{-1}	7.85 (3.13–17.7)	6.98 (3.81–20.7)	5.90 (2.65–13.4)	5.70 (3.06–13.8)
Predose concentration, μg/L	185 (31–452)	193 (126–306)	172 (73–258)	144 (85–199)

[a] Data are median (range).

DOSAGE OF MMF

MMF was administered orally in a dose of 600 mg/m^2 twice a day up to a maximum of 2 g/day. This dose was based on a preliminary report of a dose-finding study in pediatric renal-transplant recipients *(21)*. If the dose could not be administered exactly by use of 250-mg capsules, MMF capsules were opened and the exact dose for each individual child was refilled into gelatin capsules comparable to those produced by the MMF manufacturer. Body surface area was calculated by the formula of DuBois and DuBois *(22)*.

PK PROTOCOL

Patients were studied after informed (parental) consent was obtained. Blood samples for PK assessments were drawn on days 7 and 21 posttransplant ("initial phase") and 3 and 6 months posttransplant ("stable phase"). It was mandatory that all patients had at least 2 full days of the same MMF dose given twice a day before PK investigations. The study was performed in an inpatient environment, starting in the morning. Patients were required to fast from 2200 the night before sampling until after the 75-min sample had been obtained on the following morning. Blood samples were collected at the following times: before dosing and 20, 40, and 75 min and 2, 4, 6, 8, and 12 h after dosing. All blood samples were collected in tubes containing EDTA as an anticoagulant. For determination of MPA concentrations, plasma was separated and stored at −20 °C until analysis.

MEASUREMENT OF MPA BY HPLC AND EMIT

The procedure for the determination of MPA in plasma by HPLC has been described in detail elsewhere *(5, 23)*. Plasma MPA concentrations were also measured by the Emit MPA immunoassay (Dade-Behring) on a Cobas-Mira analyzer according to the manufacturer's instructions, as described previously *(24)*. A cross-check of the calibrators used for the HPLC and Emit assays revealed no calibrator bias (<5%) between the two procedures.

PK ANALYSIS

The following PK data for MPA were determined: predose concentration (c_0); time to maximum concentration [t_{max} (h)]; maximum concentration [c_{max} (mg/L)]; AUC from 0 to 12 h (AUC_{0-12}; mg·h/L) with the linear trapezoidal rule; and the evening predose (i.e., the 12-h) concentration [c_{12} (mg/L)]. An abbreviated three-point AUC based on the sampling times 0 min, 75 min, and 4 h after MMF dosing (MPA-$AUC_{0,\,75\,min,\,4\,h}$) was calculated according to a previously published algorithm *(25)*: estimated AUC = $11.8 + 3.71 \times c_0 + 1.33 \times c_{75\,min} + 3.9 \times c_{4\,h}$. In addition, an algorithm based on an empiric equation with a limited sampling strategy up to 2 h postdose (MPA-AUC_{0-2}) was taken for the calculation of the full AUC *(11)*. The PK analysis was performed with the computer program BiAS (Epsilon-Verlag).

ACUTE REJECTION EPISODES

The clinical diagnosis of rejection was made without any knowledge of the MPA concentration. Thirteen of 50 patients experienced at least one acute rejection episode during the 6-month study period; two of these patients had two rejection episodes, leading to a total number of 15 acute rejection episodes. Eleven of 15 acute rejection episodes were confirmed by biopsy; histologic examination and classification of a core biopsy was performed according to the Banff criteria *(26)*. If a biopsy was logistically impossible or clinically contraindicated, the diagnosis of "presumed rejection" was established on the basis of clinical judgment (supported by one or more of the following clinical findings: increased body temperature, graft swelling, graft tenderness, increase in serum creatinine of >20% from the baseline concentration, or oliguria). The acute rejection episodes occurred 34 (median) days (range, 8–170 days) after renal transplantation; 12 of 15 rejections occurred within the first 70 days posttransplant.

ADVERSE EVENT MONITORING

Adverse events, defined as an abnormal change in physical signs, symptoms, or laboratory values whether or not deemed causally related to the study medication, were recorded throughout the study when reported by a patient or noted by an investigator. Thrombocytopenia was defined as a thrombocyte count $<150 \times 10^{12}$/L. Leukopenia was defined as a granulocyte count <2000/μL and graded according to its severity: mild (1600–1999 granulocytes/μL); moderate (1000–1599 granulocytes/μL); or severe (≤999 granulocytes/μL). Diarrhea was graded as follows: mild diarrhea (n = 7), transient diarrhea lasting up to 2 days; moderate diarrhea (n = 3), tolerable diarrhea lasting longer than 2 days; and severe diarrhea (n = 1), intolerable diarrhea requiring therapy. Infections were classified as moderate when requiring specific antibiotic or antiviral therapy and as severe when requiring hospitalization. The following infections were recorded: herpes labialis (n = 5), herpes zoster (n = 1), oral mucocutaneous candidiasis (n = 1), urinary tract infection (n = 8), cytomegalovirus pneumonia (n = 1), cytomegalovirus colitis (n = 1), bacterial septicemia (n = 3), pneumonia (n = 1), pharyngitis (n = 1), febrile viral infection (n = 4).

STATISTICS

The Shapiro–Wilk test was used to confirm normal distribution of data *(27)*. Because not all values were gaussian, data in Table 1 and 2 are given as median (range). For comparison between two groups, the Wilcoxon signed-rank test was used. For comparison of more than two groups, one-way ANOVA on repeated measurements followed by all pairwise comparison (Student-Newman-Keuls test) was used. Correlations between variables were assessed by univariate linear regression analysis. Differences of $P < 0.05$ were considered to be statistically significant.

ROC plots of sensitivity vs 1 − specificity were generated to determine whether a particular PK variable could discriminate patients with an acute rejection from those who experienced no rejection. Areas under the ROC curves and the 95% confidence interval limits were calculated with the method of Hanley and McNeil *(28)*. The ROC curve analysis was carried out with Analyze-It software (Ver. 1.44; Analyze-It Software).

Results

COMPARISON OF HPLC AND IMMUNOASSAY

The PK variables for MPA as calculated from either the HPLC or the Emit assay data are shown in Table 2. Both in the initial and the stable phase, the PK variables calculated from data measured by the Emit assay were consistently higher than those calculated from the HPLC data. The median Emit MPA-AUC_{0-12} values were 11.6% higher (1 week posttransplant), 17.4% higher (3 weeks), 10.3% higher (3 months), and 6.2% higher (6 months) than those obtained by HPLC. The Emit MPA c_{12} values were 28.6% higher (1 week), 23.3% higher (3 weeks), 23.9% higher (3 months), and 16.7% higher (6 months) than the corresponding HPLC values. The overestimation of the Emit assay is primarily attributable to the cross-reactivity of the acyl glucuronide metabolite with the MPA antibody *(23)*. Because the kidneys clear this metabolite, it could be expected that the overestimation by Emit is greater in patients with reduced renal function compared with the rest of the study population. Indeed, we observed an inverse curvilinear relationship between the proportional bias of MPA-AUC_{0-12} values by Emit and the respective glomerular filtration rate (Fig. 1).

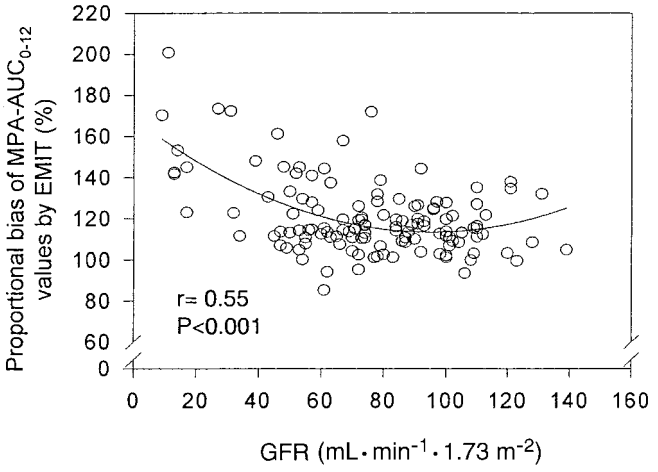

Fig. 1. Relationship between the proportional bias of MPA-AUC_{0-12} values by Emit and the respective glomerular filtration rate (*GFR*).

There was an inverse curvilinear relationship ($r = 0.55$; $P < 0.01$) that could be best described by the equation: $y = 168.7 - (1.1597x) + (0.0061x^2)$.

PK RESULTS

In agreement with our previous finding, there was a large interindividual variation of PK data, despite the fact that all patients were receiving the same body surface-adjusted MMF dosage (Table 2). For example, MPA-AUC_{0-12} values derived from Emit data at 3 months posttransplant ranged from 29.2 to 147 mg · h/L. The interindividual CV was comparable in the initial (21%) and stable phase (22%) posttransplant. Whereas the PK variables at 1 week and 3 weeks posttransplant were not significantly different, there was an increase in the PK variables c_0 (113%), c_{12} (85%), and AUC_{0-12} (97%) according to the HPLC data between the 3-week and the 3-month sampling times; the respective increases in the PK variables according to the Emit data were comparable (Table 2). There was no further statistically significant increase of MPA PK variables between the 3- and 6-month sampling times, in agreement with our previous report in a smaller cohort of patients *(17)*.

Because a full MPA-AUC that requires at least eight blood samples during a 12-h dose interval is impractical in clinical routine practice, we investigated whether a single-time point MPA concentration or an abbreviated AUC derived from a limited number of samples correlated with the respective full MPA-AUC. Only a moderate correlation was observed between either the predose

Table 2. Comparison of the PK variables for MPA at the four PK sampling time points posttransplant as calculated from either the HPLC or the Emit data.[a]

MPA PK variable	1 week		3 weeks		3 months		6 months	
	HPLC	Emit	HPLC	Emit	HPLC	Emit	HPLC	Emit
c_0, mg/L	1.01[b] (0.11–2.66)	1.24[b,d] (0.25–4.44)	0.96[b] (0.06–2.54)	1.30[b,d] (0.00–2.72)	2.07[c] (0.35–11.2)	2.38[c,d] (0.56–13.6)	2.92[c] (0.18–12.6)	3.08[c,d] (0.24–14.4)
c_{12}, mg/L	1.12[b] (0.11–4.85)	1.44[b,d] (0.28–6.04)	0.86[b,d] (0.00–7.54)	1.06[b,d] (0.00–8.04)	1.59[b] (0.28–6.89)	1.97[b,d] (0.55–8.20)	2.27[c] (0.25–9.44)	2.65[c,d] (0.52–9.62)
c_{max}, mg/L	9.74[b] (1.50–32.1)	10.7[b,d] (2.23–35.8)	14.2[b] (3.53–45.7)	14.3[b] (3.82–50.6)	24.9[c] (7.70–53.5)	25.0[c] (6.64–60.9)	26.0[c] (9.40–52.5)	27.4[c,d] (5.89–53.2)
AUC_{0-12}, mg · h/L	33.6[b] (3.12–60.6)	37.5[b,d] (6.26–76.9)	32.2[b] (12.8–58.4)	37.8[b,d] (6.26–76.9)	63.3[c] (28.6–139)	69.8[c,d] (29.2–147)	65.7[c] (21.3–117)	69.8[c,d] (26.9–152)

[a] Data are median (range).

[b,c] Intratest comparison was performed by one-way ANOVA on repeated measurements followed by all pairwise comparison (Student-Newman-Keuls test). Values sharing common superscripts are not significantly different, whereas values without common superscripts are significantly different ($P < 0.05$). Intertest comparison was performed by Wilcoxon signed-rank test.

[d] Emit vs HPLC, $P < 0.01$.

Table 3. Correlation of the MPA PK variables c_0, c_{12}, and c_{max} and the abbreviated profiles MPA-$AUC_{0,\ 75\ min,\ 4\ h}$ and MPA-AUC_{0-2} with the full-time MPA-AUC_{0-12}, as calculated from either the HPLC or Emit data, in 50 pediatric renal-transplant recipients.

PK variable	HPLC	Emit
MPA c_0	$r = 0.66$; $P < 0.0001$	$r = 0.68$; $P < 0.0001$
MPA c_{12}	$r = 0.54$; $P < 0.0001$	$r = 0.53$; $P < 0.0001$
MPA c_{max}	$r = 0.71$; $P < 0.0001$	$r = 0.73$; $P < 0.0001$
MPA-$AUC_{0,\ 75\ min,\ 4\ h}$	$r = 0.90$; $P < 0.0001$	$r = 0.92$; $P < 0.0001$
MPA-AUC_{0-2}	$r = 0.87$; $P < 0.0001$	$r = 0.89$; $P < 0.0001$

MPA trough concentration (c_0) and the respective MPA-AUC_{0-12} or the 12-h evening trough concentration (c_{12}; Table 3). Although the c_0 concentration may have been taken at a more variable time after the last dose than the c_{12} concentration, which was sampled exactly 12 h after a supervised dose, the correlations of c_{12} with the full AUC were not superior to those of c_0. There was also only a moderate correlation between the MPA peak concentration (c_{max}) and the respective MPA-AUC_{0-12}. The abbreviated MPA-$AUC_{0,\ 75\ min,\ 4\ h}$ gave a reasonable correlation with the full AUC that was somewhat superior to that seen between the abbreviated profile MPA-AUC_{0-2} and the full AUC (Table 3). There was a good agreement between the abbreviated AUC values as calculated from either the HPLC data or Emit data with the respective full AUC (Table 3).

To provide the context for an assessment of PK variables in an individual patient in relation to the pediatric population distribution, 5th to 95th percentiles for MPA-AUC_{0-12} and predose (c_{12}) concentrations were calculated according to the Emit measurements (Fig. 2).

PK/PD ANALYSIS REGARDING ACUTE REJECTIONS

There were a total of 15 acute rejection episodes in 13 of the 50 patients during the 6-month study period. The MPA-AUC_{0-12} and MPA-c_{12} values determined by Emit for the patients with an acute rejection in relation to the respective percentiles of the whole study population are shown in Fig. 2. In the initial posttransplant phase, both the AUC_{0-12} and MPA-c_{12} values were distributed in the lower PK percentiles (Fig. 2). Nine of 12 patients with rejections in the initial posttransplant phase had MPA-AUC values by Emit below the median of 37.5 mg · h/L before experiencing an acute rejection episode. The comparison with those patients who neither suffered from an acute rejection nor an adverse event yielded a relative risk of acute rejection of 42% with MPA-AUC_{0-12} values <37.5 mg · h/L compared with 15% with MPA-AUC_{0-12} values >37.5 mg · h/L. The cyclosporin A dose [rejecters, 7.5 (4.2–13) $mg \cdot kg^{-1} \cdot day^{-1}$; nonrejecters, 8.0 (3.1–14) $mg \cdot kg^{-1} \cdot day^{-1}$] and the 12-h predose whole-blood concentrations [rejecters, 187 (76–330) mg/L; nonrejecters, 190 (88–331) mg/L] were not significantly different between the two groups [data are median (range) of the respective mean data calculated from the PK-sampling time points at 1 week and 3 weeks posttransplant].

To establish which PK value is the best predictor for the risk of acute rejection, ROC curves were computed for each of the four PK variables. For these calculations, the mean values from the 1- and 3-week sampling time points were taken for each variable and patient. Diagnostic sensitivities (true-positive results) were calculated for each individual PK value as the fraction of those patients with an acute rejection who had a value below this discrimination threshold. The corresponding diagnostic specificities (false-negative results) were calculated as the fraction of patients with no rejection episode who had a value below this decision threshold. The ROC plots of sensitivity vs 1 − specificity for the PK variables AUC_{0-12} and c_{12} by HPLC and Emit are shown in Fig. 3, the statistical comparison of the areas under the ROC curves is given in Table 4. For the HPLC data, c_0, c_{12}, AUC_{0-12}, and the abbreviated AUC estimation $AUC_{0,\ 75\ min,\ 4\ h}$ were

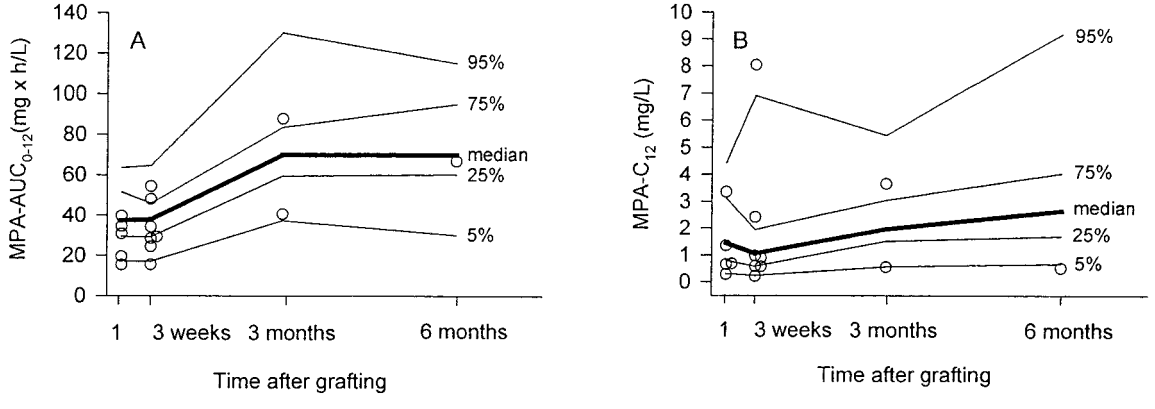

Fig. 2. Time course of MPA-AUC_{0-12} (*A*) and predose trough (c_{12}) plasma concentrations (*B*) derived from Emit measurements in the first 6 months after grafting in pediatric renal-transplant recipients.

Data are the median and the 5th to 95th percentiles of the total patient population (n = 50). All patients had received the full MMF dose of 600 mg/m^2 twice per day for at least 2 days before PK sampling. ○, episode of an acute rejection. The respective acute rejection episodes are graphically assigned to the nearest PK sampling period preceding the clinical event. The median time period between an acute rejection episode and the preceding PK sampling period was 8 days (range, 0–85 days).

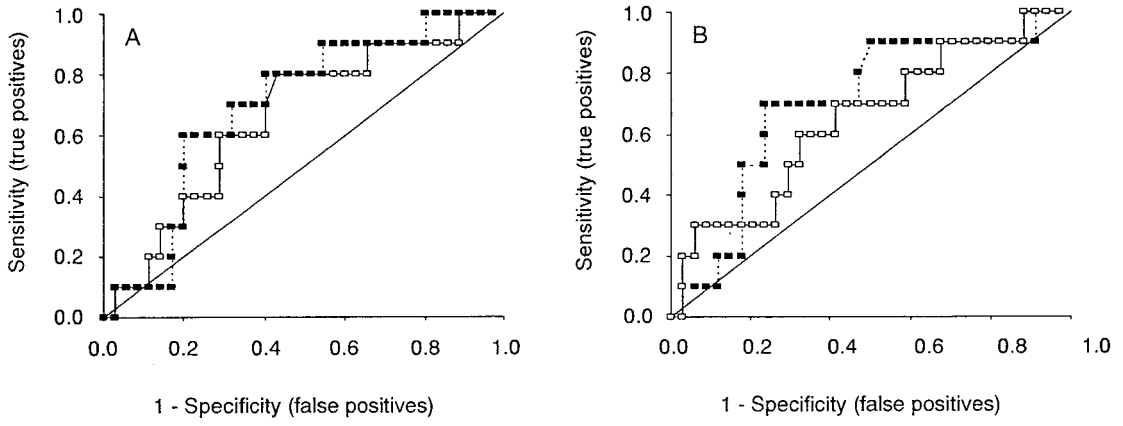

Fig. 3. ROC curves illustrating the ability of the pharmacokinetic variables MPA-AUC_{0-12} (□) and predose trough (c_{12}) concentration (■) to discriminate between patients with (n = 10) and without (n = 40) an acute rejection episode during the first 70 days posttransplant.
The PK variables were calculated from the HPLC (*A*) or Emit (*B*) data. The *solid line* represents the theoretical ROC curve for no discrimination between the two groups.

able to discriminate patients with acute rejections from patients with no rejection. The Emit data gave comparable results. As expected, the corresponding decision thresholds, below which there is an enhanced risk of acute rejection, were higher for the Emit data than those noted for the HPLC data. An AUC_{0-12} by HPLC of 33.8 mg · h/L had a diagnostic sensitivity of 80% and a diagnostic specificity of 57%. The corresponding value by Emit was 36.1 mg · h/L. For the PK variable c_{12}, a concentration of 1.2 mg/L determined by HPLC and 1.4 mg/L determined by Emit gave a sensitivity of 80% and a specificity of 60%.

PK/PD analysis regarding adverse events

PK variables of total MPA, irrespective whether derived from HPLC or Emit measurements, were not discriminatory for leukopenia and/or infections, neither in the initial (Table 5) nor the stable posttransplant phase (Table 6). There was also no association between the incidence of diarrhea, anemia, or thrombocytopenia and any of the PK values derived from measurements of MPA (results not shown).

Discussion

This is the first report in pediatric renal-transplant recipients demonstrating that the Emit assay appears to be comparable to the HPLC methodology for assessing the risk of acute rejection. We have shown that both MPA-AUC_{0-12} and predose MPA trough concentrations are significantly associated with the risk of acute rejection in this patient population irrespective of which methodology is used to measure plasma MPA. However, because of the cross-reactivity of the antibody used in the Emit assay with the active metabolite acyl-MPAG, the decision thresholds for the Emit are higher than those reported with HPLC. From our data, an MPA-AUC_{0-12} of 36.1 mg · h/L derived from Emit measurements will have a clinical efficacy comparable to a HPLC-derived threshold of 33.8 mg · h/L. In the case of predose MPA plasma concentrations, an Emit value of 1.4 mg/L is comparable to a HPLC value of 1.2 mg/L. The HPLC methodology is generally laborious and time-consuming, and therefore is less suited for routine clinical monitoring than immunoassay procedures. In contrast to the immunoassay, sample pretreatment is necessary for the HPLC determination of MPA. In our experience, up to 50 samples can be analyzed during 24 h by our HPLC method in combination with an autosampling device. The same number of samples can be analyzed within 2–3 h by the Emit procedure. Immunoassays, however, are often less specific than HPLC-based procedures because of the cross-reactivity of the antibody

Table 4. Areas under the ROC curves for PK variables of MPA as calculated from either the HPLC or the Emit data to discriminate between patients with (n = 10) or without an acute rejection episode during the first 70 days posttransplant.

	HPLC			Emit		
MPA PK variable	Area under the ROC curve	95% CI[a]	*P*	Area under the ROC curve	95% CI	*P*
c_0, mg/L	0.67	0.49–0.85	0.03	0.66	0.48–0.85	0.04
c_{12}, mg/L	0.70	0.53–0.87	0.01	0.68	0.50–0.86	0.03
c_{max}, mg/L	0.57	0.37–0.78	0.24	0.57	0.35–0.80	0.26
AUC_{0-12}, mg · h/L	0.66	0.47–0.84	0.04	0.64	0.45–0.84	0.04
$AUC_{0,\ 75\ min,\ 4\ h}$	0.72	0.54–0.90	0.009	0.72	0.53–0.91	0.01
AUC_{0-2}	0.64	0.46–0.82	0.06	0.65	0.45–0.85	0.08

[a] CI, confidence interval.

Table 5. Areas under the ROC curves for PK variables of MPA as calculated from either the HPLC or the Emit data to discriminate between patients with or without adverse events of infections (n = 20) and/or leukopenia (n = 2) during the first 70 days posttransplant.

	HPLC			Emit		
MPA PK variable	Area under the ROC curve	95% CI[a]	*P*	Area under the ROC curve	95% CI	*P*
c_0, mg/L	0.62	0.45–0.79	0.09	0.57	0.39–0.75	0.23
c_{12}, mg/L	0.63	0.45–0.81	0.07	0.59	0.41–0.77	0.18
c_{max}, mg/L	0.53	0.31–0.75	0.40	0.58	0.37–0.79	0.23
$AUC_{0–12}$, mg · h/L	0.54	0.35–0.73	0.34	0.61	0.42–0.79	0.13
$AUC_{0,\ 75\ min,\ 4\ h}$	0.54	0.35–0.73	0.35	0.59	0.40–0.78	0.17
$AUC_{0–2}$	0.52	0.33–0.71	0.42	0.56	0.36–0.77	0.27

[a] CI, confidence interval.

with structurally similar drug metabolites that may be pharmacologically inactive. In the case of the Emit immunoassay for MPA, cross-reactivity occurs with only one of the known metabolites of MPA, AcMPAG *(6, 24)*, and this metabolite is potentially pharmacologically active *(7, 8)*. The Emit assay, therefore, has the theoretical advantage to measure both MPA and its pharmacologically active metabolite. One could therefore have expected the PK variables derived from the Emit measurements to be superior to the HPLC measurements for the discrimination of acute rejection episodes. However, this was not the case. The fact that the cross-reactivity of the acyl glucuronide is concentration dependent *(24)* and that the AcMPAG-$AUC_{0–12}$ values are relatively low in relation to the MPA-$AUC_{0–12}$ values (mean 16% of MPA-$AUC_{0–12}$) *(29)* may explain these findings.

The incidence of the MMF-related adverse events diarrhea, vomiting, or abdominal pain was not associated with MPA PK variables, consistent with previous findings in adult renal-transplant recipients *(10)*. The adverse events leukopenia and/or infections were also not associated with any of the PK variables derived from total MPA measurements. This finding is an agreement with the results of the Randomized Concentration Controlled Study in adult renal-transplant recipients *(10)*, but in contrast to the recent report of Mourad et al. *(30)* from an unicenter trial in 31 adult renal-transplant recipient. In this study, a MPA concentration 30 min after the oral dose of MMF (c_{30}) was associated with an increased risk for MMF-related side effects. This difference may be attributable to the high preponderance of leukopenia (60% of all side effects) in the latter study presumably as a consequence of induction therapy with anti-thymocyte globulin, whereas in our study without induction of anti-thymocyte globulin, the incidence of leukopenia was rather low (four episodes during the observation period of 6 months). Recently, Mourad et al. *(31)* observed a relationship between total plasma MPA concentrations and toxicity of either gastrointestinal (42%) or hematologic origin (58%) in a patient population on a tacrolimus-based immunosuppressive regimen. However, because the incidence of gastrointestinal side effects is higher in patients on tacrolimus vs cyclosporin A *(32)* and PK variables of MPA are differentially influenced by coadministration of tacrolimus vs cyclosporin A *(33, 34)*, the report of Mourad et al. *(31)* is not necessarily contradictory to our observations. In this context, it is noteworthy that data from our group *(19)* and other investigators *(35)* suggest that measurement of free MPA by HPLC appears to be more appropriate for the assessment of the toxic risk of MMF regarding leukopenia and/or infections.

There is currently only limited information on the clinical utility of the Emit immunoassay. Meiser et al. *(13)* used serial daily measurements of predose MPA concentrations determined with the Emit assay (target range, 2.5–4.5 mg/L) to individualize MMF dosage in heart-

Table 6. Areas under the ROC curves for PK variables of MPA as calculated from either the HPLC or the Emit data to discriminate between patients with or without adverse events of infections (n = 7) and/or leukopenia (n = 2) in the stable posttransplant phase.

	HPLC			Emit		
MPA PK variable	Area under the ROC curve	95% CI	*P*	Area under the ROC curve	95% CI	*P*
c_0, mg/L	0.52	0.33–0.72	0.41	0.58	0.38–0.78	0.22
c_{12}, mg/L	0.53	0.33–0.73	0.38	0.53	0.32–0.74	0.39
c_{max}, mg/L	0.58	0.38–0.77	0.22	0.52	0.32–0.72	0.42
$AUC_{0–12}$, mg · h/L	0.57	0.37–0.77	0.23	0.58	0.38–0.77	0.22
$AUC_{0,\ 75\ min,\ 4\ h}$	0.64	0.45–0.83	0.09	0.61	0.52–0.88	0.06
$AUC_{0–2}$	0.54	0.35–0.73	0.34	0.58	0.39–0.77	0.21

transplant recipients on concomitant therapy with tacrolimus. In comparison to a previous fixed dose regimen of MMF, they found a reduced incidence of acute rejection in the collective with dosage individualization. Similarly, Yamani et al. *(36)*, in a study of 215 heart-transplant recipients, observed in the presence of therapeutic cyclosporin A or tacrolimus blood concentrations a significantly decreased incidence of acute rejection when MPA trough concentrations determined by Emit was ≥2 mg/L compared to samples with MPA trough concentrations <2 mg/L.

The results of our study illustrate that the Emit immunoassay will be applicable for monitoring MMF therapy in renal transplantation. It has to be emphasized that the value of therapeutic drug monitoring of MPA has not been formally tested. However, given the association between MPA PK variables and the risk of acute rejection, therapeutic drug monitoring of MPA in the initial posttransplant phase could be helpful in selected high-risk patients. It would be reasonable to aim at achieving MPA-AUC values or predose MPA concentrations somewhat higher than those achieved in the lower percentiles by individual adjustment of the MMF dose. For such an approach, the percentiles specific to patients and immunosuppressive regimens for MPA-AUC and MPA predose concentrations from this study, according to both HPLC and Emit immunoassay measurements, could serve as guidelines for optimization of MMF therapy in pediatric renal-transplant recipients. Data from this and other studies indicate that a consensus is arising that for minimizing the risk of rejection after transplantation, total MPA-AUC values in the early posttransplant stages should be maintained within the therapeutic window of 30–60 mg · h/L, according to HPLC data, or 35 to 70 mg · h/L, according to Emit data. The target predose concentration should be maintained within the range of 1–3.5 mg/L, according to HPLC data, and 1.3–4.5 mg/L, according to Emit data. Because some fluctuations are to be expected as a consequence of the enterohepatic circulation of MPA, extreme values or a large change should be verified by repeat measurement before a change in the MMF dose is undertaken.

The question arises as to which PK variable of MPA is best suited for therapeutic drug monitoring of MMF therapy in clinical practice. In this study, the variables MPA-AUC_{0-12}, MPA-$AUC_{0,\ 75\ min,\ 4\ h}$, MPA c_{12}, and MPA c_0 were comparable for assessing the risk of acute rejection episodes, whereas MPA-AUC_{0-2} had a somewhat poorer predictive value (Table 4). In two previous studies in adult renal-transplant recipients, the MPA-AUC was a better predictor of outcome than the predose concentration *(11, 37)*. Predose concentrations are more convenient than measurements of AUC, which are complex and affect the cost of routine clinical monitoring. However, the predose c_0 concentration may be sampled at a more variable time after the last dose. Hence, the calculation of AUC with a limited sampling strategy of up to 4 h after MMF dosing (MPA-$AUC_{0,\ 75\ min,\ 4\ h}$) appears be a more precise approach for the assessment of the risk of acute rejection than the predose c_0 concentration.

In conclusion, we have established that the Emit assay is comparable to the HPLC methodology for assessing this risk of acute rejection in pediatric renal-transplant recipients on an immunosuppressive triple-drug therapy with cyclosporin A, MMF, and corticosteroids. We have established putative therapeutic ranges for these PK variables according to the Emit immunoassay measurements in this specific patient population. These data could facilitate the therapeutic drug monitoring of MPA for optimization of MMF efficacy by steering patients away from the extreme values of MPA PK variables.

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