Population Pharmacokinetics of Mycophenolic Acid in Kidney Transplant Pediatric and Adolescent Patients

S. Payen, D. Zhang,† A. Maisin,‡ M. Popon,† A. Bensman,§ F. Bouissou,^ C. Loirat,‡ R. Gomeni,*# F. Bressolle,* and E. Jacqz-Aigrain†*

Abstract: Current data on mycophenolate mofetil (MMF) suggest that there is a pharmacokinetic/pharmacodynamic relationship between the mycophenolic acid (MPA) area under the curve (AUC) during treatment and both the risk of acute rejection and the occurrence of side effects. The aim of this study was to characterize the population pharmacokinetics of MPA in kidney transplant patients between the ages of 2 and 21 years and to propose a limited sampling strategy to estimate individual MPA AUCs. Forty-one patients received long-term oral MMF continuous therapy as part of a triple immunosuppressive regimen, which also included cyclosporine or tacrolimus (n = 3) and corticosteroids. Therapy was initiated at a dose of 600 mg/m^2 twice daily. The population parameters were calculated from an initial group of 32 patients. The data were analyzed by nonlinear mixed-effect modeling using a 2-compartment structural model with first-order absorption and a lag time. The interindividual variability in the initial volume of distribution was partially explained by the fact that this parameter was weight-dependent. Fifteen concentration–time profiles from 13 patients were used to evaluate the predictive performance of the Bayesian approach and to devise a limited sampling strategy. The protocol, involving two sampling times, 1 and 4 hours after oral administration, allows the precise and accurate determination of MPA AUCs (bias −0.9 μg·h/mL; precision 6.02 μg·h/mL). The results of this study combine the relationships between the pharmacokinetic parameters of MPA and patient covariates, which may be useful for dose adjustment, with a convenient sampling procedure that may aid in optimizing pediatric patient care.

Key Words: mycophenolate mofetil, population pharmacokinetics, pediatric population, limited sampling strategy

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From the *Clinical Pharmacokinetics Laboratory, Faculty of Pharmacy, University Montpellier I, Montpellier, France; †Department of Paediatric Pharmacology and Pharmacogenetics, Robert Debré Hospital, Paris, France; ‡Department of Paediatric Nephrology, Robert Debré Hospital, Paris, France; §Department of Paediatric Nephrology, Trousseau Hospital, Paris, France; and ^Department of Paediatric Nephrology, Children Hospital Toulouse, Toulouse, France. #Present address: GlaxoSmithKline, Verona, Italy.

Reprints: Dr. Françoise Bressolle, PhD, Laboratoire de Pharmacocinétique Clinique, Faculté de Pharmacie, BP 14491, 34093 Montpellier Cedex 5, France (e-mail: Fbressolle@aol.com).

Solid organ and bone marrow transplantation has long been considered a viable therapeutic option, and the number of such procedures is continually increasing. Continuous adequate immunosuppression is necessary to prevent acute rejection of the graft, whereas overimmunosuppression can lead to serious toxicity, infections, and an increased risk of lymphoproliferative disease.

Mycophenolate mofetil (MMF) is the 2-morpholinoethyl ester prodrug of mycophenolic acid (MPA). This drug has been isolated from a *Penicillium* species[1–3] and has been approved for immunosuppressive therapy following renal transplantation in both adults and children. MPA targets the de novo purine biosynthesis pathway by inhibition of inosine monophosphate deshydrogenase, thus acting against the proliferation of T and B lymphocytes. It blocks the synthesis of guanosine nucleotide, which is required as a building block for DNA and RNA synthesis.[4,5] MMF was used in place of azathioprine, in combination with cyclosporine or tacrolimus and corticosteroids. Controlled large-scale multicenter double-blind randomized trials of oral MMF in renal allograft transplantation have shown the effectiveness of this regimen in suppressing acute rejection.[6–11]

MMF is rapidly and completely absorbed, undergoing extensive presystemic deesterification to MPA. The pharmacokinetics of this drug are complicated because (1) MPA is first metabolized to MPA glucuronide (MPAG) by hepatic and renal glucuronyl transferases,[12] (2) MPAG undergoes enterohepatic recycling (appearance of a secondary plasma MPA peak, 6–12 hours after MMF intake), and (3) MPA is 97% bound to plasma proteins. In addition, there are large variations in the pharmacokinetic parameters between patients. It was found that there was a high degree of intraindividual variability in the AUC_{0-12h} in the early posttransplantation period but that this variability decreased approximately 3 months after the start of treatment.[13–15] In comparison with the immediate posttransplantation period, a 2-fold increase of the AUC_{0-12h} of total MPA and a 35% decrease in the MPA free fraction were observed in the stable posttransplantation period. The mean "apparent elimination half-life" of MPA after oral administration was 16–18 hours.[2,16]

Therapeutic drug monitoring (TDM) in pediatric and adult patients may help to reduce both short- and long-term adverse effects and to minimize the risk of acute rejection during the early posttransplantation period.[13–15,17–26] Indeed, a significant association was found between AUC_{0-12h} of total MPA and acute rejection in both adult and pediatric patients. It has been reported that the risk of rejection is increased in

adults when the AUC_{0-12h} of total MPA is less than 30–40 μg·h/mL.[15,23,27,28] However, the corresponding figures have not yet been defined for pediatric patients.

Some authors have proposed a limited sampling strategy to calculate the AUC.[24,29,30] The best 3 time-point requirements for determining the MPA AUC appeared to be at 1, 2, and 6 hours.[24,29] However, for practical purposes, it is essential to limit the number of samples to 1 or 2, particularly in the pediatric population. Moreover, because most of the patients receiving MMF are treated on an outpatient basis, the duration of hospitalization is limited to a few hours. Dose adjustment using Bayesian forecasting, which enables therapeutic drug concentrations to be arrived at more rapidly and thus improving patient clinical outcomes,[31] could play an important role in the optimization of drug dosage regimens in this vulnerable population of patients, which has a large interpatient variability. Recently, Le Guellec et al[32] and Shum et al[33] reported population pharmacokinetic data for MPA in renal adult transplant patients. The first authors concluded that an accurate prediction of AUC can be obtained by the Bayesian approach and 3 blood samples (at 20 minutes, 1 hour, and 3 hours).

The present study was carried out to provide data on MMF pharmacokinetics to optimize both pediatric and adolescent patient care. It was performed on 41 patients aged between 2 and 21 years. The first objective of this study was to determine accurate population pharmacokinetic parameters of MPA using a 2-compartment open model in a population of patients covering a wide age range. This model takes into account the dependence of the initial volume of distribution on body weight. A nonlinear mixed-effect modeling approach (NONMEM software) was used to determine population pharmacokinetic parameters from an initial group of 32 patients (population group). The predictive performance of the Bayesian procedure was evaluated using an independent group of patients (test group), and thus, the predicted MPA concentrations were compared with measured concentrations. The second objective of the study was to propose a limited sampling strategy to determine the pharmacokinetic parameters of MPA.

PATIENTS AND METHODS

Patients

A total of 41 patients (29 male, 12 female) were selected for the study. Renal transplant patients, aged 10.8 ± 5.1 years at the time of transplantation and treated with corticosteroids, cyclosporine (or tacrolimus, n = 3), and mycophenolate mofetil, were included in this study. The mean age of the patients was 12.9 ± 4.9 years at the time of the first pharmacokinetic study. All had normal liver function tests. Patient characteristics are given in Table 1, and the age distribution frequency in the studied population is presented Figure 1.

For all patients, anamnesis data, a physical examination, and standard laboratory analyses, including hematological and biochemical tests, were performed during the study period.

Written permission to collect data, available as part of routine care or clinical trial reporting, was obtained from the institutional review board. The study protocol was performed in accordance with the legal requirements, with the Declaration of Helsinki, and with current European Community and US Food and Drug Administration guidelines for good clinical practice. For pediatric patients, written consent was obtained from the patients and/or their parents or legal guardians.

TABLE 1. Patient Characteristics

	Weight (kg)	Age (years)	Creatine Clearance (mL/min)
Patients at the time of the first pharmacokinetic study (n = 41; 29 M, 12 F)			
Mean	39.4	12.9	128
CV%	35.4	38.2	48.1
Min	12.0	2.0	32
Max	68.6	21.0	248
Population group (n = 32 patients; 24 M, 8 F)			
Mean	39.5	12.5	139
CV%	33.3	36.6	44.2
Min	12.0	2.0	45
Max	61.9	21.0	248
Validation group* (n = 15 concentration–time profiles; 9 M, 6 F)			
Mean	37.0	12.2	105
CV%	46.6	51.4	54.8
Min	11.4	2.0	32
Max	68.6	19.0	214

*Nine new patients not included in the population group plus 6 concentration–time profiles from the 4 patients included in the population group for whom additional pharmacokinetic evaluations were performed (8 blood samples) at different intervals (21 to 204 days) during long-term treatment.

Drug Administration and Data Collection

Pharmacokinetic, demographic, and covariate data were collected from patient medical records, clinical trial case folders, and therapeutic drug monitoring databases.

The patients received long-term oral MMF continuous therapy as part of a triple immunosuppressive regimen, which also included cyclosporine (or tacrolimus, n = 3) and corticosteroids. MMF was initially prescribed in place of azathioprine for patients receiving a second renal transplant following acute rejection of the first graft, and for patients presenting

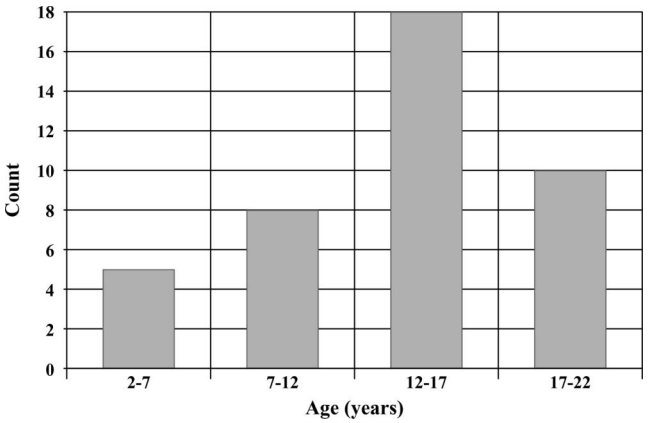

FIGURE 1. Age frequency of distribution in the studied population.

nephrotoxicity and requiring a reduction in the dose of cyclosporine. In the second stage of the study, MMF was associated with cyclosporine and corticosteroids as the drug of first choice. Therapy was initiated at a dosage of 600 mg/m^2 twice daily. Doses were adjusted empirically on the basis of clinical evidence of efficacy and toxicity and to maintain MPA trough blood concentrations between 1 and 5 μg/mL in the first months after transplantation. Patients were instructed to take their MMF dose at the same time each morning and evening on an empty stomach. Corticosteroid therapy was initiated at the dose of 60 mg/m^2, then progressively reduced to 15–30 mg/m^2 1 month after transplantation, to 7.5 mg/m^2 between 3 and 6 months after transplantation, and finally to 5 mg/m^2. This dose was then maintained for life.

During treatment, at least 1 full concentration–time profile was determined for all patients; the first profile was taken at a median of 183 (12–3754) days posttransplantation and 40 (10–1538) days after starting MMF treatment. In 4 patients in whom the first pharmacokinetic evaluation was performed 10–20 days after the start of MMF treatment, ie, early in the posttransplantation period, additional concentration–time profiles were performed during treatment to accurately adjust the dosage. Two of the patients, aged 15 and 14 years, underwent 2 additional pharmacokinetic evaluations. These were conducted 21 days after the first evaluation for 1 patient and after 97 days for the other patient, and at intervals of 42 and 204 days, respectively, between the second and the third evaluations. A further 2 patients, both aged 2 years, underwent a second pharmacokinetic evaluation after 126 and 146 days, respectively. Because there are significant changes (1) in the clinical status of the patients after the graft, (2) in drug disposition in the first few years of life, and (3) in MPA pharmacokinetics in the immediate posttransplantation period, these 6 concentration–time profiles were considered as having been obtained from 6 new patients and were included in the test group. On the day of the evaluation, blood samples were collected as follows: before administration (to determine predose trough concentration) and at 1, 2, 4, 6, 8, 10, and 12 hours after drug intake. Additional blood samples were taken periodically during treatment before drug administration (to determine trough concentrations). The number of collected samples per patient ranged from 7 to 17.

Blood samples were collected by means of an indwelling catheter in EDTA-coated polypropylene tubes; the samples were rapidly centrifuged, and the plasma was frozen at −20°C until required for analysis.

Analytic Methodology

Drug analysis was carried out at the Clinical Pharmacology Unit of the Robert Debré Hospital. The plasma MPA concentrations were measured by high-performance liquid chromatography (HPLC) using a previously published method with some modifications.[34] The HPLC system consisted of a quaternary P1000 XR pump (ThermoQuest TQ, FL), a TQ autosampler, and a TQ UV 6000 detector (254 nm) linked to a TQ Spectranet for recording and storing throughout analysis. The system used a Zorbax SB-C8 analytic column (250 × 4.6 mm ID) packed with 5-μm-diameter particles as the stationary phase and a mobile phase of acetonitrile/0.05% aqueous phosphoric acid (38:62, vol/vol). The assay showed linearity from 0.1 to 40 μg/mL. Intra- and interassay precision determined from the method validation (using quality control samples at 0.25, 1.8, and 35 μg/mL) was less than 10%, and accuracy ranged from 90% to 110%. The lower limit of quantification was 0.1 μg/mL.

No interference was found with MPAG or with potentially coadministered drugs (and their metabolites). In some patients involved in this study, both MPA and MPAG were determined.[20]

Data Splitting

The subjects enrolled in the study were randomly assigned to a model-building set (population group, 32 patients) or to a model-validation set (test group, 9 patients not included in the population group, plus the 6 concentration–time profiles from the 4 abovementioned patients, for whom additional concentration–time profiles were performed). The following covariates were considered pertinent to this study: patients' age, body surface area, weight, creatinine clearance, and comedication. Relationships between patient covariates and pharmacokinetic parameter estimates were explored.

Structural Model Development and Population Pharmacokinetic Analysis

Pharmacokinetic model-building and model-validation analyses were performed using NONMEM software (version 5.1.1, Globomax LLC, Andover, MD)[35] through the Visual-NM graphical interface.[36]

The population characteristics of the pharmacokinetic parameters (fixed and random effects) were estimated using the subroutines ADVAN-2, ADVAN-3, ADVAN-4, or ADVAN-6 from the library of programs provided with the NONMEM-PREDPP package. Both first-order (FO) and first-order conditional estimation (FOCE) methods were used to determine population pharmacokinetic parameters. The FO method was used to fit the models because it markedly improved the fit. The following structural models were tested (Fig. 2): (1) a 1-compartment model with first-order absorption rate and a lag time (model 1), (2) a 2-compartment model with first-order absorption rate with or without a lag time (models 2 and 3), (3) a 2-compartment model with a zero-order absorption rate, the absorption duration being estimated from the data (model 4),[32] and (4) a 2-compartment model plus enterohepatic recycling with a first-order absorption rate and a lag time (model 7).[37–40] The structural model was chosen on the basis of changes in −2 log likelihood and on graphic analyses of the goodness-of-fit. Because −2 log likelihood is approximately χ^2 distributed, and the addition of 1 compartment increases the degrees of freedom by a factor of 2, a change of 5.99 in −2 log likelihood was required at the 5% significance level to select the more complex model. Interindividual variability in pharmacokinetic parameters was modeled by use of a proportional error model as follows:

$$P_j = P_{mean} \cdot \exp(\eta_P) \quad (1)$$

where P_{mean} is the population mean, and η_P is the difference between the population P_{mean} and the P value in the subject j; η_P is assumed to be a Gaussian random variable with mean zero and variance $\sigma^2\eta$.

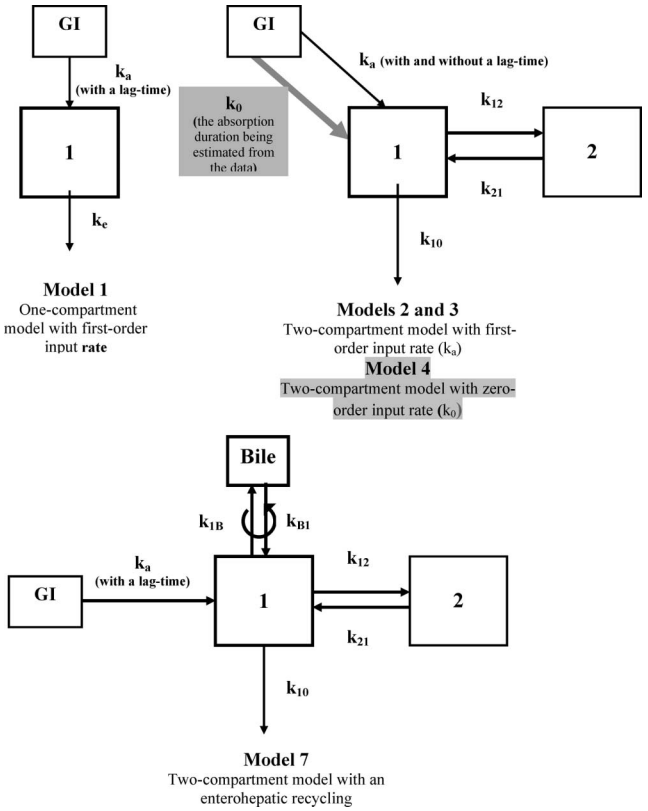

FIGURE 2. Different models tested to fit MPA data. GI, gastrointestinal tract; 1, central compartment; 2, peripheral compartment; k_a, first-order input rate; k_0, zero-order input rate; k_{12} and k_{21}, intercompartmental rate constants; k_{10}, elimination rate constant; k_{1B} and k_{B1}, transfer rate constants between the central compartment and bile; ↻, enterohepatic recirculation.

Various error models were also tested. At each step of model building, diagnostic plots were analyzed for similarity and randomness along the line of identity on the observed versus the predicted concentration plot, as well as randomness along the residual and weighted residual zero line on the predicted concentrations versus residuals or weighted residuals plot. The error on the concentration measurements of the individual j was best described by a combined additive and proportional model given below:

$$C_{ijk}(t) = f(p_j, D_{ij}, t_{ij}) \cdot \exp(\varepsilon_{1ij}) + \varepsilon_{2ij} \qquad (2)$$

where p_j is the pharmacokinetic parameter of the subject j; t_{ij} is the time of the i^{th} measurement; D_j is the dosing history of the subject j; f is the pharmacokinetic model; ε_{1ij} and ε_{2ij} represent the residual departure of the model from the observations and contain contributions from intraindividual variability, assay error, and model misspecification for the dependent variable. ε_1 and ε_2 are assumed to be random Gaussian variables with mean zero and variances of $\omega^2\varepsilon_1$ and $\omega^2\varepsilon_2$.

The predicted plasma concentrations (C_{IPRED}) were calculated for each individual by means of the empirical Bayes estimate of pharmacokinetic parameters using the POSTHOC option in the NONMEM program.

Data analysis was performed using a 3-step approach:

1. The population parameters, fixed and random effects, together with the individual posterior estimates were calculated assuming that pharmacokinetic parameters and covariates were not mutually dependent.
2. Following the selection of the basic structural and statistical models, the influence of covariates was investigated. The first approach involved graphic exploration of each covariate and each of the estimated pharmacokinetic parameters. Where a relationship appeared to exist, the selected covariates were included individually in the model and tested for statistical significance. The change in the NONMEM objective function produced by the inclusion of a covariate term (asymptotically distributed as χ^2 with degrees of freedom equal to the number of parameters added to the model) was used to compare alternative models. A change in objective function of at least 3.8 ($P < 0.05$ with 1 degree of freedom) was required for statistical significance at the initial covariate screening stage. Finally, accepted covariates were included in the model and the population pharmacokinetic parameters were estimated. To demonstrate that the retained covariates contributed to an improved fit in the population pharmacokinetic model, each covariate was deleted sequentially from the final model (backward elimination) to confirm the statistical significance (χ^2 test). If the variation in the objective function was not statistically significant, the relationship between the covariate and the pharmacokinetic parameter was ignored.
3. Only covariates that provided a significant change in the objective function when introduced into the model were retained for analysis. The population parameters were estimated, taking into account their relationship with the covariates. Closeness to and randomness along the line of unity on the observed versus the predicted concentration plot, as well as randomness along the residual and weighted residual zero line on the predicted concentration versus residual or weighted residual plot were considered qualitative evidence of the goodness of fit.

Structural Model Validation and Limited Sampling Strategy

First, in the validation group, a noncompartmental pharmacokinetic analysis of MPA was carried out to obtain the area under plasma concentration versus time curve (AUC, log-linear trapezoidal rule) from the full profile (ie, 8 blood samples between 2 drug intakes). This analysis was performed using Pk-fit software.[41,42]

Second, individual pharmacokinetic parameters of the patients in the validation group were calculated on the basis of the Bayesian approach, combining prior knowledge of the mean and dispersion of the population pharmacokinetic parameters with 8 blood samples collected at fixed time points in the interval between 2 drug administrations.

Third, individual pharmacokinetic parameters (only the AUC, because of its clinical relevance, was considered for this purpose) of the patients in the validation group were estimated on the basis of the Bayesian approach (as previously described), but in a limited number of samples. The rationale for the sampling strategy used was to select the concentrations in

the plasma that would be most sensitive to differences in the volume of distribution (the peak concentration) and elimination (the concentration peak and subsequent trough).[43–46] Moreover, for practical purposes, it is useful to limit the number of samples to 1. Thus, schedules using 1 (each of the 8 available samples), 2 (12 combinations: predose-1 hour and predose-2 hours, 1-4, 1-6, 1-8, 1-10, 1-12, 2-4, 2-6, 2-8, 2-10, and 2-12 hours), and 3 samples (1-2-4, 1-2-6, 1-4-6, 1-4-8, and 1-4-12 hours), respectively, were tested.

In the final step, the AUC was computed using the multiple linear regression (MLR) equation (with sampling times at 1, 2, and 6 hours) proposed by Fillet and Mai.[24]

Statistical Analysis

Performance of Bayesian Individual Parameter Estimates

The performance of estimation by the Bayesian methodology was assessed for the validation group (15 concentration–time profiles) by comparing the observed concentrations (DV) to the ones estimated by the Bayesian approach and all the available concentration–time points (IPRED) by using the following criteria[47,48]:

The bias or mean predictor error:

$$Bias = \frac{1}{N}\sum_{i=1}^{i=N}[DV - IPRED] \quad (3)$$

The precision or root mean square error:

$$\text{Precision} = \sqrt{\frac{1}{N}\sum_{i=1}^{i=N}[DV - IPRED]^2} \quad (4)$$

In these expressions the index i refers to the concentration number, and N is the sample size. The 95% confidence interval for bias was calculated, and the *t* test was used to compare the bias to 0.

Moreover, AUCs estimated using the Bayesian approach (considering all data concentrations) were compared with those obtained by noncompartmental analysis.

Computing of a Limited Sampling Strategy

To determine the reliability of the parameter estimates using a limited sampling protocol for each combination, AUCs determined using the Bayesian approach and a limited sampling strategy were compared with those estimated using Bayesian estimation and all data concentrations (reference values). These comparisons were performed by calculating the bias and the precision.

RESULTS

Population Modeling

The population database consisted of 449 MPA concentrations from 32 patients. The basic population pharmacokinetic model (before inclusion of covariates) was best represented by a 2-compartment model with a lag time (model 2, Table 2). The model was parameterized in terms of the apparent rate constant of distribution ($\alpha = \theta_1$), the apparent rate constant of elimination ($\beta = \theta_2$), the transfer rate constant from tissue compartment to central compartment ($k_{21} = \theta_3$), the initial volume of distribution ($V_1/F = \theta_4$), the absorption rate constant ($k_a = \theta_5$), and the lag time (θ_6).

TABLE 2. Model-Building Steps

Models	No. of Parameters	Objective Function	Difference in the Objective Function
Model 1: One-compartment model with a first-order input rate and a lag time	4	929.3	—
Model 2: Two-compartment model with a first-order input rate and a lag time	6	773.0	—
Model 3: Two-compartment model with a first-order input rate but without a lag time	5	777.1	—
Model 4: Two-compartment model with a zero-order input rate, the absorption duration being estimated from the data	5	842.8	—
Model 5: Two-compartment model with a lag time including the following relationship between V_1/F and body weight: $V_1/F = \theta_4 \cdot \text{Weight} + \theta_5$	8	713.4	59.6
Model 6: Two-compartment model with a lag time including the following relationship between V_1/F and body weight: $V_1/F = \theta_4 \cdot \text{Weight}$	7	713.4	59.6
Model 7: Two-compartment model plus enterohepatic recycling and a lag time	8	773.6	—

From the individual (Bayesian estimates) primary pharmacokinetic parameters, the following secondary pharmacokinetic parameters were calculated: the apparent oral clearance (CL/F) and the area under plasma concentration versus time curve (AUC).

The parameter estimates given by this model are summarized in Table 3. From the population parameter determined in step 1, interindividual variability of the α parameter was fixed at 0. In step 2, during covariate testing, body weight was identified as a source of variability for V_1/F (Fig. 3; $r^2 = 0.72$, $P = 4.2 \times 10^{-9}$) and was retained in the NONMEM analysis. Thus, in step 3, final population parameters were calculated, taking the relationship between V_1/F and body weight into account. The inclusion of this second-stage model (model 6) significantly improved both the relationship between model-predicted and observed concentrations (the objective function decreased from the baseline model value of 773 to 713.4, $P < 0.001$, Table 2) and the plot of weighted residuals versus model-predicted concentrations. Moreover, this model substantially decreased interindividual variability (44.3% to 29.7%) and residual error compared with the baseline model. Population pharmacokinetic parameters are presented in Table 3. The mean V_1/F value was 4.75 L (CV = 29.7%) and the apparent

TABLE 3. Population Pharmacokinetic Parameters of MPA

Parameters	Population Parameters Before Covariate Inclusion (Population Group)		Population Parameters After Covariate Inclusion (Population Group)		Population Parameters Determined From 41 patients (47 Concentration–Time Profiles)*	
	Population Mean	Interindividual Variability CV (%)	Population Mean	Interindividual Variability CV (%)	Population Mean	Interindividual Variability CV (%)
α (h^{-1})	θ_1 = 7.70	—	θ_1 = 7.50		θ_1 = 7.50	—
β (h^{-1})	θ_2 = 0.00759 (30.2%)	25.1	θ_2 = 0.00746 (28.9%)	23.2	θ_2 = 0.0072 (28.6%)	32.1
k_{21} (h^{-1})	θ_3 = 0.0182 (33.4%)	25.3	θ_3 = 0.0172 (32.2%)	26.6	θ_3 = 0.017 (31.3%)	22.4
V_1/F (L)	θ_4 = 4.55 (19.0%)	44.3	θ_4 = 0.121 (13.0%)†	29.7	θ_4 = 0.120 (16.9%)‡	35.1
k_a (h^{-1})	θ_5 = 0.71 (18.0%)	55.8	θ_5 = 0.61 (20.5%)	43.1	θ_5 = 0.63 (19.2%)	44.1
Lag time (h)	θ_6 = 0.71 (4.2%)	104.4	θ_6 = 0.65 (5.1%)	97.5	θ_6 = 0.69 (4.7%)	99.5
Intraindividual variability	20.4%; 0.849		18.5; 0.56		26.5%; 0.573	

Values in parentheses are the error of estimate expressed as coefficient of variation.

*Thirty-two patients from the population group plus patients from the test group (ie, 9 new patients not included in the population group plus 6 concentration–time profiles from the 4 patients of the population group for whom additional pharmacokinetic evaluations were performed (8 blood samples) at different intervals (21 to 204 days) during the long-term treatment).

†$V_1/F = \theta_4 \times$ weight = 4.75 L; ‡$V_1/F = \theta_4 \times$ weight = 5.00 L.

CV, coefficient of variation; α, apparent rate constant of distribution; β, apparent rate constant of elimination; V_1/F, volume of distribution of the central compartment; k_{21}, transfer rate constant; k_a, absorption rate.

The other relevant pharmacokinetic parameters can be calculated as follows: rate constant of elimination (k_{10}) = ($\alpha \times \beta$)/k_{21}; total clearance (CL) = $k_{10} \times V_1/F$; transfer rate constant (k_{12}) = $\alpha + \beta - k_{21} - k_{10}$.

oral clearance averaged 16.0 L/h (CV = 50.8%). AUCs ranged from 11.1 μg·h/mL after a 100-mg dose to 90.9 μg·h/mL after a 500-mg dose.

A plot of model-predicted versus observed concentrations for the final model, based on population parameter estimates, is shown in Figure 4. Various statistical tests were carried out that showed (1) that there was no significant difference when the regression line of individual predicted concentrations versus observed concentrations (slope = 1.02, SE = 0.0204; intercept = −0.10 μg/mL, SE = 0.086) was compared with the reference line (slope = 1 and intercept = 0) (Fig. 5A); (2) the bias (−0.035 μg/mL with 95% confidence interval of −0.18, 0.09) was not statistically different from zero (as shown by a *t* test), and (3) the frequency of distribution histogram of the normalized residuals was as expected (normal with zero mean and unitary variance) (Fig. 5B,C). Most of the weighted residuals lay within 2 units of perfect agreement and were symmetrically distributed around the zero ordinate.

Typical posterior individual fits are presented in Figure 6.

Evaluation of the Bayesian Pharmacokinetic Parameter Prediction

Individual pharmacokinetic parameters for the patients in the validation group (139 MPA concentrations, 15 concentration–time profiles) were determined using the population characteristics and all the available concentration–time points. Mean pharmacokinetic parameters were: α = 7.50 $hours^{-1}$; β = 0.0077 $hours^{-1}$ (CV = 14.5%); k_{21} = 0.018 $hours^{-1}$ (CV = 37.1%); k_a = 0.75 $hours^{-1}$ (CV = 36.0%); V_1/F = 4.45 L (CV = 52.7%); lag time = 0.51 hours (CV = 69.3%); and CL/F = 16.2 L/h

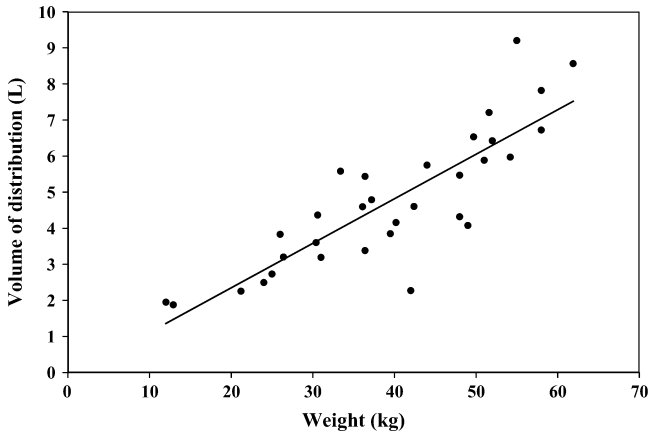

FIGURE 3. Scatter plot of individual V_1/F (Bayesian estimates) versus body weight.

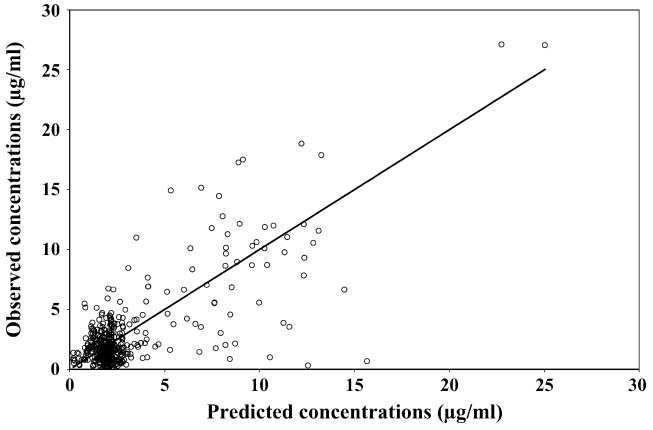

FIGURE 4. Model-predicted versus observed concentrations obtained with the final model based on population parameter estimates (n = 32 patients; 449 MPA concentrations). The solid line represents the line of identity.

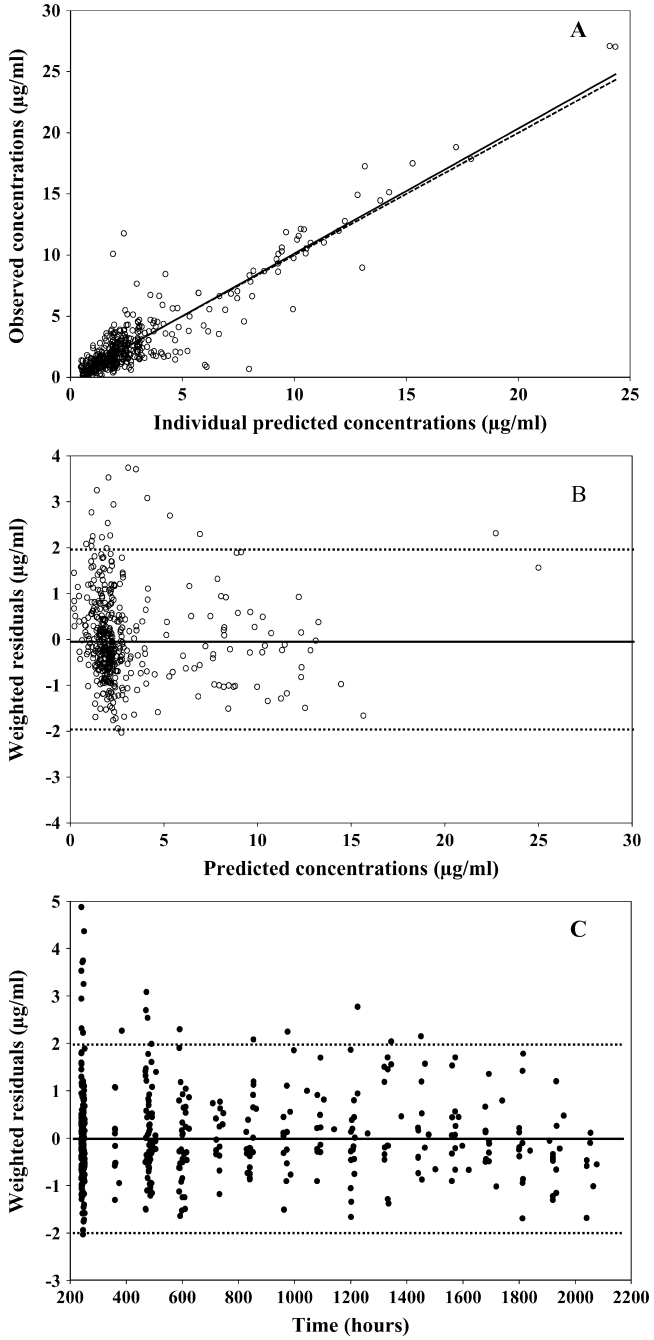

FIGURE 5. Model performance and diagnostic plots (n = 32 patients; 449 MPA concentrations). (A) Model-predicted versus observed concentrations obtained for the final model based on estimated individual parameters. The solid line represents the line of identity, and the dotted line the linear regression line. No significant difference was found between the regression line (slope = 1.02, SE = 0.0204; intercept = −0.10 µg/mL, SE = 0.086) and the line of identity (*t* test). (B) Weighted residuals versus predicted concentrations. (C) Weighted residuals versus time.

(CV = 66.2%). AUCs ranged from 13.5 µg·h/mL (500 mg dose) to 63.5 µg·h/mL (750 mg dose). The mean AUC ratio [AUC (Bayesian estimate)/AUC (trapezoidal rule)] was 1.06 ± 0.12 (Table 4). The regression line of empirical Bayes-predicted values and individual observed MPA concentrations did not differ significantly from the reference line of slope = 1 and intercept = 0. The bias (−0.12 µg/mL) was not statistically different from zero (*t* test), and the 95% confidence interval included the zero value (−0.21/0.11).

Validation of a Limited-Sampling Strategy

Validation consisted of comparing AUCs determined by Bayesian estimation and all data concentrations (considered as the reference) to AUCs determined by Bayesian estimation and limited-sampling strategies. Bias and precision values corresponding to the best of each schedule (ie, 1-, 2-, and 3-sample schedules) are given in Table 4. Precision was good for all strategies, none of which were biased, though the 2-sample schedule (1 and 4 hours after drug administration) and the 3-sample schedule (1, 4, and 6 hours after drug administration) give the best performances. However, to limit the number of samples for both ethical and practical reasons, the 2-sample schedule, which combines accurate prediction and convenience, was selected. The performances of this limited strategy were compared with those obtained by using the MLR equation published by Fillet and Mai,[24] who proposed sampling times at 1, 2, and 6 hours following drug administration. Results are presented in Table 4. Figure 7 shows the correlation between AUCs determined using the population characteristics based on all sampling points and AUCs calculated (1) by Bayesian estimation based on times 1 and 4 hours after drug administration and (2) by using the MLR equation.[24]

Final Population Pharmacokinetic Parameters

In the final step, MPA pharmacokinetic parameters were determined for all patients (n = 41). The calculated population parameters (Table 3) were similar to those calculated from the patients in the population group. The mean CL/F value was 17.2 L/h (CV = 51.0%).

DISCUSSION

Therapeutic drug monitoring of MMF is not generally employed in the treatment of adult patients. However, preliminary results obtained from adult and pediatric patients suggest that there is a pharmacokinetic/pharmacodynamic relationship between total MPA AUC during treatment (ie, between 2 drug intakes, $AUC_{0\text{-}12h}$) and the risk of acute rejection and the occurrence of side effects (especially leukopenia and/or infections).[13,14,17–26,49] Moreover, Weber at al.[15] clearly showed that the free rather than the total AUC is related to adverse events. Thus, the determination $AUC_{0\text{-}12h}$ may be useful in monitoring patients treated with immunosuppressive therapeutic regimens containing MMF. This is particularly true in the pediatric transplant population, where, despite standardized dosages, there is wide inter- and intraindividual variability in pharmacokinetic parameters, especially in the early post-transplantation period.[13–15,49] This variability may contribute to the differences in the patients' clinical outcome. In clinical

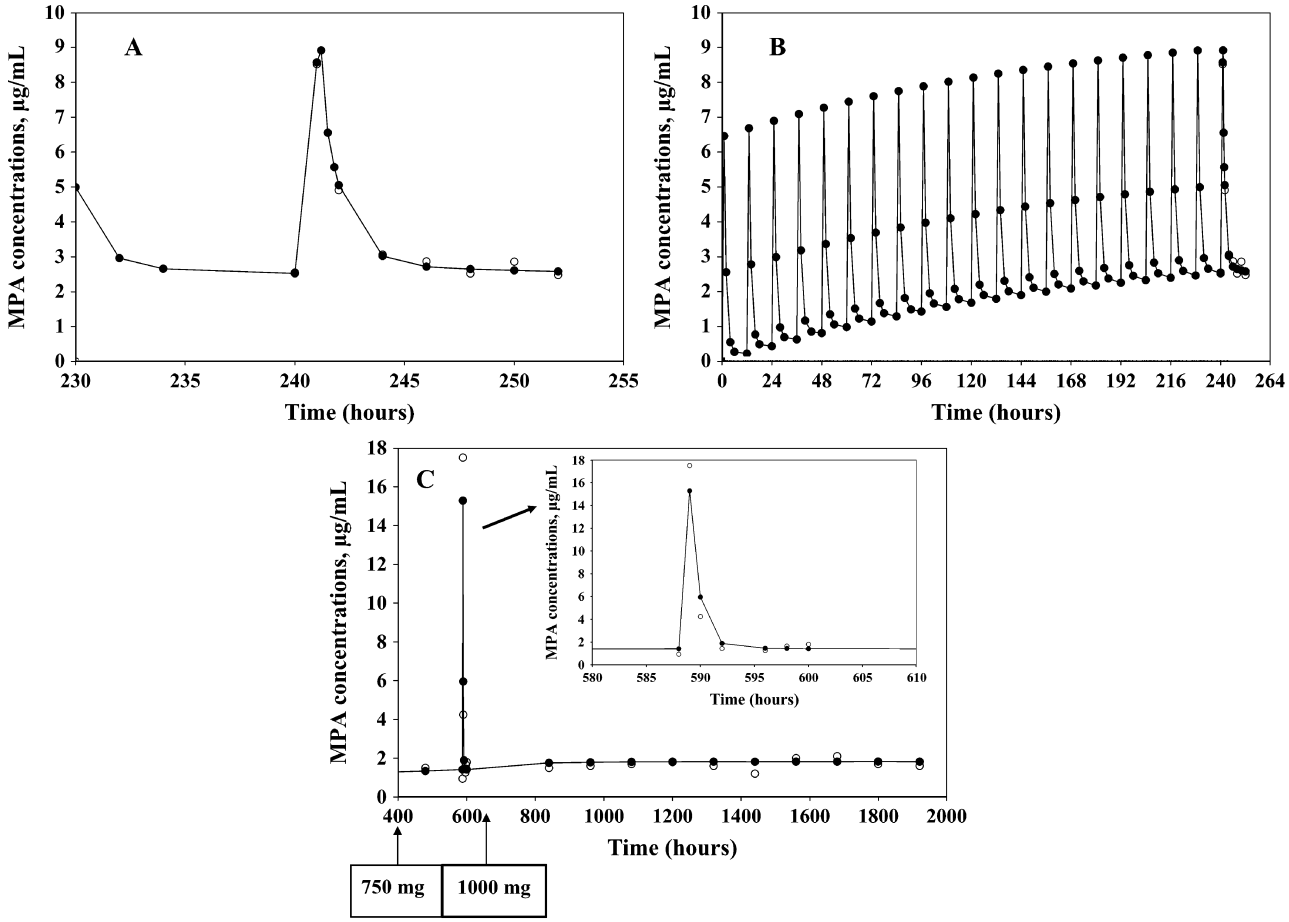

FIGURE 6. Typical posterior individual fits. (A,B) Age, 10 years; weight, 31 kg; administered dose, 500 mg. (C) Age, 15 years; weight, 54 kg. Patients received MMF on a twice-daily basis. Individual predicted concentrations (●); observed concentrations (○). Lines are obtained from individual predicted concentrations connected point by point.

practice, the use of a limited sampling strategy to determine MPA AUC is useful, especially in pediatric patients. Three-point models (predose, 1.25, and 6 hours or 1, 2, and 6 hours) that allow adequate determination of AUCs have been described.[24,29,30] However, a blood sample taken 6 hours after dosing may be considered unacceptable for ambulatory patients in some centers.

The present study was undertaken in light of limited information regarding the pharmacokinetics of MPA in pediatric patients. Different pharmacokinetic models were tested; the structural model was chosen on the basis of the changes in −2 log likelihood and qualitative assessment of diagnostic plots. Contrary to the findings reported by Le Guellec for stable adult renal transplanted patients, a 2-compartment model with a first-order input rate and a lag time was found to fit the data satisfactorily. This model was in agreement with that published by Shum et al.[33] The discrepancies between the measured and model-predicted concentrations presented in Figures 4 and 5 can probably be attributed, at least in part, to a rebound effect, ie, an increase in the plasma concentration of MPA caused by enterohepatic recycling. Although enterohepatic recycling of MPAG to MPA (6–12 hours after oral intake) has been reported, frequent sampling between 6 and 12 hours after dose administration would be necessary to incorporate enterohepatic recycling into the model. Second, the inclusion of enterohepatic recycling would require a vastly increased number of model parameters, and third, enterohepatic circulation occurs at unequal time intervals. Moreover, the secondary concentration peaks seen in most of our patients were relatively small, but such peaks may have represented a significant proportion of the AUC in other groups of individuals. However, current literature suggests that the coadministration of cyclosporine and MMF may inhibit the enterohepatic recirculation of MPA.[50–52] This study identified body weight as a source of variability for V_1/F but was unable to characterize the effects of comedications.

The present study confirms the large interindividual variability in MPA pharmacokinetic parameters. The mean elimination half-life of MPA after oral administration found in this study was very high at 96 hours. This value includes the effect of enterohepatic recycling and therefore does not reflect metabolic elimination alone; however, this parameter is important for the determination of drug accumulation during repeated dosing. The mean CL/F value of 17.2 L/h (CV = 51.0%) calculated from the whole group of patients was similar to that reported by Jacqz-Aigrain et al.[20,53]

TABLE 4. Predictive Performance of AUC Estimation, Using Different Limited Sampling Strategies and Models

	Sampling Time, Hours		AUC, μg·h/mL		
			Mean	Bias	Precision
Noncompartmental approach	1, 2, 4, 6, 8, 10, and 12	Mean Min–max CV, %	33.9 12.5–57.5 38.1	—	—
Bayesian estimation	1, 2, 4, 6, 8, 10, and 12	Mean Min–max CV, %	36.0 13.5–63.4 41.4	—	—
MLR equation proposed by Filler and Mai	1, 2, and 6	Mean Min–max CV, %	38.7 22.7–72.5 34.3	−1.98 (−6.74/−2.79)	9.21
Bayesian estimation	1, 2, and 4	Mean Min–max CV, %	37.4 15.1–64.1 38.9	−1.48 (−5.16/−2.21)	7.10
Bayesian estimation	1, 2, and 6	Mean Min–max CV, %	36.4 15.1–66.0 40.6	− 0.51 (−5.53/4.51)	9.48
Bayesian estimation	1, 4, and 6	Mean Min–max CV, %	37.6 14.8–67.3 33.0	−0.045 (−2.70/2.79)	5.18
Bayesian estimation	1 and 2	Mean Min–max CV, %	35.0 16.5–61.3 41.0	0 0.021 (−6.90/6.94)	12.4
Bayesian estimation	1 and 4	Mean Min–max CV, %	36.4 15.8–63.4 35.0	−0.90 (−3.72/2.91)	6.02
Bayesian estimation	4	Mean Min–max CV, %	34.6 9.5–54.1 37.1	−1.28 (−5.42/6.98)	11.9

The values in parentheses represent the 95% confidence intervals.
MLR, multiple-linear regression.

The Bayesian approach developed in this study accurately predicts concentrations of MPA in plasma. This was demonstrated in patients of the validation group. For these patients, AUCs computed by Bayesian approach and all concentration time points were compared with those computed by log-linear trapezoidal rule. The mean ratio (AUC [Bayesian estimate]/AUC [trapezoidal rule]) was 1.06. Because few time points were drawn in the first 2 hours postdose, the plasma peak was not correctly estimated in some patients, and the AUC computed by the noncompartmental approach could be imprecise. This was illustrated in Figure 6A. In this paper, different limited sampling strategies with 1, 2, or 3 blood samplings for determining MPA AUC in clinical routine with minimal constraints for patients were tested. The schedules with sampling times of 1 and 4 hours and 1, 4, and 6 hours after oral administration gave the best results when compared with the reference values. However, for both ethical and practical reasons, and as suggested by the international guidelines for drug evaluation in children,[54] it is essential to select a strategy that allows the reduction of the number of samples and the time spent in the hospital for sampling. Thus, the 2-sample schedule based on times 1 and 4 hours after drug intake, (1) allowing a better quality of life for the child and (2) decreasing cost, was selected. Three mathematical equations based on stepwise linear regression analysis have previously been proposed to estimate MPA AUC; these required 3 samples.[24,29,30] However, the methodology based on Bayesian estimation using NONMEM is more flexible because the MLR model requires accurate control of the time at which the samples are obtained. MPA AUCs determined using the MLR equation published by Filler and Mai[24] and modified by Filler et al[29] were compared

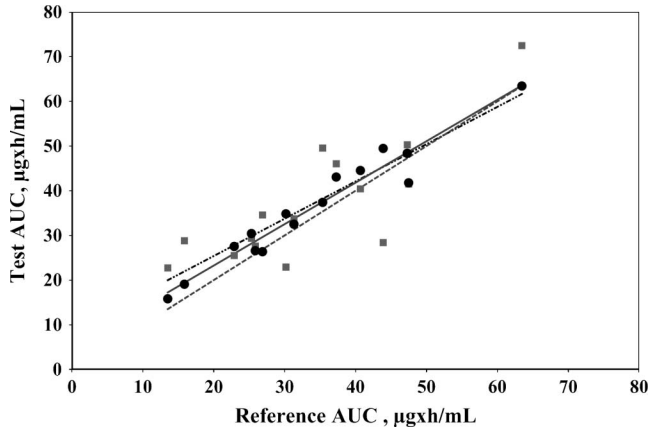

FIGURE 7. Relationships between AUCs determined using the population characteristics based on all sampling points (reference) and AUCs calculated (i) by Bayesian estimation based on times 1 and 4 hours after drug administration (●) and (ii) by using the MLR equation[24] (■); (- - - -) line of identity; (——) linear regression line ($AUC_{reference}$ versus $AUC_{1\text{-}4\ hours}$); (·····) linear regression line ($AUC_{reference}$ versus AUC_{MLR}).

with those obtained using the present Bayesian approach and 2-sample schedule (ie, 1 and 4 hours). A better estimation of low MPA AUC values was obtained using the Bayesian methodology (Fig. 7).

The pharmaceutical industry and drug regulatory bodies, such as the Food and Drug Administration and the Therapeutic Goods Administration, are increasingly recognizing the importance of population pharmacokinetic studies for the development and evaluation of new drug therapies and for optimal dosing of existing drugs in specific groups of patients.[55] The NONMEM software program is regarded as the criterion standard program in this area.

The patient group included in this study is representative of "real-life" infant kidney transplant recipients. The dose of MMF given to the patients, and also the course of each individual's therapy, varied considerably. The data collected characterized those that are normally available for transplant subjects. Thus, findings from this study should be applicable to the larger kidney transplant community.

In conclusion, this is the first time that the population pharmacokinetics of MPA has been described in pediatric and adolescent renal transplant patients. The limited sampling strategy proposed in this paper to estimate individual MPA AUC may be useful in optimizing pediatric patient care.

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