Population pharmacokinetics of mycophenolic acid in pediatric renal transplant patients using parametric and nonparametric approaches

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ABSTRACT

Mycophenolic acid (MPA) is an immunosuppressive drug widely used in the prevention of acute rejection in pediatric renal transplant recipients and is characterized by a wide inter-individual variability in its pharmacokinetics. The aim of this study was to compare population pharmacokinetic modeling of MPA in pediatric renal transplant recipients given mycophenolate mofetil, the ester prodrug of MPA, using parametric and nonparametric population methods. The data from 34 pediatric renal transplants (73 full pharmacokinetic profiles obtained on day 21, months 3, 6 and 9 post-transplant) were analyzed using both the nonlinear mixed-effect modeling (NONMEM) and nonparametric adaptive grid (NPAG) approaches, based on a two-compartment model with first order lagged time absorption and first order elimination. The predictive performance of the two models was evaluated in a separate group of 32 patients. Higher mean population parameter values and ranges of individual pharmacokinetic parameters were obtained with NPAG, especially for the elimination constant ke: mean $1.16\,h^{-1}$ (0.26–$4.33\,h^{-1}$) and $0.78\,h^{-1}$ (0.66–$1.15\,h^{-1}$) with NPAG and NONMEM, respectively. With NPAG, the skewness and kurtosis values for ke (2.03 and 7.80, respectively) were far from the theoretical values expected for normal distributions. Such a non-normal distribution could explain the high value of shrinkage (35%) obtained for this parameter with the parametric NONMEM method. Bayesian forecasting of mycophenolic acid exposure using the NPAG population pharmacokinetic parameters as priors yielded a better predictive performance, with a significantly smaller bias than with the NONMEM model (-1.68% vs -9.53%, $p < 0.0001$). In conclusion, in the present study, NPAG was found to be the most adequate population pharmacokinetic method to describe the pharmacokinetics of MPA in pediatric renal transplant recipients.

1. Introduction

Mycophenolate mofetil (MMF), the ester prodrug of mycophenolic acid (MPA) has been approved for immunosuppressive therapy in combination with calcineurin inhibitors following renal transplantation, both in adults and children. MMF, which is dosed based on body surface area in the pediatric population, was shown to be effective in the prevention of acute rejection in pediatric renal transplant recipients, and is now widely used for maintenance immunosuppressive therapy in this population [1–4].

As in adults, the pharmacokinetics (PK) of MPA in pediatric renal transplant recipients is characterized by a large inter- and intra-individual variability, particularly in the early post-transplant period [5,6]. This variability is clinically relevant as shown by the association between the risk of acute rejection episodes and MPA AUC_{0-12h} values [5,7]. The pharmacokinetics of MPA is also characterized by a large inter-individual variability and a gradual increase in dose-normalized exposure over time in the first three months post-transplantation [6,8]. In a population analysis based on a two-compartment model with time-lagged first order absorption, van Hest et al. [9] found that this time dependency could be due to a combination of improving creatinine clearance,

progressively increasing albumin, hemoglobin and decreasing cyclosporine predose concentrations (C0) during the first 6 months after transplantation.

A decreased incidence of acute rejection has been found as a result of therapeutic drug monitoring (TDM) of MPA in adult [10] renal allograft recipients, and suggested in pediatric [11] recipients. However, establishing such a strategy requires characterization and modeling of MPA pharmacokinetics in the target population. Sophisticated pharmacokinetic models able to describe the complex plasma concentration profiles observed in adult allograft recipients in the early post-transplantation period [12], or the enterohepatic recirculation often observed when MMF is combined with tacrolimus [13–16] or sirolimus [17], have been reported.

Some authors have proposed algorithms based on a limited number of sampling time points, able to predict MPA interdose area under the curve (AUC_{0-12h}) in pediatric kidney graft recipients on MMF and cyclosporine therapy [18–20]. Bayesian forecasting methods based on population PK models represent an alternative approach to predict the individual MPA PK parameters, hence concentration profiles. Using Bayesian estimators developed in populations of adult renal transplants at early and late post-transplantation periods, and based on a limited strategy of three time points (20 min, 1 h and 3 h post-dose) [21], the APOMYGRE trial showed the feasibility, efficacy, safety and cost-effectiveness of MMF dose individualization in adult renal transplant patients using such Bayesian estimators [10,22,23]. However, these Bayesian estimators were developed using the iterative two-stage Bayesian method and an in-house computer program. A more common approach is one-stage population pharmacokinetic modeling. Payen et al. [24] reported a population pharmacokinetic analysis of MPA in pediatric and also adolescent renal transplant patients performed using NONMEM (NONlinear Mixed-Effect Modeling software), and the development of a Bayesian estimator based on this population model to predict MPA exposure. Most of the pharmacokinetic profiles were collected in stable patients as more than half of the patients were grafted for more than six months.

To the best of our knowledge, the published one-stage population pharmacokinetic analyses of MPA were based on a parametric approach, mostly using NONMEM [13,15,24–29]. Usually, following such a parametric approach the influence of covariates on the PK parameters is analyzed and the remaining, unexplained inter-individual variability of the PK parameters is assumed to follow a predefined distribution e.g., a normal or log-normal distribution. However, when such a parametric approach is inappropriate to describe the unexplained inter-individual variability, implementation of Box–Cox or Logit transformations in NONMEM have been recently proposed [30]. These methods have been called "semi-parametric" as the shape of the transformed distribution is no more Gaussian. Non-parametric methods can describe the actual distribution of random effects whatever its shape [31–33]. These methods are able to directly discover, in the population itself, unsuspected subpopulations of individuals (i.e., without external help such as descriptors, covariates or individual Bayesian posterior parameter estimates), which is not possible with parametric techniques. Non-parametric methods also provide the traditional estimates of the parameter: mean, standard deviation, mode, median, correlation and covariance.

The aim of this study was to characterize MPA PK in pediatric renal transplant patients using several population approaches, namely nonlinear mixed-effect modeling (NONMEM) with or without semi-parametric transformations of the inter-individual variability, and the nonparametric adaptive grid (NPAG) method.

2. Materials and methods

2.1. Patients

The population analysis was performed using pharmacokinetic data issued from two different cohorts of pediatric renal transplant recipients on a comparable immunosuppressive regimen consisting of mycophenolate mofetil (600 mg/m^2 body surface area twice a day, up to a maximum of 2 g/day), cyclosporine and corticosteroids. The two patient cohorts have been described in detail in a previous paper [18]. 44 patients (28 boys) of the Cohort of the German Study group on MMF therapy, with a median age of 10.7 years (range, 3.2–15.9) and 22 patients (14 boys) of the Tricontinental MMF suspension trial, with a median age of 7 years (range, 1–16) were analyzed. The parents of the patients gave informed consent for the participation of their children and the clinical trials were designed in accordance with legal requirements. One to three profiles were collected in each patient, so that the 66 pediatric patients provided 129 full pharmacokinetic profiles (of 9 blood samples collected at: predose, 20 min, 40 min, 75 min, 2, 4, 6, 8 and 12 h after dosing; or of 8 blood samples collected at: predose, 30 min 1, 1.5, 2, 4, 8 and 12 h after dosing) on day 21 and months 3, 6, and 9 post-transplant.

The whole dataset was randomly divided into 2 groups: a reference group made of 34 patients (73 pharmacokinetic profiles) was used to develop the population pharmacokinetic models and a test group of the remaining 32 patients (56 pharmacokinetic profiles) was used to evaluate the predictive performance of the population models.

2.2. MPA analysis

MPA plasma concentrations were measured using a reverse-phase high-performance liquid chromatography method with ultraviolet detection previously described in details [34]. The error pattern of the assay was determined by measuring several assay samples (which cover the full working range of the assay) in at least quadruplicate leading to find standard deviation (SD) for each of the samples. Then, the overall relationship between the plasma concentration and the SD with which it has been measured was expressed by the following polynomial equation:

$$SD = 0.963 + 0.0227C + 0.008C^2,$$

where SD is the assay standard deviation and C is the measured plasma concentration.

2.3. Pharmacokinetic population modeling

The plasma concentration *vs* time data of the index group were analyzed using two population pharmacokinetic approaches in parallel: NONMEM and nonparametric adaptive grid (NPAG) methods.

2.3.1. NONMEM analysis

Data were analyzed using NONMEM version VI [35] and Wings for NONMEM® version 614 (N. Holford, Auckland, New Zealand). All population pharmacokinetic analyses were performed using the first order conditional estimation (FOCE) method with interaction.

A two-compartment model with first-order, lagged time absorption and first-order elimination best described the data.

Unexplained inter-individual variability (IIV) was initially modeled following an exponential error model. The semi-parametric approach proposed by Petersson et al. [30] was also tested: two transformations with estimated shape parameter (Box–Cox and logit) were explored to evaluate if they could improve data fitting.

Inter-occasion variability (IOV) was modeled using an exponential error model. Diagonal matrix and full variance/covariance matrix (using the Block( ) command) was successively tested to estimate inter-patients random variabilities (ETAs). The variance/covariance matrix was also tested for certain parameters only, if appropriate. Additive, proportional and combined (i.e., additive and proportional) error models were tested to describe the residual variability.

The influence of gender, age and weight (the only covariates available) on the absorption and elimination parameters and on the apparent central volume of distribution was investigated. Covariates selected to enter the intermediate model simultaneously had to lead individually to a significant ($p < 0.01$) decrease in the objective function from the covariate-free model. Finally, in a backward-deletion strategy, each covariate was independently removed from the full model to confirm its relevance. If the increase in the objective function was not statistically significant ($p < 0.001$), the relationship between this covariate and the respective pharmacokinetic parameter was not taken into account in the final model. The final model thus only included the significant covariates. At this stage, the clinical relevance of the covariate(s) was also appraised taking into account the reduction in IIV.

Potential over-parameterization was tested taking into account the condition number (calculated as the square root of the ratio of the smallest to the largest eigenvalue) and the extreme eigenvalues. The stability and performance of the final population model were evaluated using the bootstrap approach. Thousand bootstrap sets were drawn by resampling from the original dataset, each providing population PK parameter estimates. The mean, median and 95% confidence interval values estimated from the 1000 bootstrap sets for each PK parameter were compared to those obtained with the original dataset.

The adequacy of the final population model was evaluated by a Visual Predictive Check (VPC) performed using the 'R for NONMEM' package [36]. A total of 1000 datasets were simulated from the final model using the original data set. Plots of the median and 90% confidence interval of the simulated concentration *vs* time profiles were generated to check that the distribution of the observed concentration–time profiles was reasonably contained within this confidence interval. Because the MMF dose (in mg) was different in each patient, the predictions and the observations were dose normalized on the basis of the mean dose administered in the reference group (thereby the simulations were done using any dose). Moreover, the extent of shrinkage on each parameter (η-shrinkage) and on individual predictions (ε-shrinkage) was computed following Karlsson and Savic recommendations [37]. η-Shrinkage was calculated using the following formula: $1 - \text{SD}(\eta_{\text{parameter}})/\omega$, where $\text{SD}(\eta_{\text{parameter}})$ is the standard deviation of individual estimates of η for one parameter and ω the population estimated standard deviation of the corresponding random effect. Large values of shrinkage (higher than 20–30%) would generally be associated with poor individual estimates of that parameter. ε-Shrinkage was calculated as $1 - \text{SD (IWRES)}$, where IWRES are the individual weighted residuals, and will thus increase from zero toward one as data become less informative.

2.3.2. NPAG analysis

The nonparametric population analysis was performed using BigWinPops version 0.03 (R. Jeliffe, Laboratory of Applied Pharmacokinetics, USC Keck School of Medicine, Los Angeles, USA). One- and two-compartment models with first-order elimination and first-order absorption with or without a lag-time were tested. The best model was selected on the basis of the log-likelihood of the results.

The iterative two-stage Bayesian (IT2B) method was first used to obtain initial estimates of the PK parameter ranges. Then, nonpara-

metric population analysis was performed using the NPAG program [29]. Combined with an interior point rather than an expectation-maximization algorithm, NPAG constitutes an improved version of the nonparametric expectation maximization program, which computes the discrete joint probability density functions of the population pharmacokinetic parameters. NPAG also computes a so-called γ parameter, representing an overall measure of all the other sources of intra-individual variability besides the assay error, such as the errors in preparation and administration of the doses, errors in times of administration, misspecification of the pharmacokinetic model (residual error). Gamma is used as a multiplier of the assay error polynomial (total error SD = gamma × assay standard deviation). A value of gamma of 1.0 suggests that there is no other source of variability than the assay. A value of gamma > 1.0 suggests that the environmental noise needs to be taken into account in the overall error by multiplying the value of gamma by the analytical error pattern.

2.3.3. Evaluation of the performance of the population models

The performance of NONMEM or NPAG population models was evaluated both in the index and in the test patient groups using Bayesian forecasting.

Individual Bayesian estimates of individual pharmacokinetic parameters and AUCs were calculated for each patient included in the index group, using as priors the mean values and variability of the population pharmacokinetic parameters previously obtained with the best models retained with NONMEM and NPAG, respectively. Goodness of fit of the population models was evaluated by comparing Bayesian estimated and observed MPA plasma concentrations.

Individual Bayesian estimates of MPA exposure and concentration values were also calculated for the 36 patients included in the test group (56 concentration–time profiles) using the population characteristics of the index group and all the concentration–time points available.

With NONMEM, the individual Bayesian estimates were obtained using the POSTHOC Bayesian option. With NPAG, Bayesian estimation of individual parameter values in independent patients was not available. Consequently, the final population model (i.e., mean parameter vector and full covariance matrix) was inserted into the PRIOR subroutine of the ADAPT II software package [38], to allow for maximum a posteriori Bayesian estimation. Two hypotheses related to the prior distribution model (normal or lognormal) were tested.

2.4. Statistical analyses

All statistical analyses were performed using the R software [39].

Comparisons of patients' characteristics and MPA exposure indices between the index and test groups were performed using the Student *t*-test.

Estimated and observed MPA concentrations and AUCs were compared using the mean relative error (MRE%) as a measure of bias, and root mean squared prediction error (RMSE) as a measure of precision. Statistical significance of the MRE was evaluated using the paired *t*-test.

3. Results

3.1. Data description

The characteristics of the patients included in the reference and test groups are summarized in Table 1. No significant differences were observed between the two groups. Fig. 1 shows the individual concentration–time curves observed during the four post-transplantation periods studied. For some profiles (less

Table 1
Demographic characteristics of the patients and mycophenolic acid (MPA) dose-normalized AUC_{0-12} (mg·h/mL)/mg at the different sampling periods.

	Reference group (n = 34)	Test group (n = 32)
Age (years)[a]	11.2 ± 3.2	8.9 ± 4.9 (NS)
Body weight (kg)[a]	32.2 ± 11.5	30.5 ± 16.1 (NS)
Sex ratio M/F[a]	23/11	19/13
Mycophenolate mofetil dose (mg)[a]	625 ± 178	569 ± 213 (NS)
MPA dose-normalized AUC_{0-12} (mg·h/mL)/mg[b]		
All periods	106 (18–415)	91 (32–251)
Week 3	53 (18–176)	54 (37–122)
Month 3	104 (45–415)	91 (42–228)
Month 6	97 (35–183)	124 (78–251)
Month 9	–[c]	106 (32–217)

NS: no significant difference with respect to the reference group.

[a] Data are given as mean ± SD.
[b] Data are given as median (range).
[c] Reference group did not include data on month 9 post-transplantation.

than 10 out of 129) a second concentration peak was observed at approximately 6 h postdose, but most profiles exhibited a single concentration peak. The dose-normalized MPA AUC_{0-12} [(AUC/dose)], calculated using the linear trapezoidal rule, was not significantly different between the two groups ($p = 0.126$). A wide distribution of these dose-normalized MPA AUC_{0-12} was observed in each group at each period (Table 1), with some extremely high values, i.e., a very low apparent clearance. The Shapiro-Wilk normality test showed a significant deviation from the normal distribution of AUC/dose in both the reference ($p < 0.001$) and the test ($p = 0.0018$) groups, reflecting a non Gaussian distribution of the apparent clearance.

3.2. Population modeling

3.2.1. NONMEM analysis

A two-compartment model with first-order, lagged time absorption and first-order elimination adequately described the concentration data. The model parameters were the absorption rate constant (ka), the apparent central volume of distribution (Vc/F), the rate constant from central to peripheral compartments (k12), the rate constant from peripheral to central compartments (k21), the elimination rate constant (ke) and the absorption lag-time. Introduction of IIV on ka, Vc/F, k12, ke and lag-time significantly improved the fit of the model. As the Box–Cox and logit transformations failed to improve model fitting and shrinkage, the IIV was modeled using an exponential error model. Inclusion of IOV on ka and Vc/F led to a significant decrease of the objective function value, but to negligible IIV values (1.04E − 5% and 0.17%, respectively). Consequently, the final model included IOV on ka and Vc/F, IIV on k12, ke and lag-time, and a combined, additive and proportional, residual error. Finally, a simple diagonal matrix was selected. The population pharmacokinetic parameters (popPK) obtained are summarized in Table 2.

Among the covariates investigated, only the post-transplantation period influenced ke with a statistically significant decrease of the objective function of 12 units ($p < 0.01$). However, introduction of this covariate led to a high value of shrinkage estimates for ke (40%) and did not seem to significantly reduce ke unexplained inter-individual variability that still reached 14.3% (CI95%: 0–27.4%) vs 19.9% (CI95%: 0–31.5%) with the covariate-free model. Therefore, the covariate-free model was finally retained. Diagnostic plots are shown in Fig. 2. The scatter plot of predicted (PRED) and individual model-predicted concentrations (IPRED) vs observed concentrations (DV) showed no major bias (Fig. 2), while 91% of weighted residuals (WRES) were in the (−2; +2) range.

Estimates of the shrinkage for ETAs of ka, k12, ke, Vc/F and lag-time were 0.096, 0.093, 0.352, 0.067 and 0.199, respectively. The condition number was 14.7. The residual error was fairly high (38.7% and 0.08 mg/L for the proportional and additive parts, respectively), but not shrunk (0.124). The mean parameter estimates obtained using the bootstrap procedure (1000 runs) were similar to those previously obtained with the original dataset (Table 2).
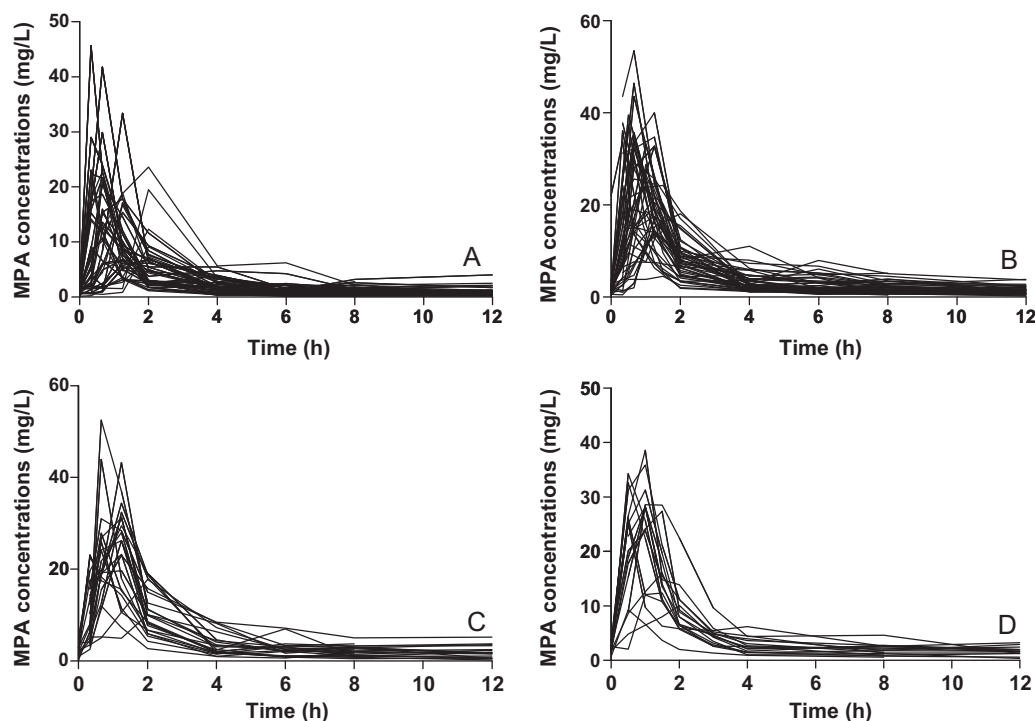

Fig. 1. Mycophenolic acid (MPA) concentration–time profiles of patients in the reference and test groups obtained at (A) week 3 (n = 42), (B) month 3 (n = 47), (C) month 6 (n = 23) and (D) month 9 (n = 17) post-transplant.

Table 2
Mycophenolic acid (MPA) population pharmacokinetic parameters, inter-individual and inter-occasion variabilities, range of individual estimates obtained with the POSTHOC option, and results of the bootstrap internal validation procedure. Bias and precision of Bayesian estimates of MPA exposure in the test group (32 patients, $n = 56$ concentration–time profiles) based on NONMEM population model.

Parameter	Final model original dataset	Range of individual estimates	Bootstrap[a]			Bayesian forecasting	
	Mean (SE)		Mean ± SE	Median	5th–95th percentiles		
Fixed effect parameters						AUC_{0-12}	
ka (h^{-1})	2.28 (0.51)	0.22–20.41	2.04 ± 0.53	1.88	1.42–3.06	Bias, MRE %	−9.53[b]
Vc/F (L)	17.7 (1.84)	4.09–64.97	17.31 ± 2.90	17.10	12.60–22.12	Precision, RMSE %	14.51
K12 (h^{-1})	0.58 (0.10)	0.17–1.55	0.60 ± 0.12	0.57	0.46–0.80	Range of relative error	−24.13%; +36.83%
K21 (h^{-1})	0.007 (0.001)	–	0.009 ± 0.01	0.006	0–0.018		
ke (h^{-1})	0.78 (0.07)	0.66–1.15	0.85 ± 0.14	0.83	0.65–1.10	Plasma concentrations	
Lag-time (h)	0.26 (0.01)	0.21–0.33	0.24 ± 0.03	0.25	0.18–0.28	Bias, MRE %	13.67[b]
Inter-individual variability						Precision, RMSE%	77
ω(k12) (%)	62 (CI 95 11–87)		62 ± 13	63	40–80		
ω(ke) (%)	20 (CI 95 0–31)		27 ± 19	20	0–69		
ω(lag-time) (%)	15 (CI 95 0–23)		31 ± 23	18	0–70		
Inter-occasion variability							
ω(ka) (%)	109 (CI 95 68–139)		99 ± 17	100	67–125		
ω(Vc/F) (%)	53 (CI 39–63)		53 ± 7	53	42–63		
Residual variability							
σ_1 (%)	38.7		38 ± 3	38	34–42		
σ_2 (mg/L)	0.08		0.11 ± 0.08	0.11	0–0.23		

ka, absorption constant; Vc/F, apparent central volume of distribution; k12 and k21, inter-compartment transfer constants, ke, elimination rate constant, lag-time, absorption lag-time.
[a] Data from 1000 bootstrap analyses.
[b] $p < 0.05$.

Fig. 3 shows the results of the VPC for the final model. Simulations were based on dose-normalized concentrations, using the mean MMF dose of 625 mg observed in the index group. The results are presented separately for profiles observed at early and stable post-transplant periods. The observations were within the 90% confidence interval but not distributed symmetrically relative to the median prediction. This shows the tendency of the model to underestimation.

3.2.2. NPAG analysis

The two-compartment model with first-order, time-lagged absorption and first-order elimination provided the best description of the data. The pharmacokinetic model included the same 6 pharmacokinetic parameters as previously described for the parametric analysis: ka, Vc/F, k12, k21, ke and lag-time.

The initial IT2B analysis led to a value of γ of 0.89 suggesting that there was no other source of variability than the assay SD to
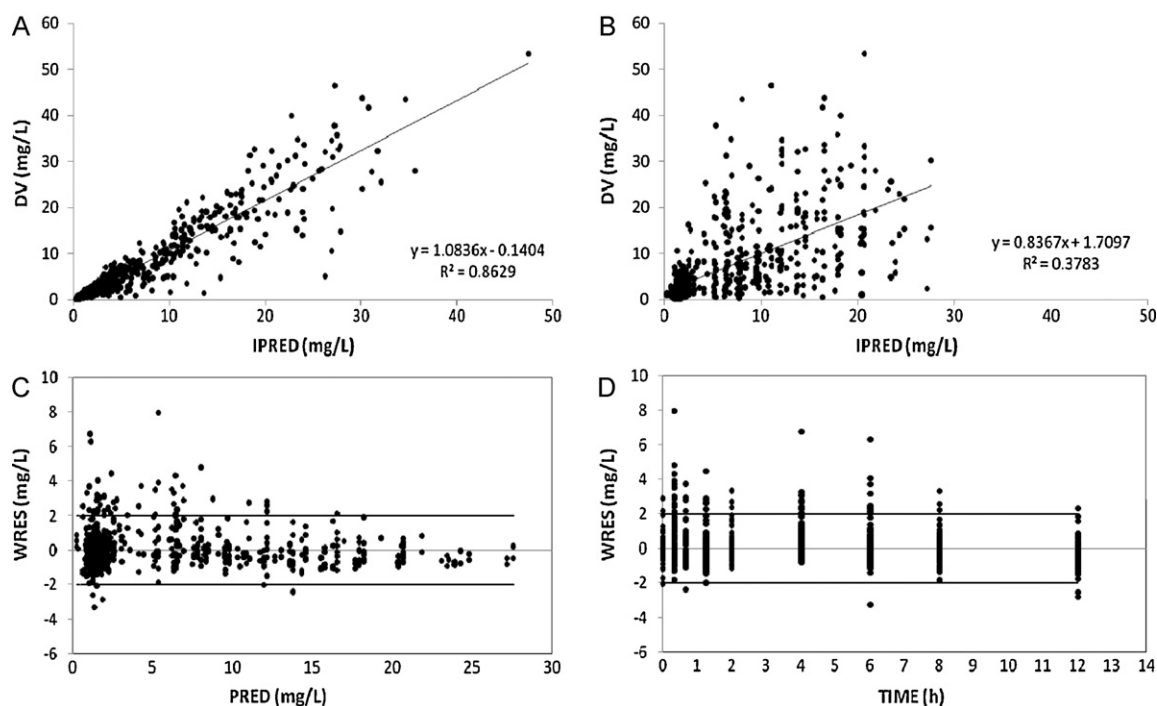

Fig. 2. Scatter plots of (A) observed concentrations (DV) vs population model-predicted concentrations (PRED), (B) observed concentrations (DV) vs individual model-predicted concentrations (IPRED), (C) weighted residuals (WRES) vs model-predicted concentrations (PRED) and (D) weighted residuals (WRES) vs time.
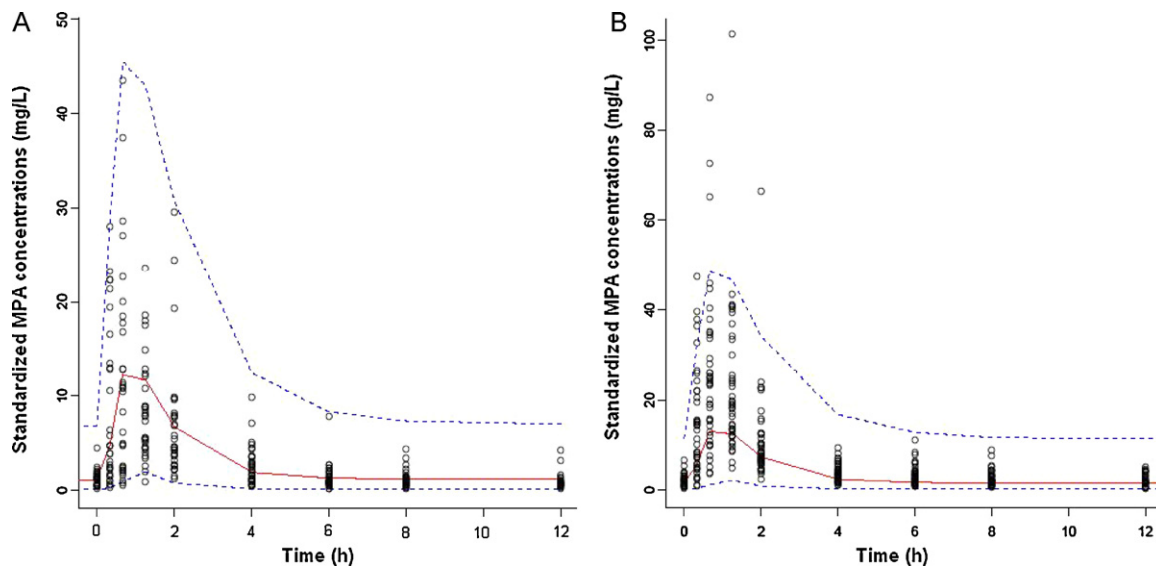
Fig. 3. Visual predictive check results of mycophenolic acid (MPA) for (A) early (W3) and (B) stable (M3 and M6) post-transplant periods. Symbols denote observation. Solid line and dashed lines represent the median and the 90% tolerance interval, respectively. Concentrations are corrected to a mycophenolate mofetil dose of 625 mg.

consider in the model. Consequently, γ was set to 1, meaning that the total error (both assay and environmental) SD was equal to the error assay pattern SD.

MPA pharmacokinetic parameter estimates in the index group are summarized in Table 3. Mean and median values of ka, lag-time and k21 were far apart, showing the heterogeneity and the asymmetry of their distribution. The results of the NPAG predictive performance evaluation are shown in Fig. 4. The scatter plot of observed concentrations *vs* individual plasma concentrations predicted using the median parameter values of each patient's non-parametric Bayesian joint density (Bayesian posterior predicted levels) showed no major bias.

3.2.3. Comparison between NONMEM and NPAG population models

Except for Vc/F, the mean population pharmacokinetic parameter values obtained with NPAG were higher by at least 50% than with NONMEM. The two methods led to a similar high value of ka in an "outlier" patient, characterized by a very fast absorption of the drug. The range of the individual ke and lag-time values was much narrower with NONMEM (0.66–1.15 h^{-1} *vs* 0.26–4.33 h^{-1} and 0.21–0.33 h *vs* 0–1.85 h, respectively). Interestingly, the ke and lag-time skewness (2.03 and 1.70) and kurtosis (7.80 and 5.38) were not in favour of a normal distribution for these parameters.

3.3. Evaluation of the predictive performance of the population models in the test group

The predictive performance of the two final population models developed according to the NONMEM and NPAG methodologies is reported in Tables 2 and 3. The NPAG model resulted in more accurate and precise prediction of MPA AUC and concentrations than the NONMEM model, as shown by the lower and non significant bias and RMSE values.

Using the nonparametric model, the best results were obtained with the lognormal prior distribution model option in ADAPT II. This model provided significantly lower mean AUC bias than the parametric model on M3 (0.79% *vs* −12.34%, $p < 0.001$), M6 (7.50% *vs* −11.35%, $p < 0.05$) and M9 (−0.25% *vs* −12.39%, $p < 0.001$) but not on W3 (3.56% *vs* 2.97%, NS).

4. Discussion

To the best of our knowledge, this study is the first to report population pharmacokinetic analysis of MPA using both parametric and nonparametric approaches (NONMEM and NPAG methods, respectively) in renal transplant children (from 1 to 18 years) and to show the superiority of the nonparametric modeling method.

All the previous MPA population pharmacokinetic models developed both in adults and in children were based on paramet-
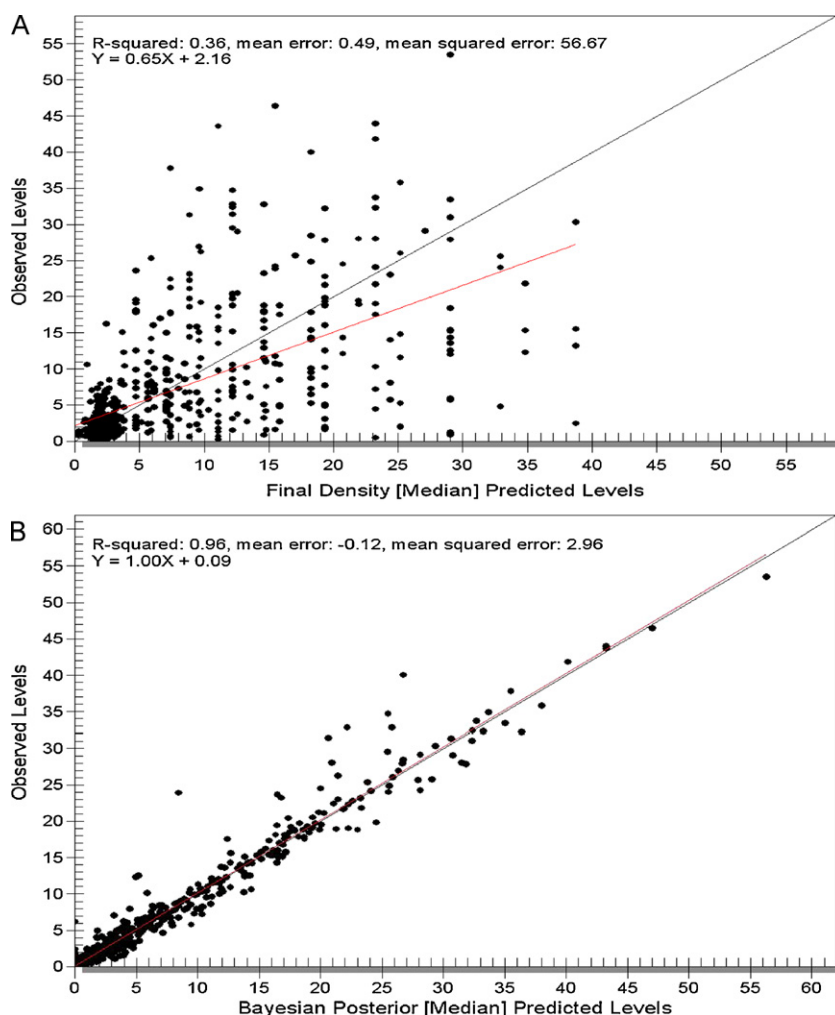
Table 3
Mycophenolic acid (MPA) population pharmacokinetic parameters obtained in NonParametric Adaptive Grid (NPAG). Bias and precision of Bayesian estimates of MPA exposure in the test group (32 patients, $n = 56$ concentration–time profiles) based on NPAG population model.

NPAG population model in reference group					Bayesian forecasting in test group using NPAG model as PRIORS in ADAPTII		
Parameter	Mean	Median	Inter-individual variability (CV%)	[Range]		Normal[a]	Lognormal[a]
ka (h^{-1})	5.21	2.87	92.20	[0.10–20.10]	AUC$_{0-12}$		
Vc/F (L)	16.11	12.13	74.80	[1.80–56.92]	Bias, MRE %	1.40	−1.68
K12 (h^{-1})	1.30	1.05	82.16	[0.002–5.00]	Precision, RMSE %	12.10	6.87
K21 (h^{-1})	0.69	0.03	207.83	[0.005–4.99]	Range of relative error	−11.46%; +65.35%	−11.22%; +26.29%
ke (h^{-1})	1.16	1.05	56.40	[0.26–4.33]	Plasma concentrations		
Lag-time (h)	0.40	0.28	98.54	[0.0001–1.85]	Bias, MRE %	11.53	13.6
					Precision, RMSE%	44.71	50.02

ka, absorption constant; Vc/F, apparent central volume of distribution; k12 and k21, inter-compartment transfer constants; ke, elimination rate constant, lag-time, absorption lag-time.

[a] Prior distribution model used in ADAPT II.

Fig. 4. Scatter plot of (A) observed and predicted plasma mycophenolic acid (MPA) concentrations based on the population median parameter values and (B) observed and predicted plasma MPA concentrations based on the median value of each subject's individual nonparametric Bayesian posterior parameter distributions obtained using NonParametric Adaptive Grid (NPAG).

ric methods. Two population pharmacokinetic studies in pediatric renal transplant recipients were previously reported [24,40]. Unlike the current study, Payen et al. [24], analyzed data collected in children and young adults, mainly in the stable post-transplantation period. Recently, Zhao et al. [40] reported a population pharmacokinetic model developed in children mainly focused on the period from before dosing to 3 h after (91% of the samples collected within this period). However, this sampling design did not allow describing the concentration–time curves beyond 3 h post-dosing, i.e., when the MPA PK profiles are the most complex and probably the most variable. Therefore, different modeling strategies have been proposed in adults: (i) structural sophisticated models allowing to describe profile with two concentration peaks [12]; (ii) enterohepatic circulation [13,17] partly inhibited when cyclosporine is co-administered [41,42]; (iii) time-dependent clearance [9,15]. These approaches were tested herein and failed to improve the data fitting. In adult patients, the MPA pharmacokinetic modeling studies investigating the first weeks post-transplantation failed to estimate with precision the concentration profiles or yielded very high residual variability [15,26,27].

In the present study, the observed MPA concentration–time profiles mostly exhibited a single peak concentration and were best described by a two-compartment model with time-lagged first-order absorption. This is consistent with previous reports where MMF was co-administered with cyclosporine [15,17,24,25,27]. The

analysis performed with NPAG led to higher values of MPA population PK parameters (except for Vc/F) and to a wider inter-individual variability than with NONMEM (especially for the elimination constant). The differences observed between the two approaches can be explained by the different calculation methods used by NONMEM and NPAG. Indeed, NONMEM is a parametric method in that it assumes a normal or log-normal distribution of the PK parameters and tends to put less weight on extreme values. This tendency can be highlighted by the η-shrinkage expressing the shrinkage of the variance of empirical Bayes estimates distribution toward zero.

The high value of the calculated ke η-shrinkage (35%), illustrates the trend to estimate individual ke values close to the population mean (η expressing the deviation of the individual parameter estimates from the typical parameter value in the population) which may be a problem since the estimates of this parameter are used for TDM. Indeed, with such high shrinkage values, posthoc η_s cannot be considered as informative about the true η distribution and the individual Bayesian estimates would be obtained with a large imprecision, which is hardly compatible with the development of Bayesian estimators for TDM. This result could be expected as the initial non-compartmental analysis showed a non-gaussian distribution of the dose-normalized AUC in the reference group.

One consequence of η-shrinkage is that covariate relationships may be hidden, falsely induced, or the shape of the true relationship

distorted [37]. This may partly contribute to the fact that, in the present study, no covariate was found significant. However, very few potential covariates were registered in the database used.

The high value of shrinkage obtained for the elimination constant rate prompted us to ask ourselves about the relevance of standard parametric modeling (using fixed exponential transformation) and its ability to describe the shapes of the parameter distribution. Thus, two semi-parametric transformations (i.e., Box–Cox and logit transformations) [30] were investigated but failed to improve the model fit. Finally, a nonparametric approach was used. In NPAG, no assumption is made about the shape of the parameters distribution which allows for many possible distributions and enables detection of the full diversity in the distribution of population parameters. This approach could reveal the true nature of the distribution of the elimination rate constant ke, allowing the description of subpopulations and the extreme values [43]. However, it is not possible (in the current state of the art) to determine the inaccuracy of estimates obtained using such a nonparametric approach. Consequently, shrinkage cannot be calculated.

This study performed in children is the first focusing on a non normal distribution of MPA PK parameters. Such deviation from normality was not reported in adults. The high heterogeneity of the elimination constant values in children suggests the requirement of a wide range of doses to reach the target exposure. In clinical practice, this could result in a higher variability of administered doses in children than in adults. The mechanisms underlying this age-related difference in MMF dose requirements are largely unknown but could be related to developmental changes (also known as ontogeny of drug disposition) of the UDP-glucuronosyltransferases, the enzyme family mainly responsible for MPA metabolism [40].

The results of the Bayesian estimation of MPA AUC and concentrations in the test patient group showed that the NPAG had a better predictive performance than the NONMEM model, as it yielded smaller and non-significant bias. The biased estimates obtained with the NONMEM model in the present case make it unsuitable for TDM. This result supports the assumption that NPAG would be preferable to NONMEM in case of multimodal or non-normal distribution of any one of the PK parameters in the model. However, the limit of the present results lies in the fact that Bayesian estimation of individual parameter values in the independent group of patients was not feasible in BigWinPops and was performed using ADAPT II, i.e., under the usual normality assumptions, which is not optimal in the case of non-normally distributed PK parameters.

Another limitation is the lack of relevant covariates in our database. Indeed, while few demographic covariates, available for all patients, could be included in the population analysis, no major clinical variables, such as renal function or serum albumin which have been previously found related to MPA exposure in adults [13], were investigated due to many missing data. This is not a barrier to nonparametric analysis as, due to no assumption about the shape of the parameter distributions, unsuspected subpopulations of subjects can be detected without additional aid from covariates or other descriptors [32,43]. Finally, the population model analysis reported in the present study dealt with pediatric renal transplant patients receiving a MMF/cyclosporine regimen and cannot be extrapolated to another immunosuppressive regimen like the MMF/tacrolimus association which is becoming more common in prevention of acute rejection.

In conclusion, NPAG was a more adequate population pharmacokinetic method to describe the pharmacokinetics of MPA in pediatric renal transplant recipients. This finding could be linked to the studied database which included very few covariates able to explain the pharmacokinetic variability of MPA (such as serum albumin, blood hemoglobin and cyclosporine concentrations).

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