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Population Pharmacokinetics and Bayesian Estimation of Mycophenolic Acid Concentrations in Stable Renal Transplant Patients

Chantal Le Guellec,[1,2] *Hélène Bourgoin*,[1,2] *Matthias Büchler*,[2,3] *Yann Le Meur*,[4] *Yvon Lebranchu*,[2,3] *Pierre Marquet*[5] and *Gilles Paintaud*[1,2]

1 Department of Pharmacology, Tours University Hospital, Tours, France
2 Haematopoïetic Stem Cells and Haemostasis in Transplantation Research Group (EA 3249 and IFR 120), Tours University, Tours, France
3 Department of Nephrology and Clinical Immunology, Tours University Hospital, Tours, France
4 Department of Nephrology, Limoges University Hospital, Limoges, France
5 Department of Pharmacology and Toxicology, Limoges University Hospital, Limoges, France

Abstract

Background: Therapeutic drug monitoring of mycophenolic acid (MPA) may minimise the risk of acute rejection after transplantation. Area under the curve (AUC) rather than trough concentration-based monitoring is recommended and models for AUC estimation are needed.

Objectives: To develop a population pharmacokinetic model suitable for Bayesian estimation of individual AUC in stable renal transplant patients.

Patients and methods: The population pharmacokinetics of MPA were studied using nonlinear mixed effects modelling (NONMEM) in 60 patients (index group) receiving MPA on a twice-daily basis. Ten blood samples were collected at fixed timepoints from ten patients and four blood samples were collected at sparse timepoints from 50 patients. Bayesian estimation of individual AUC was made on the basis of three blood concentration measurements and covariates. The predictive performances of the Bayesian procedure were evaluated in an independent group of patients (test group) comprising ten subjects in whom ten blood samples were collected at fixed timepoints.

Results: A two-compartment model with zero-order absorption best fitted the data. Covariate analysis showed that bodyweight was positively correlated with oral clearance. However, the weak magnitude of the reduction in variability (from 34.8 to 28.2%) indicates that administration on a per kilogram basis would be of limited value in decreasing interindividual variability in MPA exposure. Bayesian estimation of pharmacokinetic parameters using samples drawn at 20 minutes and 1 and 3 hours enabled estimation of individual AUC with satisfactory accuracy (bias 7.7%, range of prediction errors 0.43–15.1%) and precision (root mean squared error 12.4%) as compared with the reference value obtained using the trapezoidal method.

Conclusion: This paper reports for the first time population pharmacokinetic data for MPA in stable renal transplant patients, and shows that Bayesian estimation can allow accurate prediction of AUC with only three samples. This method provides a tool for therapeutic drug monitoring of MPA or for concentration-effect studies. Its application to MPA monitoring in the early period post-transplantation needs to be evaluated.

Mycophenolic acid (MPA), the active moiety of mycophenolate mofetil, is widely used in the prevention of acute organ rejection in combination with calcineurin inhibitors. Its use is associated with gastrointestinal and haematological adverse effects, which are more frequent with the highest doses (3 g/day). In combination with ciclosporin, mycophenolate mofetil is generally administered at a fixed dose of 1g twice daily, but dosage modifications can be made in relation to clinical effects. Relationships between MPA concentrations and effects have been investigated in heart and renal transplant patients, based on single-timepoint samples such as peak (C_{max}) or trough (C_0) concentrations or on area under the concentration-time curve (AUC).[1-13] In renal transplant patients, the incidence of rejection was influenced by MPA AUC and, although less strongly, by C_0.[11,14] Concentration-controlled trials in renal transplant patients have confirmed that the risk of biopsy-proven acute rejection was higher in the group allocated to low MPA concentrations.[5,15] In these studies, the relationship was stronger with median AUC_{12}, obtained from repeated determinations over 6 months, than with median C_0. Conversely, more frequent withdrawals for adverse events occurred in the high-AUC groups, also corresponding to the highest doses. Other authors also found higher MPA concentrations (AUC and concentrations at specific times, including C_0) in patients with toxicity than in those who did not experience adverse effects.[6,10,16] However, in most studies conducted in renal transplant patients, the incidence of mycophenolate mofetil-related adverse events was not associated with any MPA pharmacokinetic variable.[15,17] Some authors advocated a relationship between free MPA AUC and the occurrence of adverse effects.[12,13] There is now a consensus for therapeutic drug monitoring of MPA in the initial post-transplant phase, primarily to detect patients with low concentrations in order to prevent under-immunosuppression,[18-21] whereas its role in the control of adverse effects has to be evaluated further.[22,23]

Elements in favour of an AUC-based rather than a C_0-based monitoring of MPA are: (i) the existence of a stronger pharmacokinetic-pharmacodynamic relationship between acute rejection and AUC as compared with C_0; and (ii) the observation of a lower intra-individual variability for AUC than for C_0.[12,14] However, to obtain multiple samples in a patient has evident limitations, and AUC estimation procedures would be needed for routine therapeutic drug monitoring. Two different methods can be used to estimate individual AUC with a limited number of samples. The first approach is based on multilinear regression models using three to five drug concentrations. This approach, known as the limited sampling strategy, is increasingly used in the monitoring of immunosuppressive drugs[24] and a number of such strategies have been published for MPA.[25-30] Bayesian estimation of individual pharmacokinetic parameters involves more complex calculations than limited sampling but is more flexible, in that the sampling times are less strict.[31] More important, this approach is based on a population pharmacokinetic analysis that allows patient characteristics explaining interindividual pharmacokinetic variability to be identified.[32-34]

The goal of our study was to develop a Bayesian model for the estimation of individual MPA pharmacokinetics in stable renal transplant patients using only three samples.

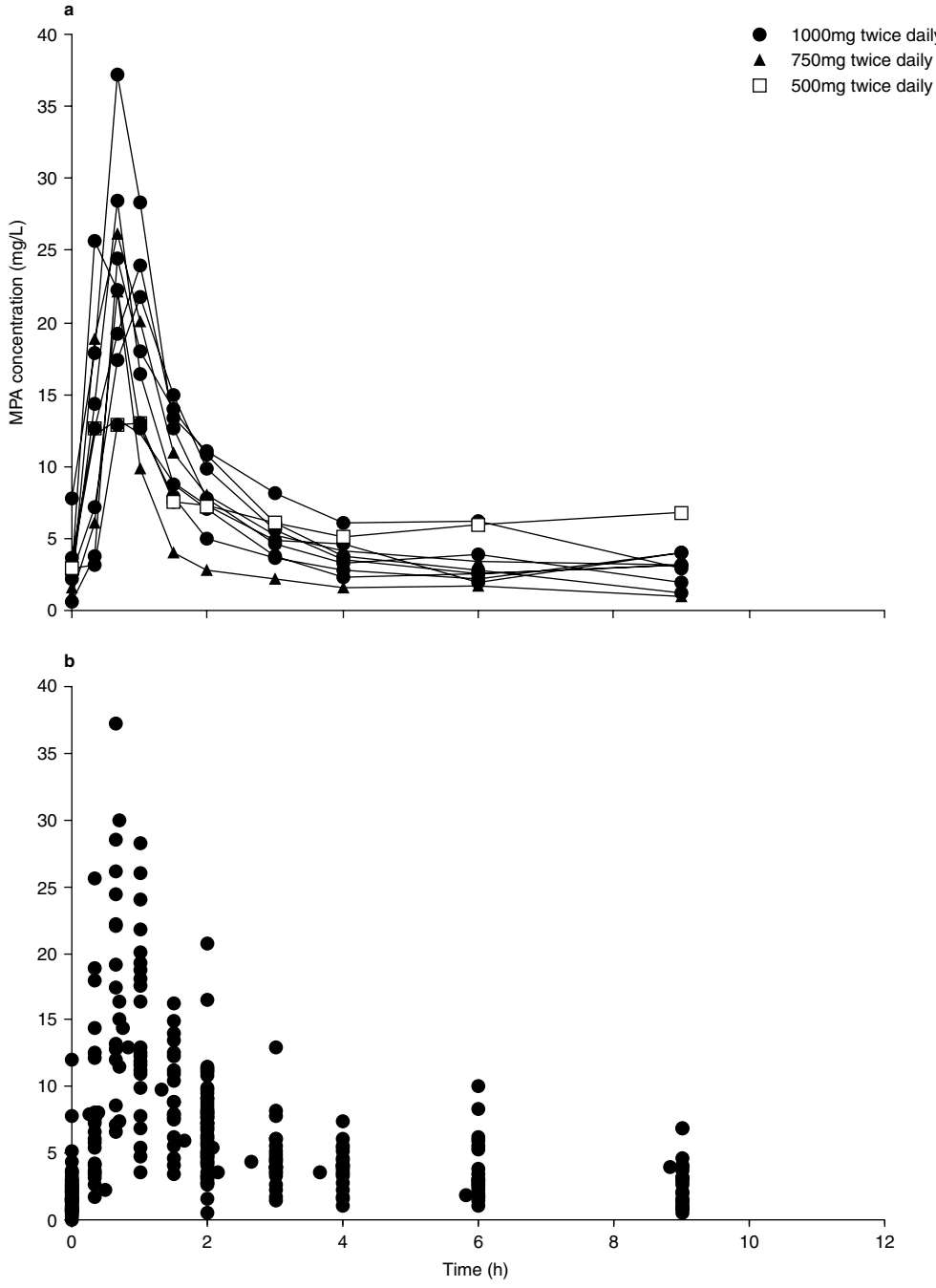

Fig. 1. Concentrations of mycophenolic acid (MPA) in (**a**) 20 renal transplant patients participating in the full pharmacokinetic study based on ten blood samples, and (**b**) in the index data set used for model building, including ten of the 20 subjects in the full pharmacokinetic study and 50 subjects with four samples.

Table I. Patient characteristics in the index and the test groups. Values are given as median (range). There was no significant difference between the groups in any of the characteristics

Characteristic	Index (n = 60)	Test (n = 10)
MMF daily dose (mg)	2000 (500–2000)	2000 (1000–2000)
Age (years)	46 (18–71)	47 (27–66)
Weight (kg)	64 (39–97)	62 (50–130)
Height (cm)	167 (144–190)	169 (155–184)
AST (U/L)	14 (6–30)	14 (9–27)
ALT (U/L)	13 (5–36)	10 (7–25)
Serum creatinine (μmol/L)	115 (69–191)	130 (78–217)
Bilirubin (μmol/L)	8 (3–24)	10 (3–16)
Corticosteroids (on/off)	29/31	3/7
Interacting drugs[a]	None	None
Reference AUC (mg • h/L)[b]	54 (28–73)	42 (26–65)

a Antacids, metronidazole or cholestyramine.

b Obtained using the trapezoidal method in full pharmacokinetic profiles.

AUC = area under the curve; **h** = hour; **MMF** = mycophenolate mofetil; **n** = number of patients.

Patients and Methods

Treatment and Patient Characteristics

Seventy recipients of a first renal allograft, transplanted in one of two centres (Tours and Limoges University Hospitals, France) at least 6 months previously, were included. They had stable renal function with serum creatinine <220 μmol/L. All patients received induction therapy with antithymocyte globulin (Thymoglobuline®[1], SangStat, Fremont, CA, USA) and were treated with ciclosporin (Neoral®, Novartis, East Hanover, NJ, USA) and mycophenolate mofetil (Cellcept®, Roche, Nutley, NJ, USA) with or without corticosteroids. Ciclosporin and mycophenolate mofetil were taken simultaneously every 12 hours, with ciclosporin dosage adjusted to maintain a morning blood C_0 of 150–200 μg/L and a mycophenolate mofetil dose of 1g, adapted if necessary according to individual tolerance. At the time of the study, drug dosages had been unchanged for at least 1 month and patients had stable ciclosporin C_0. Any change in drug treatment during the 15 days preceding the study precluded participation. All concomitant medications were recorded for each patient. The protocol was approved by the regional ethics committee (University Hospital of Limoges) and all the patients gave informed consent before inclusion.

Twenty patients participated in a full pharmacokinetic study involving ten blood samples. In 50 patients, blood samples were obtained before (C_0) and 2 hours after (C_2) administration and at two other times, randomly selected from those of the full pharmacokinetic study. The randomisation procedure ensured an equal number of samples at each timepoint other than C_0 and C_2.

Study Design

The analysis was conducted in two steps. A population pharmacokinetic model was first developed in a subset of patients and a Bayesian model was subsequently evaluated in an independent validation group. Sixty patients were assigned to the index group to build the population pharmacokinetic model using nonlinear mixed effects modelling. This group included ten patients randomly selected from the full-pharmacokinetic group and the 50 patients from the sparse sampling group. The last ten patients of the full-pharmacokinetic group were assigned to the validation group, in which the Bayesian procedure was evaluated.

Sample Collection and Analytical Method

Blood samples were collected in EDTA tubes and the plasma was kept frozen at –20°C until analysis. The full pharmacokinetic study involved samples drawn before (C_0) and 20 and 40 minutes and 1, 1.5, 2, 3, 4, 6 and 9 hours after the morning dose. Mycophenolate mofetil was administered under fasting conditions, and unstandardised meals were given 0.5 (breakfast) and 4 (lunch) hours later.

Plasma MPA was analysed by an enzyme-multiplied immunoassay technique (EMIT; Dade-Behring Diagnostics, Marburg, Germany), with intra-assay variability of 2.8% and 4.8% and interassay variability of 7.4% and 6.3% at low (0.5–2.0 mg/L) and high (10–15 mg/L) concentrations, respectively.

1 The use of trade names is for product identification purposes only and does not imply endorsement.

The assay allows quantification of MPA in a 0.5–15 mg/L range and appropriate dilution of the samples was made using blank plasma when necessary. The crossreactivity with MPA glucuronide is <0.2%.[17] Intra- and interlaboratory quality controls were used during all the study period.

Model Building

The data of the index group were analysed by nonlinear mixed effects modelling using NONMEM (version V, level 1.0) and Visual-NM (RDPP, Montpellier, France) a Windows™-based interface to NONMEM containing graphical and statistical tools. The First Order method was used during the model-building process and concentration steady state was assumed. One and two-compartment models with first-order or zero-order absorption were fitted to the data. Between-subject variability was modelled with additive, exponential or proportional error models, and residual variability with additive, proportional or combined error models.

Estimates of the individual pharmacokinetic parameters were obtained by means of the NONMEM 'posthoc' option. Graphical analyses were conducted to study relationships between each parameter and covariates (age, sex, weight, height, serum creatinine, creatinine clearance, ALT, AST and bilirubin). The influence of comedications was also studied by comparing parameter values in patients with or without drugs known to interact with MPA pharmacokinetics (corticosteroids, aciclovir, antacids, metronidazole and cholestyramine). If present, any relationship was included in the pharmacokinetic model. Final inclusion of a covariate in the model was considered if a decrease in the objective function of at least five units was observed.

Model selection was based on the objective function and on graphical analyses of the goodness-of-fit. The overall fitting was evaluated on the basis of plots of predicted versus observed concentrations, absolute and weighted residuals versus observed concentrations and individual weighted residuals versus individual predicted concentrations. The choice of the absorption model (first-order or zero-order) was based on plots of residual concentrations versus time to analyse which model fitted best the data during the absorption phase.

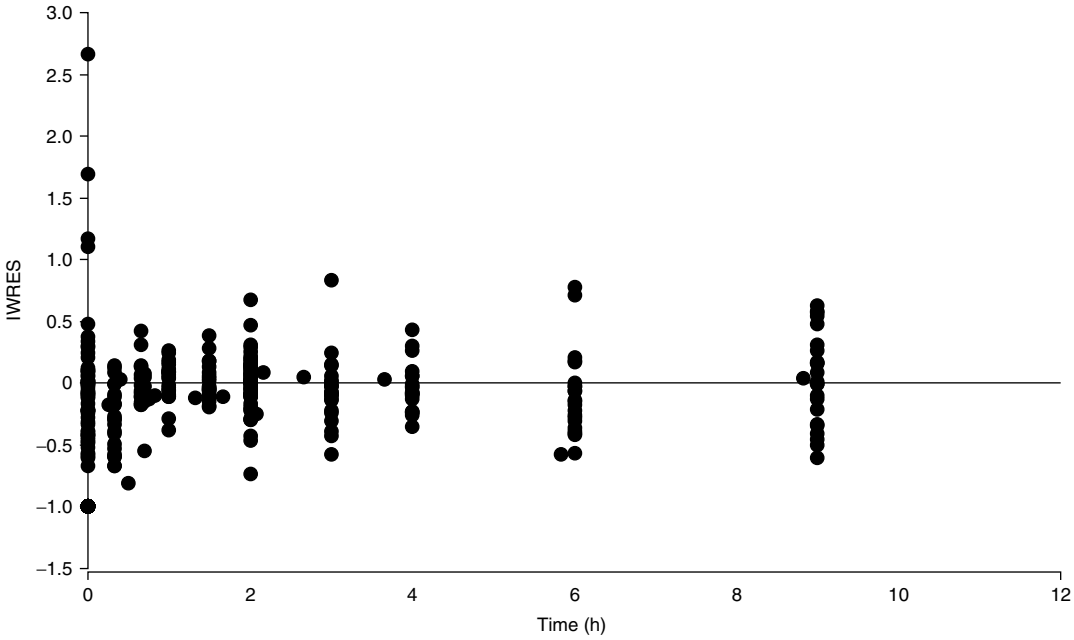

Fig. 2. Quality of pharmacokinetic fitting in the index data set. Individual weighted residuals (IWRES) versus sampling time.

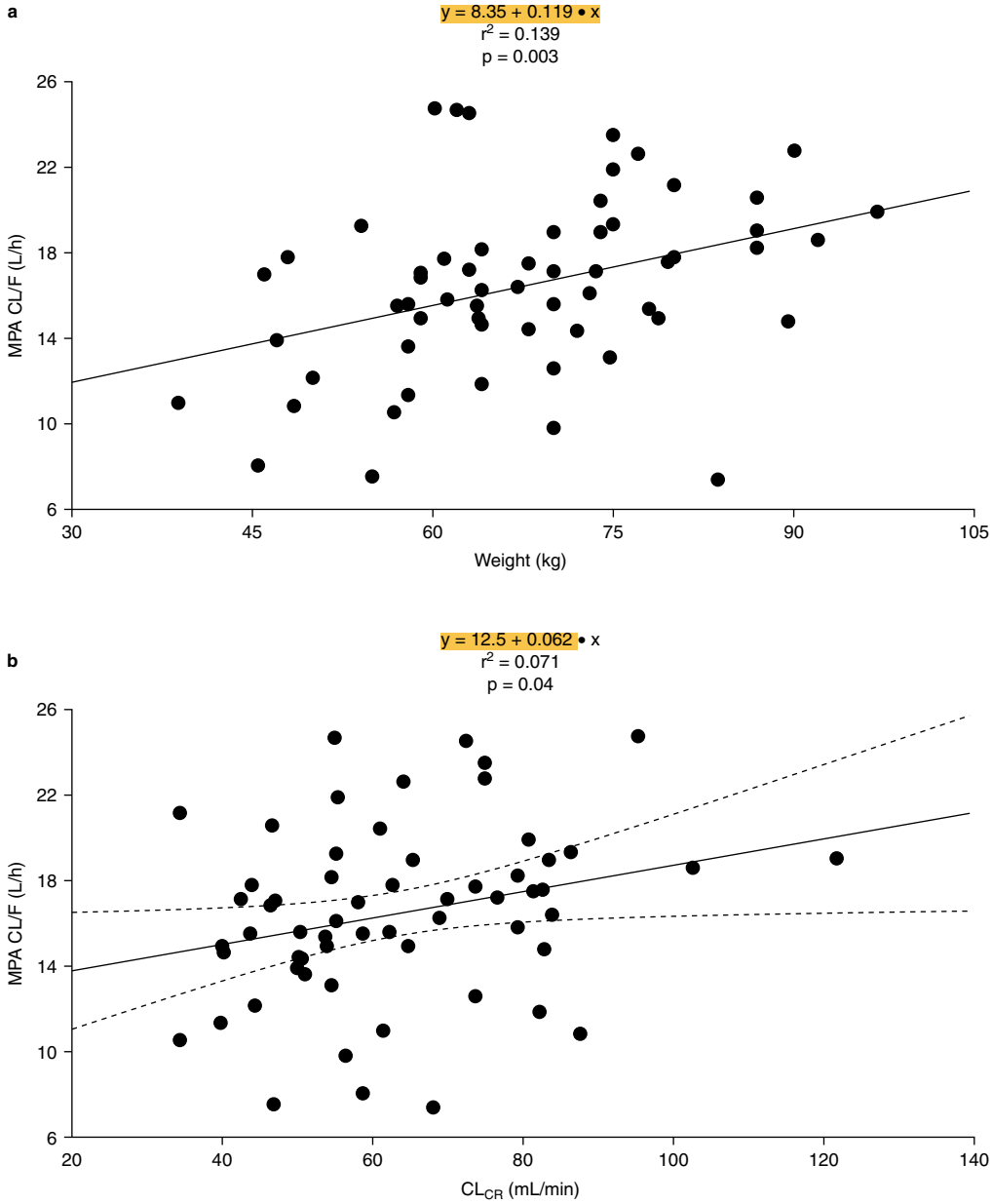

Fig. 3. Relationship between apparent ('oral') clearance (CL/F) of mycophenolic acid (MPA) and (**a**) bodyweight or (**b**) creatinine clearance (CL_{CR}) in the index group.

Bayesian Estimation in the Test Group

Bayesian estimation was made using a combination of three concentrations (20 minutes and 1 and 3 hours), selected according to a previously published multiple linear regression model.[26] Individual pharmacokinetic parameters of each subject of the test group were obtained using the 'posthoc' subroutine of NONMEM without estimation step (MAXEVAL

= 0) and setting mean parameter values, interindividual and intraindividual variabilities to population values previously obtained. These parameters were then implemented on spreadsheet software (Excel®) to simulate the steady-state concentration-time curve.

The quality of the Bayesian estimation was assessed by checking the goodness-of-fit for all concentrations in each of the ten patients in the full pharmacokinetic study. Bayesian estimated individual AUCs were compared with the reference AUCs obtained by the trapezoidal method and predictive performances were evaluated by comparing predicted and observed concentrations in the whole test data set using correlation analysis. Bias and precision of predicted versus observed concentrations at each sampling time and of estimated versus reference AUCs were calculated as mean absolute error (ME) and root mean squared error (RMSE), respectively.[35]

Results

Data Description

In the 20 subjects with a full pharmacokinetic profile, MPA concentrations showed an early and sharp increase with a peak occurring at a median time of 40 minutes, followed by a rapid decrease (figure 1a). Some patients displayed a second increase, probably related to enterohepatic cycling of MPA by secretion and deconjugation of MPA-glucuronide, and occurring between 6 and 9 hours after the dose. The highest concentrations were obtained in patients taking the highest doses but a marked interindividual variability was observed for the same dose. A total number of 300 concentrations from 60 patients were available in the index dataset for model building (figure 1b). Patient characteristics were comparable in the index and the test groups (table I).

Population Pharmacokinetic Modelling

A two-compartment model with zero-order absorption (i.e. an intravenous infusion model, ADVAN3), the absorption duration (parameter D_1) being estimated from the data, provided the best fitting (figure 2). The model was parameterised in terms of apparent ('oral') clearance (CL/F), central volume (V_1/F), peripheral volume (V_2/F) and intercompartmental clearance (Q) using TRANS4. An exponential error model was selected to describe the interindividual variability in the parameters. However, whatever the error model chosen, the value of V_2/F did not vary between subjects and no value of η was allocated to this parameter. A combined model provided the best results for residual variability but was associated with a high value for the constant component ε (2.04 mg/L).

Covariate analysis showed that bodyweight, but not creatinine clearance, was positively correlated with CL/F (figure 3) and its inclusion in the final population pharmacokinetic model decreased the objective function from 878 to 865 and interindividual variability from 34.8 to 28.2%. Median [range] CL/F was not different in patients with (15.5 [7.58–23.6] L/h) or without (16.5 [7.95–25.3] L/h) corticosteroids. The influence of other known interacting drugs could not be studied because no patients were taking them. The duration of absorption had a mean of 0.69 hour and a low between-subject variability (coefficient of variation 10.9%). A summary of parameter values, between-subject variability and precision of estimates is presented in table II.

Bayesian Estimation in the Test Group

Individual pharmacokinetic parameters were estimated in the ten subjects of the test group using three concentrations per subject and their current

Table II. Final population pharmacokinetic parameters

Parameter	Mean value	Between-subject variability (CV%)	Precision of estimates (%)
CL/F (L/h)	0.246 • WGT	28.2	4.9
V_1/F (L)	36	63.1	18.9
V_2/F (L)	137		16.6
Q (L/h)	25.9	44.7	36.2
D_1 (h)	0.69	10.9	6.7

CL/F = apparent ('oral') clearance; **CV%** = percentage coefficient of variation; **D_1** = duration of absorption; **h** = hour; **Q** = intercompartmental clearance; **V_1/F** = apparent volume of the central compartment; **V_2/F** = apparent volume of the peripheral compartment; **WGT** = bodyweight (kg).

bodyweight. Examples of concentration prediction using parameters estimated by the Bayesian method are displayed in figure 4 for two patients, corresponding to the best and the worst fit, respectively.

Comparison of predicted and observed concentrations in the entire data set indicated a close correlation (figure 5). Predictions were unbiased, except at sampling times 0 and 9 hours, and satisfactory precision was obtained for most sampling times (table III).

With respect to the main objective of the Bayesian method, i.e. estimation of individual exposure, results were very satisfactory with a correlation coefficient between estimated and reference AUCs of 0.973 and a slope of the correlation line very close to 1 (figure 6). The method provided accurate estimation of AUC, although a bias was observed leading to slight overprediction of AUC (bias 7.7%, range of prediction errors 0.43–15.1%). A very satisfactory precision was observed (RMSE 12.4%).

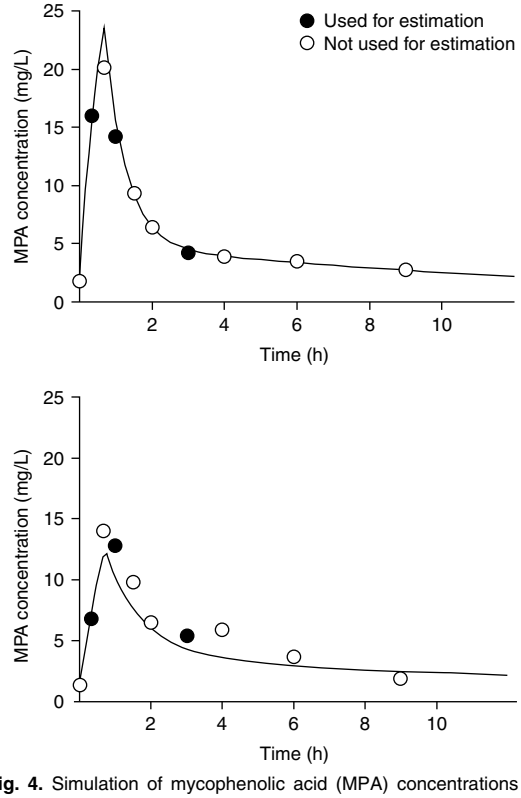

Fig. 4. Simulation of mycophenolic acid (MPA) concentrations in two individual patients with individual parameters estimated by the Bayesian model. Filled circles are the concentrations used for Bayesian estimation of individual parameters and open circles are the observed concentrations not used during the estimation process.

Discussion

AUC rather than C_0 monitoring is recommended for MPA,[18] and models for AUC estimation are needed. A number of limited sampling strategies have been published for MPA AUC estimation using multiple linear regression. They were developed either in children[27,36] or in adults[26,29,30,37] and generally involved C_0 together with two other time points. Most of them included a sample drawn 6 hours after administration, which is not very suitable for routine use. The differences in the timepoint combinations found in these studies may arise from variable conditions of the patients between studies. Indeed, a number of factors have been thought to modify the shape of the MPA profile, affecting in particular C_{max} and the time that this occurs, AUC itself being modified or not. These variables are: concomitant immunosuppressant (tacrolimus or ciclosporin), food intake, analytical technique for MPA measurement, age (child versus adult) and post-transplant period. Furthermore, constrained inclusion of C_0 in the limited sampling strategy in some studies introduces another source of discrepancy between results. Testing and validation are thus recommended before using a model developed in other patients.

Bayesian forecasting is a tool for therapeutic drug monitoring based on the estimation of individual pharmacokinetic parameters subsequently used to simulate concentrations under any administration regimen. This approach has been applied to numerous drugs, and user-friendly programs are available for the monitoring of drugs having a narrow therapeutic window, including aminoglycosides, vancomycin, theophylline and digoxin.[38] Bayesian models are particularly useful if population databases including patients with different characteristics are available. Population databases are still lacking for some drugs (e.g. methotrexate and immunosuppressants) and some population subtypes (e.g.

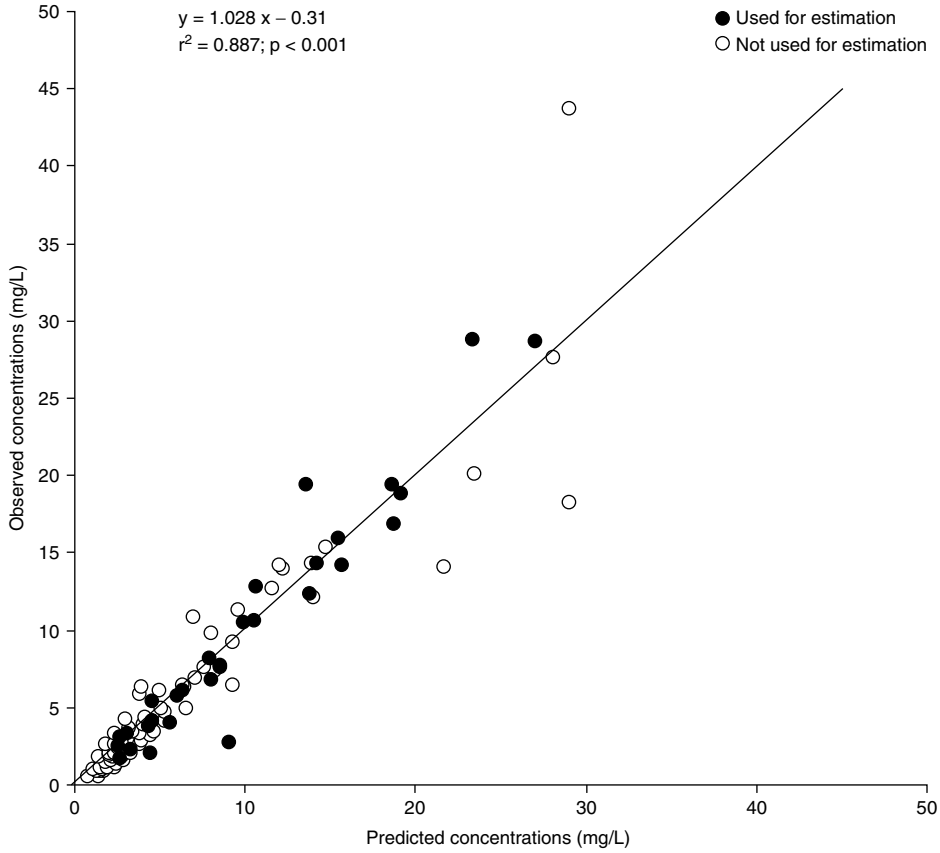

Fig. 5. Plot of observed versus individual predicted concentrations of mycophenolic acid in the test group. Filled circles are the concentrations used for Bayesian estimation of individual parameters and open circles are the observed concentrations not used during the estimation process.

children), and users may have to build their own Bayesian programs. In the field of immunosuppressant drugs, Bayesian models have been proposed for ciclosporin in its original formulation[39-41] and with the new microemulsified formulation.[39,42,43] The present report is the first application to MPA.

Population pharmacokinetic analysis is the first step in the development of a Bayesian method. It allows the estimation of mean pharmacokinetic parameters and also offers the advantage of considering individual characteristics that may help in interpreting different pharmacokinetic behaviours between patients. Our population pharmacokinetic analysis was conducted in a large enough number of patients to provide relevant information about MPA pharmacokinetics in the specific subgroup of stable renal transplant patients. Mean pharmacokinetic parameters were similar to those estimated using conventional methods.[1] We found that CL/F was positively correlated with weight, suggesting that calculation of dose on a mg/kg basis might decrease interpatient variability in MPA exposure. However, considering weight in the pharmacokinetic model only reduced the variability in CL/F by one-fifth, so administration on a per kilogram basis should not contribute to MPA dose optimisation to a large extent. No correlation was found between CL/F and any other of the covariates tested, particularly creatinine clearance.

A time-dependent increase in MPA AUC is observed during the early post-transplant period,

which may be caused by: (i) renal function and increase of free fraction via competition with MPA-glucuronide favouring MPA elimination; (ii) interaction with ciclosporin, whose concentrations are decreasing over time; or (iii) variable gastrointestinal absorption.[36,44] Impairment of renal function has been shown to influence MPA disposition.[44,45] Patients with acute impairment of renal function have a higher MPA CL/F than do patients with normal renal function.[45] This phenomenon is associated with an increase in MPA free fraction and MPA-glucuronide concentrations. The likely explanation of the changes in MPA pharmacokinetics is reduced binding of MPA to serum albumin by a direct effect of MPA-glucuronide, which competes with MPA for binding sites. These changes are maximum during the first two weeks following renal transplantation and normalise as renal function returns to normal. The temporary impairment of renal function after renal transplantation may then lead to a decrease in MPA AUC. However, these pharmacokinetic modifications may be observed only in a subset of patients with delayed graft function. Stabilisation of MPA pharmacokinetics occurs around the third month post-transplantation and renal function no longer regulates MPA CL/F.[1] Our results confirmed the absence of influence of renal function on MPA CL/F in stable patients, transplanted for more than 6 months, with moderate impairment of renal function. In patients with chronic renal insufficiency receiving long-term mycophenolate mofetil therapy, Meier-Kriesche et al. demonstrated an increase in MPA free fraction attributed to hypoalbuminaemia, elevated MPA-glucuronide concentration or uraemia. Total MPA AUC was not increased as compared with patients with better renal function, but the risk of haematological toxicity due to free MPA remains an issue of concern in the clinic.[44]

Table III. Predictive performances of the Bayesian estimation at each sampling time

Sampling time (h)	ME (CI) [mg/L]	ME (%)	RMSE (%)
0	0.62 (0.37, 0.86)	52.4	60.6
0.33	0.87[a] (−2.00, 4.64)	35.8[a]	80.7[a]
0.66	−0.19 (−5.11, 4.72)	2.4	30.8
1	−1.04 (−2.96, 0.87)	−3.9	14.0
1.5	−0.35 (−1.39, 0.69)	−2.7	18.9
2	0.24 (−0.61, 1.09)	4.6	22.2
3	0.27 (−0.25, 0.78)	11.5	26.5
4	0.21 (−0.5, 0.95)	19.6	44.7
6	−0.03 (−0.54, 0.49)	−1.2	23.0
9	0.52 (0.27, 0.76)	35.5	44.4
Overall	0.11	13.1	31.7

a One patient had a very sharp absorption phase, resulting in a large prediction error at 0.33 hours.

ME = mean absolute error; **RMSE** = root mean squared error.

Pharmacokinetic interactions with frequently used comedications have been described for MPA. Increased AUC is observed in patients receiving tacrolimus as compared with ciclosporin, the latter probably decreasing MPA biliary secretion and thus reducing the enterohepatic cycling of the drug. Decreased AUC is observed with antacids and cholestyramine due to decreased absorption. It has recently been reported that corticosteroid withdrawal in renal transplant patients increases MPA AUC by approximately 20%.[19] A possible explanation is that corticosteroids enhance MPA metabolism by induction of hepatic glucuronyltransferase activity. Such a difference was not seen in our patients, CL/F being similar in patients on and off corticosteroids, indicating that systematic modification of MPA dosage is not necessary during the corticosteroid tapering phase. These discrepant results may arise from the additional impact of decreasing ciclosporin exposure, which could be different between the two studies.

Our pharmacostatistical model indicated a large residual variability. In fact, individual concentration-time data often exhibited erratic profiles that could not be described by any realistic pharmacokinetic model. Sampling errors cannot be involved, since the prospective nature of this study guaranteed rigorous sampling according to procedures. On the other hand, measurement errors could be involved since the EMIT assay that we used crossreacts with acyl-MPA glucuronide, whose concentrations vary over the administration interval.[46] Rebound of MPA plasma concentration due to enterohepatic cycling of MPA is probably the main reason for the

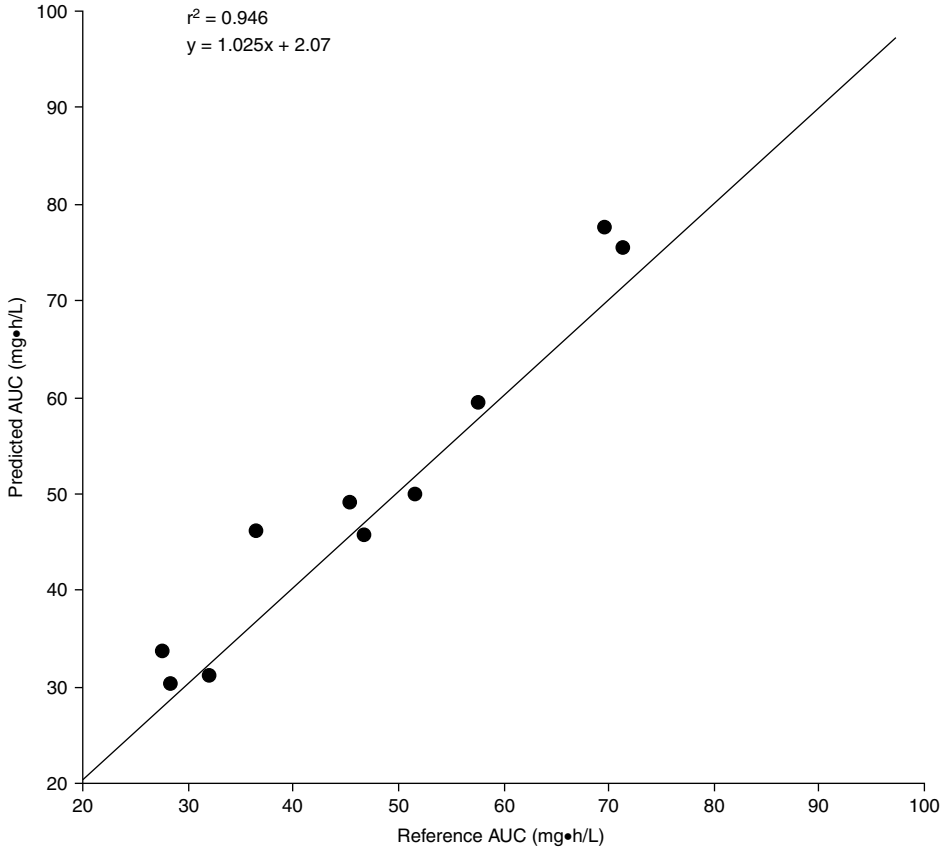

Fig. 6. Correlation of predicted area under the concentration-time curve (AUC) of mycophenolic acid versus reference AUC obtained by the trapezoidal method.

differences between individually predicted and observed concentrations. A pharmacokinetic model for enterohepatic cycling has previously been proposed for MPA[47] but, following the principle of parsimony, we decided not to use such a complex model. The secondary peaks seen in most of our patients were relatively small, but such peaks may have represented a significant proportion of the AUC in others. The use of an enterohepatic cycling pharmacokinetic model would be useful in studies focusing on the analysis of factors that influence this phenomenon, such as, for example, the differential effect of ciclosporin and tacrolimus, the former being associated with reduced cycling.[48]

The choice of the sample combination used in our Bayesian procedure was based on clinically and practically relevant considerations. A maximum of three sampling times was allowed, each sample being taken during the few hours post-dose to comply with outpatient care. The three-point combination tested (20 minutes and 1 and 3 hours) was selected according to a previously published multiple linear regression model.[26] The accuracy of our Bayesian procedure was satisfactory, as shown by the good agreement between estimated and observed concentrations and by a low prediction error at most of the sampling times. A positive value for prediction error indicates that the concentration is overpredicted by the Bayesian model. Systematic overprediction and poor precision occurred at 0 and 9 hours. This result is not consistent with enterohepatic cycling of the drug at late time points, which would be accompa-

nied by an underestimation of concentrations. Poor precision of our Bayesian model at low concentrations might be responsible for these results.

There is still debate on the clinical situations justifying MPA monitoring. Monitoring is now recommended during the early period after renal transplantation, and a consensus has emerged on the superiority of AUC as compared with C_0 in those cases. Other situations could certainly benefit from MPA therapeutic drug monitoring, and a Bayesian method allowing estimation of individual exposure is essential to conduct prospective pharmacokinetic-pharmacodynamic studies. Our Bayesian model provides a useful tool for MPA AUC estimation in stable renal transplant patients, as confirmed by the high correlation between estimated and reference AUCs. The trend to overpredict AUC that was observed would not be clinically relevant when considering the relative imprecision of the therapeutic range of MPA AUC.

There are also concerns about the preferable analytical method to be used for MPA measurement. Our Bayesian model is based on concentrations measured by an EMIT technique. Slight discrepancies between high-performance liquid chromatography (HPLC) and EMIT have been described, arising from crossreactivity with the acyl-glucuronide, which is pharmacologically active.[46,49,50] From this viewpoint, the EMIT assay should be as effective as HPLC for MPA monitoring. This was recently confirmed by a study evaluating pharmacokinetic-pharmacodynamic relationships of mycophenolate mofetil in paediatric renal transplant patients.[17] The authors found that MPA AUCs obtained by the EMIT technique were as effective as those obtained by HPLC to predict the risk of acute rejection. They proposed a slightly higher therapeutic range for the EMIT assay (35–70 mg • h/L) than for HPLC (30–60 mg • h/L).

Whether or not our Bayesian model could be applied to patients having other characteristics in terms of age, time post-transplantation or comedication must be evaluated. It will be implemented in the future with such data to take into account time-dependent and other sources of variability in MPA pharmacokinetics.

Conclusion

This paper reports for the first time population pharmacokinetic data for MPA in stable renal transplant patients, and shows that Bayesian estimation can allow accurate prediction of AUC with only three samples. This method provides a tool for therapeutic drug monitoring of MPA or for concentration-effect studies. Its application to MPA monitoring in the early period post-transplantation needs to be evaluated.

Acknowledgements

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References

1. Bullingham RE, Nicholls AJ, Kamm BR. Clinical pharmacokinetics of mycophenolate mofetil. Clin Pharmacokinet 1998; 34 (6): 429-55
2. Cantin B, Giannetti N, Parekh H, et al. Mycophenolic acid concentrations in long-term heart transplant patients: relationship with calcineurin antagonists and acute rejection. Clin Transplant 2002; 16 (3): 196-201
3. DeNofrio D, Loh E, Kao A, et al. Mycophenolic acid concentrations are associated with cardiac allograft rejection. J Heart Lung Transplant 2000; 19 (11): 1071-6
4. Dubrey SW, Holt DW, Banner N. Measurement of mycophenolate mofetil plasma levels after heart transplantation and a potential side effect of high levels. Ther Drug Monit 1999; 21 (3): 325-6
5. Hale MD, Nicholls AJ, Bullingham RE, et al. The pharmacokinetic-pharmacodynamic relationship for mycophenolate mofetil in renal transplantation. Clin Pharmacol Ther 1998; 64 (6): 672-83
6. Hubner GI, Eismann R, Sziegoleit W. Relationship between mycophenolate mofetil side effects and mycophenolic acid plasma trough levels in renal transplant patients. Arzneimittel Forschung 2000; 50 (10): 936-40
7. Pillans PI, Rigby RJ, Kubler P, et al. A retrospective analysis of mycophenolic acid and cyclosporin concentrations with acute rejection in renal transplant recipients. Clin Biochem 2001; 34 (1): 77-81
8. Sanquer S, Breil M, Baron C, et al. Trough blood concentrations in long-term treatment with mycophenolate mofetil [letter]. Lancet 1998; 351 (9115): 1557
9. Shaw LM, Nowak I. Mycophenolic acid: measurement and relationship to pharmacologic effects. Ther Drug Monit 1995; 17 (6): 685-9

10. Smak Gregoor PJ, Hesse CJ, van Gelder T, et al. Relation of mycophenolic acid trough levels and adverse events in kidney allograft recipients. Transplant Proc 1998; 30 (4): 1192-3
11. Takahashi K, Ochiai T, Uchida K, et al. Pilot study of mycophenolate mofetil (RS-61443) in the prevention of acute rejection following renal transplantation in Japanese patients. RS-61443 Investigation Committee: Japan. Transplant Proc 1995; 27 (1): 1421-4
12. Oellerich M, Shipkova M, Schutz E, et al. Pharmacokinetic and metabolic investigations of mycophenolic acid in pediatric patients after renal transplantation: implications for therapeutic drug monitoring. German Study Group on Mycophenolate Mofetil Therapy in Pediatric Renal Transplant Recipients. Ther Drug Monit 2000; 22 (1): 20-6
13. Weber LT, Shipkova M, Armstrong VW, et al. The pharmacokinetic-pharmacodynamic relationship for total and free mycophenolic acid in pediatric renal transplant recipients: a report of the German study group on mycophenolate mofetil therapy. J Am Soc Nephrol 2002; 13 (3): 759-68
14. Nicholls AJ. Opportunities for therapeutic monitoring of mycophenolate mofetil dose in renal transplantation suggested by the pharmacokinetic/pharmacodynamic relationship for mycophenolic acid and suppression of rejection. Clin Biochem 1998; 31 (5): 329-33
15. van Gelder T, Hilbrands LB, Vanrenterghem Y, et al. A randomized double-blind, multicenter plasma concentration controlled study of the safety and efficacy of oral mycophenolate mofetil for the prevention of acute rejection after kidney transplantation. Transplantation 1999; 68 (2): 261-6
16. Mourad M, Malaise J, Chaib Eddour D, et al. Pharmacokinetic basis for the efficient and safe use of low-dose mycophenolate mofetil in combination with tacrolimus in kidney transplantation. Clin Chem 2001; 47 (7): 1241-8
17. Weber LT, Shipkova M, Armstrong VW, et al. Comparison of the Emit immunoassay with HPLC for therapeutic drug monitoring of mycophenolic acid in pediatric renal-transplant recipients on mycophenolate mofetil therapy. Clin Chem 2002; 48 (3): 517-25
18. Shaw LM, Holt DW, Oellerich M, et al. Current issues in therapeutic drug monitoring of mycophenolic acid: report of a roundtable discussion. Ther Drug Monit 2001; 23 (4): 305-15
19. Cattaneo D, Gaspari F, Ferrari S, et al. Pharmacokinetics help optimizing mycophenolate mofetil dosing in kidney transplant patients. Clin Transplant 2001; 15 (6): 402-9
20. Holt DW. Monitoring mycophenolic acid. Ann Clin Biochem 2002; 39 (Pt 3): 173-83
21. Wong SH. Therapeutic drug monitoring for immunosuppressants. Clin Chim Acta 2001; 313 (1-2): 241-53
22. Mourad M, Wallemacq P, Konig J, et al. Therapeutic monitoring of mycophenolate mofetil in organ transplant recipients: is it necessary? Clin Pharmacokinet 2002; 41 (5): 319-27
23. Stephan A, Masri MA, Barbari A, et al. Therapeutic drug level monitoring for mycophenolate mofetil: is it justified? Transplant Proc 2001; 33 (5): 2778-9
24. Dumont RJ, Ensom MH. Methods for clinical monitoring of cyclosporin in transplant patients. Clin Pharmacokinet 2000; 38 (5): 427-47
25. Filler G, Mai I. Limited sampling strategy for mycophenolic acid area under the curve. Ther Drug Monit 2000; 22 (2): 169-73
26. Le Guellec C, Buchler M, Giraudeau B, et al. Simultaneous estimation of cyclosporin and mycophenolic acid areas under the curve in stable renal transplant patients using a limited sampling strategy. Eur J Clin Pharmacol 2002; 57 (11): 805-11
27. Schutz E, Armstrong VW, Shipkova M, et al. Limited sampling strategy for the determination of mycophenolic acid area under the curve in pediatric kidney recipients: German Study Group on MMF Therapy in Pediatric Renal Transplant Recipients. Transplant Proc 1998; 30 (4): 1182-4
28. Weber LT, Schutz E, Lamersdorf T, et al. Therapeutic drug monitoring of total and free mycophenolic acid (MPA) and limited sampling strategy for determination of MPA-AUC in paediatric renal transplant recipients: The German Study Group on Mycophenolate Mofetil (MMF) Therapy. Nephrol Dial Transplant 1999; 14 Suppl. 4: 34-5
29. Willis C, Taylor PJ, Salm P, et al. Evaluation of limited sampling strategies for estimation of 12-hour mycophenolic acid area under the plasma concentration-time curve in adult renal transplant patients. Ther Drug Monit 2000; 22 (5): 549-54
30. Yeung S, Tong KL, Tsang WK, et al. Determination of mycophenolate area under the curve by limited sampling strategy. Transplant Proc 2001; 33 (1-2): 1052-3
31. Tanigawara Y, Yano I, Kawakatsu K, et al. Predictive performance of the Bayesian analysis: effects of blood sampling time, population parameters, and pharmacostatistical model. J Pharmacokinet Biopharm 1994; 22 (1): 59-71
32. Aarons L. Software for population pharmacokinetics and pharmacodynamics. Clin Pharmacokinet 1999; 36 (4): 255-64
33. Proost JH. Adaptive control of drug dosage regimens using maximum a posteriori probability Bayesian fitting. Int J Clin Pharmacol Ther 1995; 33 (10): 531-6
34. Vozeh S, Steimer JL, Rowland M, et al. The use of population pharmacokinetics in drug development. Clin Pharmacokinet 1996; 30 (2): 81-93
35. Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. J Pharmacokinet Biopharm 1981; 9 (4): 503-12
36. Weber LT, Lamersdorf T, Shipkova M, et al. Area under the plasma concentration-time curve for total, but not for free, mycophenolic acid increases in the stable phase after renal transplantation: a longitudinal study in pediatric patients: German Study Group on Mycophenolate Mofetil Therapy in Pediatric Renal Transplant Recipients. Ther Drug Monit 1999; 21 (5): 498-506
37. Pawinski T, Hale M, Korecka M, et al. Limited sampling strategy for the estimation of mycophenolic acid area under the curve in adult renal transplant patients treated with concomitant tacrolimus. Clin Chem 2002; 48 (9): 1497-504
38. Buffington DE, Lampasona V, Chandler MH. Computers in pharmacokinetics: choosing software for clinical decision making. Clin Pharmacokinet 1993; 25 (3): 205-16
39. Charpiat B, Falconi I, Breant V, et al. A population pharmacokinetic model of cyclosporine in the early postoperative phase in patients with liver transplants, and its predictive performance with Bayesian fitting. Ther Drug Monit 1998; 20 (2): 158-64
40. Ducharme MP, Verret L, Brouillette D, et al. Ability of a first-pass pharmacokinetic model to characterize cyclosporine blood concentrations after administrations of Sandimmune or Neoral formulations. Ther Drug Monit 1998; 20 (2): 165-71
41. Ruggeri A, Martinelli M. A program for the optimization of cyclosporine therapy using population kinetics modeling. Comput Methods Programs Biomed 2000; 61 (1): 61-9
42. Debord J, Risco E, Harel M, et al. Application of a gamma model of absorption to oral cyclosporin. Clin Pharmacokinet 2001; 40 (5): 375-82

43. Leger F, Debord J, Le Meur Y, et al. Maximum a posteriori Bayesian estimation of oral cyclosporin pharmacokinetics in patients with stable renal transplants. Clin Pharmacokinet 2002; 41 (1): 71-80
44. Meier-Kriesche HU, Shaw LM, Korecka M, et al. Pharmacokinetics of mycophenolic acid in renal insufficiency. Ther Drug Monit 2000; 22 (1): 27-30
45. Shaw LM, Korecka M, Aradhye S, et al. Mycophenolic acid area under the curve values in African American and Caucasian renal transplant patients are comparable. J Clin Pharmacol 2000; 40 (6): 624-33
46. Vogl M, Weigel G, Seebacher G, et al. Evaluation of the EMIT mycophenolic acid assay from Dade Behring. Ther Drug Monit 1999; 21 (6): 638-43
47. Funaki T. Enterohepatic circulation model for population pharmacokinetic analysis. J Pharm Pharmacol 1999; 51 (10): 1143-8
48. Brown NW, Aw MM, Mieli-Vergani G, et al. Mycophenolic acid and mycophenolic acid glucuronide pharmacokinetics in pediatric liver transplant recipients: effect of cyclosporine and tacrolimus comedication. Ther Drug Monit 2002; 24 (5): 598-606
49. Schutz E, Shipkova M, Wieland E, et al. Evaluation of an immunoassay for mycophenolic acid. Ther Drug Monit 2000; 22 (1): 141-2
50. Beal JL, Jones CE, Taylor PJ, et al. Evaluation of an immunoassay (EMIT) for mycophenolic acid in plasma from renal transplant recipients compared with a high-performance liquid chromatography assay. Ther Drug Monit 1998; 20 (6): 685-90

Correspondence and offprints: Dr *Chantal Le Guellec*, Department of Pharmacology, Bretonneau University Hospital, 2 Boulevard Tonnellé, Tours, F-37044, France.
E-mail: leguellec@med.univ-tours.fr