BJCP British Journal of Clinical Pharmacology

DOI:10.1111/bcp.12276

Population pharmacokinetics and Bayesian estimation of tacrolimus exposure in paediatric liver transplant recipients

Nastya Kassir,[1,2,3] Line Labbé,[2] Jean-Romain Delaloye,[1,4] Mohamad-Samer Mouksassi,[2,3] Anne-Laure Lapeyraque,[1,5] Fernando Alvarez,[5] Michel Lallier,[4] Mona Beaunoyer,[4] Yves Théorêt[1,6,7] & Catherine Litalien[1,5,7]

[1]Clinical Pharmacology Unit, [4]Department of Surgery, [5]Department of Paediatrics and [6]Department of Biochemistry, CHU Ste-Justine, Montreal, Quebec, Canada, [2]Faculty of Pharmacy and [7]Department of Pharmacology, Université de Montréal, Montreal, Quebec, Canada and [3]Pharsight – A Certara Company, Montreal, Quebec, Canada

Correspondence
Dr Catherine Litalien MD, FRCPC, Division of Paediatrics Critical Care, Department of Paediatrics, Director, Clinical Pharmacology Unit, Centre Hospitalier Universitaire Sainte-Justine, 3175 chemin de la Côte Sainte-Catherine, Montréal, Québec, Canada H3T 1C5.
Tel.: +1 514 345 4931 extension 2571
Fax: +1 514 345 7731
E-mail: catherine.litalien.hsj@ssss.gouv.qc.ca

Keywords
Bayesian method, liver transplant, optimal sampling strategy, paediatrics, population pharmacokinetics, tacrolimus

Received
28 June 2013

Accepted
22 October 2013

Accepted Article Published Online
6 November 2013

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- There is a need for alternative strategies to trough concentration-based tacrolimus monitoring because the relationship between trough concentration and rejection remains controversial.
- Area under the concentration–time curve (AUC)-based monitoring is impractical for clinical practice, especially in the paediatric population. Alternatively, the AUC can be predicted using an optimal sampling strategy.

WHAT THIS STUDY ADDS

- A tacrolimus population pharmacokinetic model in paediatric patients using rich sampling was developed.
- The first Bayesian estimator in paediatric liver transplant recipients was developed to estimate tacrolimus exposure accurately.
- This estimator will allow the conduct of trials aimed at identifying the tacrolimus AUC therapeutic window in this population.

AIMS

The objectives of this study were to develop a population pharmacokinetic (PopPK) model for tacrolimus in paediatric liver transplant patients and determine optimal sampling strategies to estimate tacrolimus exposure accurately.

METHODS

Twelve hour intensive pharmacokinetic profiles from 30 patients (age 0.4–18.4 years) receiving tacrolimus orally were analysed. The PopPK model explored the following covariates: weight, age, sex, type of transplant, age of liver donor, liver function tests, albumin, haematocrit, drug interactions, drug formulation and time post-transplantation. Optimal sampling strategies were developed and validated with jackknife.

RESULTS

A two-compartment model with first-order absorption and elimination and lag time described the data. Weight was included on all pharmacokinetic parameters. Typical apparent clearance and central volume of distribution were 12.1 l h^{-1} and 31.3 l, respectively. The PopPK approach led to the development of optimal sampling strategies, which allowed estimation of tacrolimus pharmacokinetics and area under the concentration–time curve (AUC) on the basis of practical sampling schedules (three or four sampling times within 4 h) with clinically acceptable prediction error limit. The mean bias and precision of the Bayesian *vs.* reference (trapezoidal) AUCs ranged from −2.8 to −1.9% and from 7.4 to 12.5%, respectively.

CONCLUSIONS

The PopPK of tacrolimus and empirical Bayesian estimates represent an accurate and convenient method to predict tacrolimus $AUC_{(0-12)}$ in paediatric liver transplant recipients, despite high between-subject variability in pharmacokinetics and patient demographics. The developed optimal sampling strategies will allow the undertaking of prospective trials to define the tacrolimus AUC-based therapeutic window and dosing guidelines in this population.

Introduction

Tacrolimus (Prograf®; Fujisawa Healthcare Inc., Deerfield, IL, USA) is a first-line immunosuppressive agent widely used in paediatric and adult solid organ transplant recipients. As it has a narrow therapeutic index and significant inter- and intra-individual pharmacokinetic (PK) variability, dosing individualization is essential to ensure graft survival and limit associated toxicities, including life-threatening complications [1].

The PK properties of tacrolimus have mainly been studied in adults. Generally, oral bioavailability is poor and extremely variable, partly due to presystemic metabolism of tacrolimus by gastrointestinal and hepatic cytochrome (CYP) P450 3A isoenzymes and removal by P-glycoprotein transport. In paediatric liver transplant patients, oral bioavailability was found to range from 5 to 77% [2]. Tacrolimus binds extensively to erythrocytes in blood, while in plasma it is principally associated with α_1-acid glycoprotein and albumin. In the liver, tacrolimus is extensively metabolized by CYP3A4 and the polymorphically expressed CYP3A5, with >95% of metabolites eliminated through biliary excretion [3].

In this context, therapeutic drug monitoring has become a standard of care for optimization of tacrolimus dosing. Trough concentration (C_{trough}) is commonly used to guide tacrolimus dose individualization despite its inadequacy in reflecting total drug exposure, as was demonstrated by numerous studies involving solid organ transplant recipients [4–6]. Although the relationship between tacrolimus exposure and patient outcome has not been precisely defined, the last consensus report on twice-daily tacrolimus concluded that there was an urgent need to evaluate alternative strategies to C_{trough}, such as area under the concentration–time curve from 0 to 12 h ($AUC_{(0–12)}$), which is widely considered as the best marker for drug exposure [1]. However, AUC-based monitoring implies the measurement of multiple concentration–time points over the entire dosing interval and is time consuming, expensive and often impractical for routine clinical practice, especially in the paediatric population [7]. Alternatively, AUC can be predicted using an optimal sampling strategy (OSS).

The OSS using maximum *a posteriori* Bayesian estimators (MAP-BE) can predict individual PK parameters using a limited number of concentration–time points, with flexibility in sampling time and consideration of patient characteristics. In addition, the MAP-BE approach can be made simple to use by clinicians with appropriate computer technology [8]. At present, an OSS using MAP-BE has been developed for tacrolimus (twice-daily formulation) in adult kidney [9, 10] and lung transplant recipients [11] but not in paediatrics.

To establish an OSS using MAP-BE, a population PK (PopPK) model is required. Few PopPK studies have been performed in paediatric liver transplant recipients (Table 1) [12–19]. In these studies, factors that may alter tacrolimus PK have been investigated but not always simultaneously. Although numerous covariates were identified, between-subject variability remained important, implicating yet unidentified factors. Furthermore, these models were derived using sparse data, mostly C_{trough}.

Therefore, the aims of this study were as follows: (i) to develop a PopPK model for tacrolimus in paediatric liver transplant patients using rich sampling; and (ii) to develop OSSs using MAP-BE that accurately predict tacrolimus PK parameters and $AUC_{(0–12)}$.

Methods

Patients and study design

This is a retrospective study analysing 12 h PK profiles performed in the paediatric liver transplant population of the Centre Hospitalier Universitaire Sainte-Justine (Montreal, Canada) between July 2006 and May 2011. The study was approved by the Institutional Research Ethics Committee of Centre Hospitalier Universitaire Sainte-Justine (identification code: 3162). The need for subject consent was waived. Thirty-eight intensive PK profiles obtained from 30 patients receiving tacrolimus orally twice daily as a capsule or suspension (0.5 mg ml^{-1}) were analysed. All PK profiles were obtained at least 3 days after receiving the same dose of tacrolimus. Concentrations were obtained as part of patient clinical management. Dosage was adjusted by the liver transplant team to keep tacrolimus C_{trough} within the suggested target range of 5–15 ng ml^{-1} according to time post-transplantation and concomitant immunosuppression. However, measurement of $AUC_{(0–12)}$ was performed at or around patient discharge and was requested in some specific situations, including the following: (i) when nephrotoxicity occurred despite C_{trough} levels within the target range; (ii) when important intra-individual C_{trough} variability occurred in the absence of dose modification; and (iii) at initiation of mycophenolate mofetil.

Medical charts were reviewed and the following data collected: bodyweight, height, age, sex, type of transplant (full or cut-down liver), age of liver donor, underlying diagnosis, liver function tests [alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), albumin and total bilirubin], serum creatinine, haematocrit, time post-transplantation, drug formulation, use of steroids and presence of clinically relevant CYP3A4 inhibitors.

Sample collection and analytical methods

Serial blood samples were collected in EDTA-containing vacutainers immediately before tacrolimus administration (C_{trough}) and after 0.5 ($C_{0.5}$), 1 (C_1), 1.5 ($C_{1.5}$), 2 (C_2), 3 (C_3), 4 (C_4), 8 (C_8) and 12 h (C_{12}). Tacrolimus whole blood concentrations were determined using the microparticle enzyme immunoassay (MEIA) IMx (Abbott Laboratories, Abbott

Table 1

Summary of published population pharmacokinetic models for tacrolimus in paediatric liver transplant recipients

Reference	*n*	Median/mean age and range (years)	Time post-transplantation	Pharmacokinetic parameters	BSV *CL* (%)	BSV *V* (%)
Garcia Sanchez *et al.* [15]*	18	9.1¶ 0.3–16.0	2.9 months¶ 1 day to 6.8 years	$CL = 10.4 \times (WT/70)^{0.75} \times e^{(-0.00032T)} \times e^{(-0.057BILI)} \times (1 - 0.079 \times ALT)$ (l h^{-1}) $F = 20\%$ (fixed)	24.3	NR
Sam *et al.* [14]*	20	3.7** 1.1–13.9	0–7 days	$CL = 1.46 \times (1 + 0.339 \times (AGE - 2.25)$ (l h^{-1}) $V = 39.1 \times [1 + 4.57 \times (BSA - 0.49)]$ (l) BILI <200 μmol l^{-1}: $F = 0.197 \times (1 + 0.0887 \times WT - 11.4)$ (%) BILI ≥200 μmol l^{-1}: $F = 0.197 \times (1 + 0.0887 \times WT - 11.4) \times 1.61$ (%)	33.5	33.0
Yasuhara *et al.* [30]*	33§	4.2** 0.3–15.0	52 days**	$CL = (0.0749 + 0.000457 \times POD) \times [15 \times (WT/15)^{0.290}]$ (l h^{-1}) $V = 2.76 \times [15 \times (WT/15)^{0.290}]$ (l) $F = 19\%$	52.1	27.4
Staatz *et al.* [13]†	35	5.7** 0.5–16.6	NR	$CL/F_{whole\ liver} = 44$ (l h^{-1}) $CL/F_{cut\text{-}down\ liver} = 5.75$ (l h^{-1}) $V/F = 617$ (l)	110–297¶¶	
Guy-Viterbo *et al.* [19]*	42	1.4¶ 0.5–10.9	From the day after transplantation until the patient experienced a rejection episode or, alternatively, until the end of the first year	$CL/F = 0.001 \times [1 + (314 \times TIME)/(17.4 + TIME)] \times [(Size/WT)/median(Size/WT)]^{0.12} \times [Hct/29]^{-0.85}$ (l day^{-1})†† $V_1/F = 253 \times (WT/10.2)^{0.9}$ (l) $V_2/F = 100$ fixed $Q/F = 115$ (l day^{-1})	54.8	77.5
Abdel Jalil *et al.* [18]*	43	5.0** 0.7–17.6	First year post-transplantation	$CL/F = 12.9 \times (WT/13.2)^{0.75} \times e^{(-0.00158 \times POD)} \times e^{(0.428 \times hFLAG)}$‡‡ (l h^{-1})	40	NR
Wallin *et al.* [17]‡	73	3.5¶ 0.4–16.9	First year post-transplantation	$CL/F = 0.148 + (1.37 \times POD^{3.78})/(5.38^{3.78} + POD^{3.78})$ (l h^{-1} kg$^{-0.75}$) $V/F = 27.2$ (l kg^{-1})	NR	90
Fukudo *et al.* [12]*	100	1.2¶ 0.1–15.0	First 50 days post-transplantation	$CL/F = (0.134 \times 1.8^{iFLAG} + 0.0181 \times 2^{hFLAG} \times XPOD) \times 8.6 \times (WT/8.6)^{0.341} \times e^{(-0.0358 \times AST/53)}$ (l h^{-1} kg^{-1})§§ $V/F = 17.1 \times 8.6 \times (WT/8.6)^{0.341}$ (l)	48.7	82.6

Abbreviations are as follows: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BILI, bilirubin concentration; BSA, body surface area; BSV, between-subject variability; *CL*, clearance; *CL/F*, apparent oral clearance; *F*, bioavailability; Hct, haematocrit; HF, hepatic function; *n*, number of patients; NR, not reported; POD, postoperative day; *Q/F*, intercompartmental clearance; *T*, time after initiation of treatment; TIME, time after transplantation; *V*, volume of distribution; *V/F*, apparent volume of distribution; V_1/F, apparent central volume of distribution; V_2/F, apparent peripheral volume of distribution; and WT, bodyweight. *Tacrolimus whole blood concentrations were measured using the microparticle enzyme immunoassay by use of an IMx analyser. †Tacrolimus whole blood concentrations were measured using a validated high-performance liquid chromatography tandem mass–spectrometry assay specific for the parent drug. ‡Tacrolimus whole blood concentrations were measured using either the EMIT 2000 assay (Siemens Healthcare) or a validated high-performance liquid chromatography tandem mass–spectrometry assay specific for the parent drug. §A total of 55 patients were included in this study, among whom 33 contributed to the development of the population pharmacokinetic model. The data regarding age and time post-transplantation pertain to the entire cohort. ¶Median value. **Mean value. ††Equation not completely provided by authors. ‡‡If the patient was a *CYP3A5*1* allele carrier, then hFLAG = 1; otherwise, 0. §§If POD was <21, then XPOD = POD; otherwise, XPOD = 21; if the donor was a *CYP3A5*1* allele carrier, then hFLAG = 1; otherwise, 0; and if the intestinal *MDR1* mRNA level was >0.22 amol (μg total RNA)$^{-1}$, then iFLAG = 1; otherwise, 0. ¶¶As reported by Staatz *et al.* in their article [13].

Park, IL, USA). The lower and upper limits of detection were 1.5 and 30 ng ml^{-1}, respectively. The between-run coefficients of variation were 14.10% at 5 ng ml^{-1}, 11.15% at 11 ng ml^{-1}, and 10.21% at 22 ng ml^{-1}. Three hundred and forty-one blood samples for determination of tacrolimus levels were obtained at steady state.

Population pharmacokinetic analysis

The PopPK analysis was performed using NONMEM version 7, level 1.2 (ICON Development Solutions, Ellicott City, MD, USA), with the first-order conditional estimation (FOCE) and the INTERACTION option. Nonlinear mixed-effects models were used to fit the concentration–time data of tacrolimus described by typical PK compartmental models (e.g. one- or two-compartmental models, first-order absorption, with linear or nonlinear elimination). For a two-compartment model, PK parameters were apparent oral clearance (CL/F), apparent volume of distribution (V_1/F), intercompartmental clearance (Q_2/F), apparent peripheral volume of distribution (V_2/F) and first-order absorption rate constant (k_a). Between-subject variability (BSV) and between-occasion variability in PK parameters were modelled as exponential random-effect models in order to constrain the individual parameter values positively, which were thus assumed to follow a log-normal distribution. Covariance between parameters was also examined. Additive, proportional and combined error structures were tested during modelling of residual random error. Model evaluation and selection were based on pertinent graphical representations of goodness of fit and on the minimization of $-2\times$Log (Likelihood), which was presented as the objective function value (OFV). A decrease in the OFV of 3.84 (P = 0.05) was considered significant to include a parameter in the model. Allometric scaling was applied to the base model [20, 21], that is:

$$CL/F=\theta_1\times(\mathrm{WT}/\mathrm{WT}_{\mathrm{median}})^{0.75},\ Q_2/F=\theta_3\times(\mathrm{WT}/\mathrm{WT}_{\mathrm{median}})^{0.75},$$

$$V_1/F=\theta_2\times(\mathrm{WT}/\mathrm{WT}_{\mathrm{median}})^{1},\ V_2/F=\theta_4\times(\mathrm{WT}/\mathrm{WT}_{\mathrm{median}})^{1}\ \text{and}$$

$$k_a=\theta_3\times(\mathrm{WT}/\mathrm{WT}_{\mathrm{median}})^{-0.25}$$

where θ is the typical value of the parameter in a child with median weight (WT) of the study population.

Covariate analysis and sources of variability

Covariate analysis was carried out using visual inspection followed by a formal evaluation in NONMEM. The latter consisted of a stepwise forward additive approach (P = 0.05) followed by backward elimination (P = 0.01). Potential covariates that were evaluated were as follows: age, sex, type of transplant (full or cut-down liver), age of liver donor, time post-transplantation, liver function tests (ALT, AST, GGT and total bilirubin), albumin, renal function (serum creatinine and creatinine clearance), haematocrit, use of steroids, presence of clinically relevant CYP3A4 inhibitors and drug formulation. Some of the continuous covariates were also tested as categorical variables (Table 2).

Table 2

Patient characteristics at baseline: categorical covariates

Characteristics	*n* (%)
Categorical variables	
Sex	
Female	13 (43.3)
Male	17 (56.7)
Type of transplant	
Cut-down liver	20 (66.7)
Full liver	10 (33.3)
CYP3A4 inhibitors*	
Yes	8 (26.7)
No	22 (73.3)
Steroids†	
Yes	20 (66.7)
No	10 (33.3)
Hepatic impairment‡	
Yes	5 (16.7)
No	25 (83.3)
Drug formulation	
Capsule	15 (50)
Solution	15 (50)
Transformation of continuous into discrete covariates (*n* at baseline)	
Time post-transplantation	
≤28 days	8
>28 days	22
ALB (g l^{-1})§	
>32	23
≤32	7
ALT (U l^{-1})§	
<45	15
≥45	15
Haematocrit (%)§	
<33	7
≥33	23

Abbreviations are as follows: ALB, albumin; ALT, alanine aminotransferase; and *n*, number of patients. *CYP3A4 inhibitors included amlodipine, lansoprazole and diltiazem. †Prednisone was the steroid used in this study. ‡Hepatic impairment was defined as total bilirubin >68.4 mmol l^{-1} and/or ALT >2 times the upper limit for the age group (2 × 45 = 90 U l^{-1}). §The cut-off ranges for these transformations were those previously published [12].

Model evaluation

The performance of the final PopPK model was evaluated with diagnostic plots and shrinkage of PopPK parameters. In order to assess whether the final model could be used to estimate individual PK parameters based on population means and sparse PK data, changes in the estimates of BSV, residual variability and shrinkage in individual random effects were evaluated [22]. Shrinkage values of

≤20% indicate good individual estimates of a parameter of interest, while larger shrinkage values show that individual Bayesian estimates 'shrunk' towards the population mean values.

In addition, the stability and precision of the model were evaluated using a nonparametric bootstrap within Perl-Speaks-NONMEM (PsN V3.4.2) [23]. The bootstrap technique involves resampling from the original data, with each individual subject considered as a sampling unit. One thousand replicates of the data were generated by bootstrap to obtain the median and 95% percentile of PK parameters and the fixed- and random-effect parameters. The bias of each parameter was calculated by computing the difference between the median value derived from the bootstrap and the final parameter estimate.

Building and validation of optimal sampling strategy using Bayesian estimator

Using the final model, the initial time points for the OSS were obtained with the WinPOPT® (version 1.2; University of Otago, Dunedin, New Zealand) software. Limited sampling strategies among combinations of a maximum of four sampling time points, including C_{trough}, and up to 4 h post-drug administration were tested. The selection of the best strategy used the determinant of the Fisher information matrix as a measure of the informativeness of the design. In addition, the best three combinations of sampling time points previously identified (C_{trough}–C_1–C_4, C_{trough}–$C_{0.5}$–C_2–C_4 and C_{trough}–C_1–C_2–C_4) using multiple regression analysis (MRA) in a subset of the real data were also selected for further evaluation [24].

Evaluation and validation of the OSS identified by WinPOPT® as well as the three MRA-derived combinations of sampling times were performed using a jackknife technique in NONMEM. This approach consists of building n reference populations of $n-1$ patients by sequentially excluding one patient at a time from the analysis. For each reference population, the four different MAP-BE sampling time points were used to predict the CL/F and the resulting $AUC_{(0-12)}$ of the excluded patient. The predictive performance was evaluated by calculating bias [relative mean prediction error, ME(%)] and precision [relative root mean squared prediction error, RMSE(%)] using the following equations [25]:

$$ME(\%) = 100 \times \frac{1}{n}\left(\sum \frac{Pred - Obs}{Obs}\right)$$

$$RMSE(\%) = 100 \times \left(\sqrt{\frac{1}{n}\sum\left(\frac{Pred - Obs}{Obs}\right)^2}\right)$$

where Pred is the Bayesian $AUC_{(0-12)}$ estimate, Obs is the reference $AUC_{(0-12)}$ value obtained using the linear trapezoidal method applied to the 12 h intensive PK profiles, and n is the number of patients.

Results

Thirty-eight 12 h intensive PK profiles obtained from 30 liver transplant recipients were available for this study. Patients' characteristics are summarized in Tables 2 and 3. Patients had a wide variety of diagnoses leading to whole or cut-down liver transplantation, including biliary atresia ($n = 12$), tyrosinaemia ($n = 8$), North American Indian childhood cirrhosis ($n = 3$), fulminant hepatitis ($n = 2$), Alagille syndrome ($n = 2$), histiocytosis X ($n = 1$), sclerosing cholangitis ($n = 1$) and autoimmune hepatitis ($n = 1$). The age of liver donor ranged from 0.58 to 66 years.

A total of 341 blood samples were available for the population PK analysis. A median of nine concentration–time points were obtained for each patient (range, 8–10). Observed concentration–time profiles of tacrolimus are plotted in Figure 1.

Population pharmacokinetics

Among the structural models tested, a two-compartment model with first-order absorption and first-order elimination and lag time adequately fitted the concentration data of oral tacrolimus. More complex absorption models were not supported by the data (transit absorption models). Between-subject variability was included on CL/F, V_1/F and Q_2/F. A proportional error model was used for residual unexplained variability. Between-occasion variability was not included in the model.

Analysis of covariates and sources of variability

Visual inspection of the relationships between individual random effects of CL/F and V_1/F and covariates showed

Table 3

Patient characteristics at baseline: continuous covariates

Continuous variable	Median (range)
Age (years)*	7.3 (0.4–18.4)
Weight (kg)	20.4 (4.5–57.8)
BSA (m^2)	0.8 (0.2–1.6)
ALB (g l^{-1})	36.0 (26.0–43.0)
AST (U l^{-1})	33.0 (16.0–68.0)
ALT (U l^{-1})	44.5 (14.0–140.0)
GGT (U l^{-1})	54.5 (8–432)
Total bilirubin (μmol l^{-1})	11.0 (4.0–25.0)
Haematocrit (%)	34.5 (25.0–44.1)
Serum creatinine (mg dl^{-1})	0.4 (0.1–0.9)
Creatinine clearance (ml min^{-1})†	155.6 (58.4–360.3)
Time post-transplantation (months)	2.5 (0.5–188.2)

Abbreviations are as follows: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSA, body surface area; and GGT, γ-glutamyl transferase. *Eight patients were <2 years old, of whom four were <1 year old. †Creatinine clearance was calculated based on the Schwartz equation for patients ≤18 years and Cockcroft–Gault for patients >18 years old.

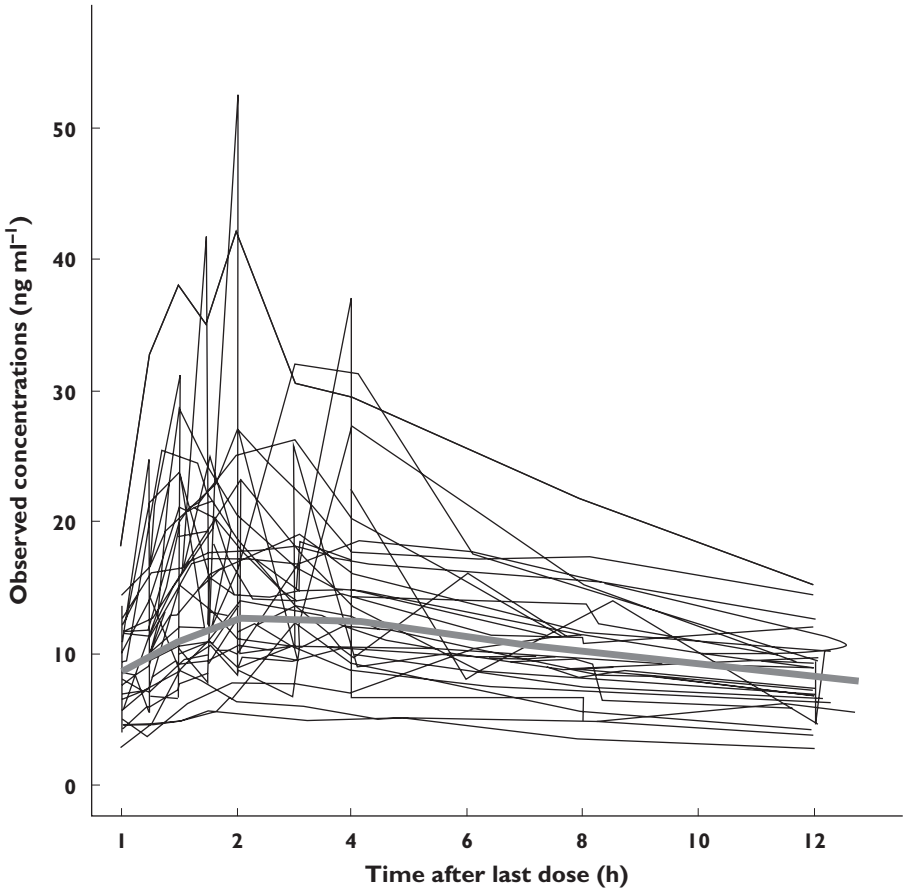

Figure 1

Whole blood tacrolimus concentrations *vs.* time after last dose in paediatric liver transplant recipients. ▬, LOESS is a locally weighted scatterplot smoothing line

trends for liver function test (ALT) and concomitant administration of CYP3A4 inhibitors. A thorough covariate analysis was performed within NONMEM, and none of the tested covariates explained the variability of PK parameters of tacrolimus in paediatric liver transplant recipients.

The final PopPK model was described by the following equations:

$$CL/F\ (\mathrm{l\,h^{-1}}) = 12.1 \times (\mathrm{WT}/20)^{0.75}$$

$$V_1/F\ (\mathrm{l}) = 31.3 \times (\mathrm{WT}/20)^{1}$$

$$Q_2/F\ (\mathrm{l\,h^{-1}}) = 30.7 \times (\mathrm{WT}/20)^{0.75}$$

$$V_2/F\ (\mathrm{l}) = 290 \times (\mathrm{WT}/20)^{1}$$

$$k_a\ (1\,\mathrm{h^{-1}}) = 0.342 \times (\mathrm{WT}/20)^{-0.25}$$

$$t_{lag}\ (\mathrm{h}) = 0.433$$

where CL/F is apparent oral clearance, V_1/F is apparent volume of distribution, Q_2/F is intercompartmental clearance, V_2/F is apparent peripheral volume of distribution, k_a is first-order absorption rate constant, t_{lag} is lag time, and WT is bodyweight.

Population parameter estimates, along with between-subject and residual variabilities, are reported in Table 4. Typical population CL/F and V_1/F values for a child weighing 20 kg were 12.1 l h^{-1} and 31.3 l, respectively.

Model evaluation

The performance of the final population PK model was evaluated with diagnostic plots and shrinkage of population PK parameters. The overall fit of observed and predicted tacrolimus concentrations based on population and

Table 4

Parameter estimates for the final model with bootstrap validation

Parameter	Parameter estimates (RSE%)*	Median	Bootstrap† 95% Confidence interval	Bias (%)
Pharmacokinetic parameter‡				
CL/F (l h^{-1})	12.1 (10.1)	12.08	10.1, 14.9	–0.17
V_1/F (l)	31.3 (42.8)	30.87	12.3, 70.3	–1.37
Q_2/F (l h^{-1})	30.7 (29.3)	28.9	13.1, 53.1	–5.86
V_2/F (l)§	290 fixed			
k_a (1 h^{-1})	0.342 (33.3)	0.342	0.142, 0.656	0.00
t_{lag} (h)	0.433 (4.2)	0.4325	0.383, 0.456	–0.12
Between-subject variability				
BSV *CL/F* (%)	55.6 (9.6)	54.39	43.82, 65.12	–4.27
BSV V_1/F (%)	126.1 (18)	120.50	69.50, 165.23	–8.68
BSV Q_2/F (%)	84.0 (21.3)	81.0	44.61, 141.77	–7.07
Residual variability				
Residual proportional error (%)	20.3 (12.1)	20.21	15.3, 24.9	–0.44

Abbreviations are as follows: BSV, between-subject variability; *CL/F*, apparent oral clearance; *F*, bioavailability; k_a, absorption rate; Q_2/F, intercompartmental clearance between the central compartment and the peripheral compartment; t_{lag}, lag time; V_1/F, apparent central volume of distribution; and V_2/F, apparent peripheral volume of distribution. *Relative standard error calculated as the standard error of parameter estimate/parameter estimate × 100. †Median of 939 successful bootstrap samples from the 1000 runs with prediction intervals (confidence intervals) calculated as the 2.5th and 97.5th percentiles. ‡Bodyweight was included in all pharmacokinetic parameters as an allometric fixed term. §V_2/F was fixed to a value estimated from a previous run in order to stabilize the model.

individual parameter estimates are presented in Figure 2A and B, respectively. Shrinkages to the mean of the individual random effects of *CL/F* and V_1/F were low (<12%). Conditional weighted residuals were homogeneously distributed around 0 (Figure 2C and D), suggesting no bias in the prediction of tacrolimus concentrations. Bootstrap resampling strategies were used to validate the PopPK model. A total of 939 runs were successfully minimized with a covariance step. Medians of PK parameters derived with the bootstrap resampling analysis were consistent with those derived from the original analysis, with bias <9% (Table 4), demonstrating accuracy of predictions as well as model stability.

Building and validation of a Bayesian estimator

The PopPK parameters obtained from the final model were used as priors for the development of the MAP-BE. The OSS schedule, selected using the Fisher information matrix, was 0, 0.5 and 2 h postdose with relative standard errors of 5.8 and 22.6% on *CL/F* and V_1/F, respectively. The $AUC_{(0-12)}$ bias and precision obtained during the validation procedure were –2.57% and 12.49%, respectively (Table 5). Typical fits for the estimation of reference parameters (considering all concentrations) and Bayesian estimation (with sampling times at 0, 0.5 and 2 h postdose) are illustrated in Figure 3. Moreover, the predictive performance of the MAP-BEs using MRA-derived combinations of sampling times are reported in Table 5. Their relative bias and precision were below ±10%. Estimation of AUC with these sampling times seemed to be more accurate based on RMSE than the schedule identified with WinPOPT®; however, the MRA-derived sampling times targeted only *CL/F* prediction, while those identified by WinPOPT® optimized for all PK parameters.

For comparison, the predictive performance of limited sampling strategies developed with MRA by Delaloye *et al.* [24] are also presented in Table 5. The precision of both MAP-BE and MRA for the same sampling times (C_{trough}–C_1–C_4, C_{trough}–$C_{0.5}$–C_2–C_4 and C_{trough}–C_1–C_2–C_4) appeared to be similar. Although bias with both approaches was low, MRA had a tendency to underestimate $AUC_{(0-12)}$.

Discussion

To the best of our knowledge, this study is the first maximum *a posteriori* Bayesian estimator for the prediction of tacrolimus exposure in paediatric liver transplant recipients.

A two-compartment model with first-order absorption and elimination best fitted the concentration–time profiles of tacrolimus at steady state in the study population. This is in contrast to other PopPK studies in paediatric liver transplant recipients, where tacrolimus PK followed a one-compartment model. The use of trough concentrations in those studies precluded the development of multicompartment models, whereas in the present analysis, full concentration–time profiles allowed a complete characterization of tacrolimus PK behaviour [12–16, 18]. Such intensive PK sampling for tacrolimus PopPK model development is a first in paediatrics. After inclusion of weight in the base model, none of the tested covariates

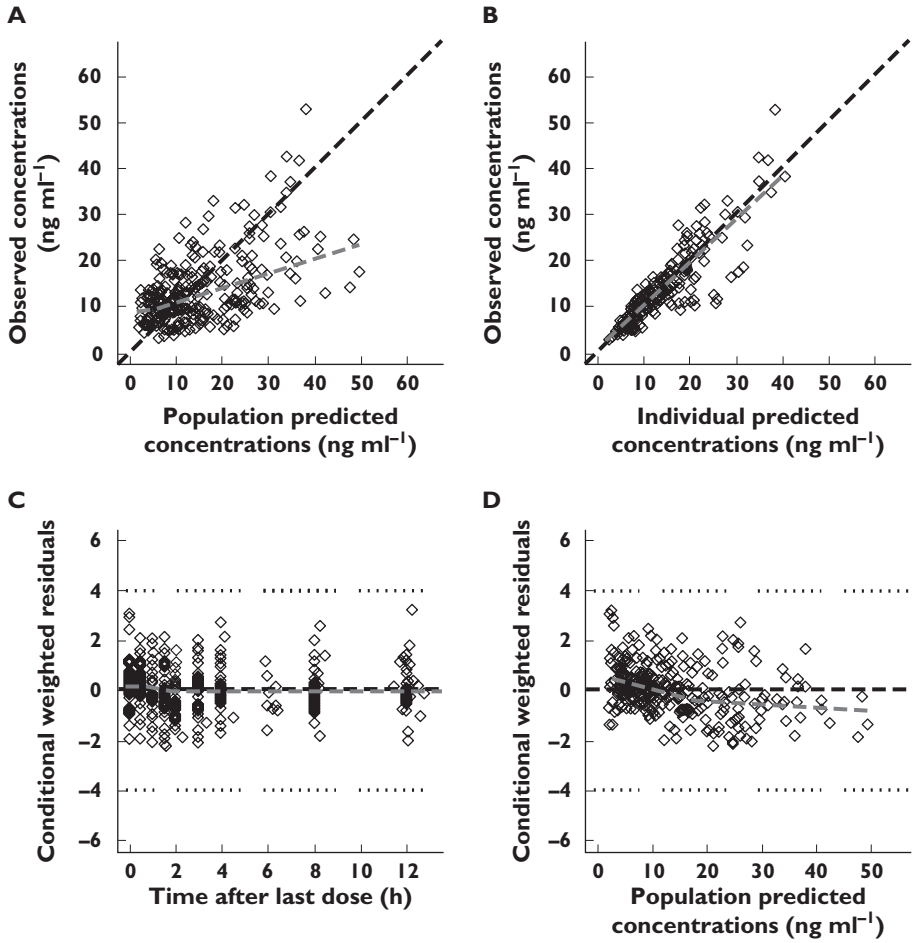

Figure 2

(A) Observed individual concentrations of tacrolimus *vs*. population predicted concentrations in the final model; – – –, line of identity; – – –, LOESS predicted. (B) Observed individual concentrations of tacrolimus *vs*. individual predicted concentrations in the final model; – – –, line of identity; – – –, LOESS predicted. (C) Conditional weighted residuals *vs*. time after last dose; – – –, zero; – – –, LOESS. (D) Conditional weighted residuals *vs*. population predicted concentrations; – – –, zero; – – –, LOESS. LOESS is a locally weighted scatterplot smoothing line

Table 5

Comparison of the predictive performance between maximum *a posteriori* Bayesian estimators and multiple regression analysis

	Maximum *a posteriori* Bayesian estimators*		Multiple regression analysis†	
Optimal sampling times (h)	ME% (95% CI)	RMSE% (95% CI)	ME% (95% CI)	RMSE% (95% CI)
C_{trough}–$C_{0.5}$–C_2	–2.57 (–30.94, 17.43)	12.49 (0.71,31.16)	—	—
C_{trough}–C_1–C_4	–1.93 (–14.90, 15.34)	8.44 (0.60,17.59)	–4.98 (–8.37, –1.59)	8.29 (3.29, 11.28)
C_{trough}–$C_{0.5}$–C_2–C_4	–1.93 (–14.66, 10.94)	7.37 (1.02,15.85)	–6.15 (–9.14, –3.16)	8.48 (4.25, 11.22)
C_{trough}–C_1–C_2–C_4	–2.78 (–14.38, 10.91)	7.42 (0.14,15.04)	–5.07 (–8.34, –1.81)	8.15 (3.22, 11.07)

Abbreviations are as follows: CI, confidence interval; ME%, relative mean prediction error; and RMSE%, relative root mean squared prediction error. *Validation in the present study was carried out by the jackknife technique. †Validation in the Delaloye *et al*. study was carried out with an independent set of patients (validation group) [24].

significantly described the variability in PK parameters. This model allowed a precise estimation of tacrolimus clearance with a relative standard error of 10%. The PopPK approach led to the development of OSSs, which allowed estimation of tacrolimus PK and AUC on the basis of practical sampling schedules (three or four sampling times within 4 h); these OSSs have a ±20% prediction error limit, which is considered clinically acceptable [26].

Growth and development, which are usually described by demographic factors such as size and age, contribute

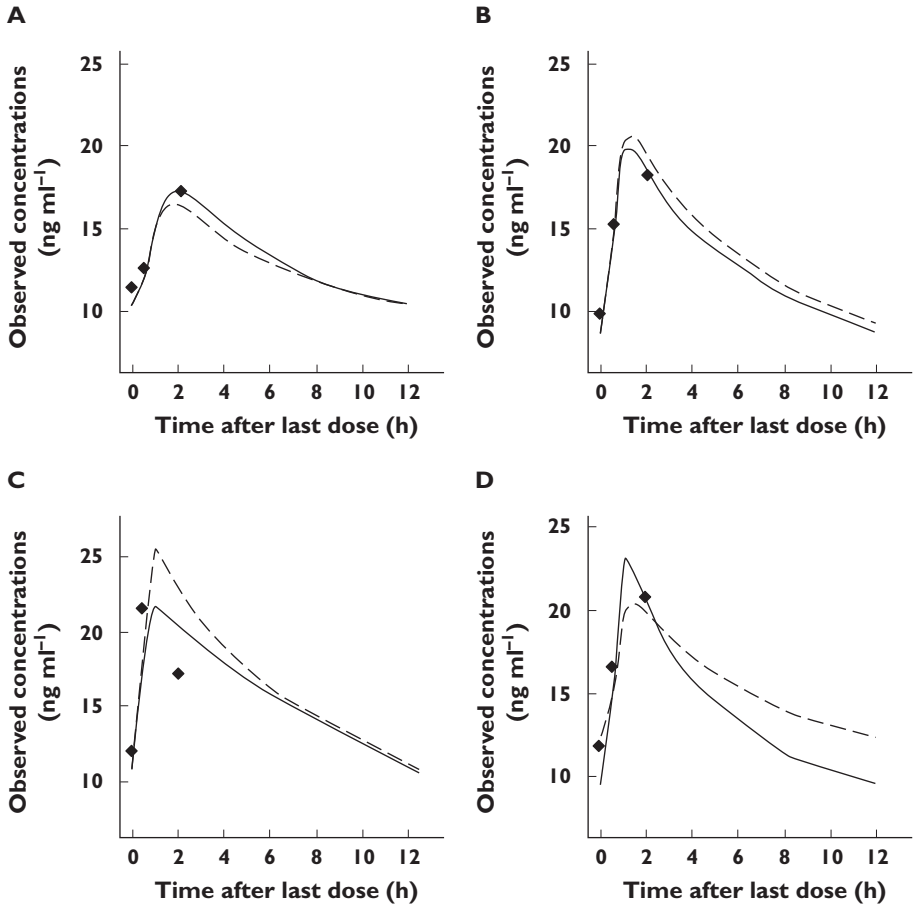

Figure 3

Simulation of tacrolimus concentrations for typical subjects with low bias (A, B) or high bias (C, D). Filled circles represent observed concentrations, continuous lines represent prediction using all available concentrations, and dashed lines represent prediction with three sampling times (C_{trough}–$C_{0.5}$–C_2) and maximum *a posteriori* Bayesian estimators

most of the variance to drug PK through the paediatric age span. In the present study, bodyweight was included on all PK parameters as an allometric fixed term to account for the effect of growth [21, 27]. The impact of maturation was more difficult to assess as tacrolimus clearance in paediatric liver transplant recipients is the result of a complex interplay between the recipient's and the donor's characteristics. These characteristics include the following: (i) the recipient's intestinal metabolism and transporter activity, which are influenced at least by maturation and the recipent's *CYP3A5* and possibly by *ABCB1* gene polymorphisms; (ii) the donor's hepatic metabolism, which depends at least on the donor's age and *CYP3A5* genotype; and (iii) the ratio of the graft size to the recipient's bodyweight [19] and to the recipient's standard liver volume [28, 29]. Also, the influence of the recipient's age on the metabolic capacity of the transplanted liver is currently unknown. Finally, considering that only four patients <1 year old were included, it was not possible to study the developmental effects using the sigmoidal E_{max} model (e.g. Hill equation) with postmenstrual age, which allows a description of the gradual maturation of clearance during the first year of life. Indeed, for all of the above reasons, the impact of hepatic and intestinal enzyme maturation on tacrolimus PK could not be evaluated appropriately. This complexity is also reflected by the lack of proper evaluation of the impact of development in all previously published paediatric liver transplant PopPK studies (Table 1).

Given that tacrolimus is mainly metabolized by CYP3A isoenzymes, hepatic dysfunction can significantly decrease its elimination; some authors have reported that liver function tests influence tacrolimus clearance [12, 14, 15]. However, the present final model does not include these covariates, which may be due to the relatively low incidence of hepatic impairment (17%) as well as the absence of severe hepatic dysfunction among the study population.

The time post-transplantation has been shown to affect tacrolimus clearance. Some authors found that

clearance increased within the immediate postoperative period, possibly due to recovery of metabolic function in the graft, induction of metabolic activity by concomitant steroid therapy and changes in haematocrit and plasma protein levels [12, 17, 30]; on the contrary, studies with longer follow-up periods have reported decreased clearance with time post-transplantation [15, 18], potentially associated with steroid weaning. In the present study, time post-transplantation had no effect; it is of interest to note that no included patients were studied during the first 2 weeks after transplantation and that the median time post-transplantation was 2.5 months.

The donor's age and transplant type were also evaluated, as they may influence tacrolimus PK in paediatric liver transplant recipients. In the present study, both covariates were found not to be significant. On the contrary, Staatz *et al*. [13] found that children who received cut-down liver from an adult exhibited an average 7-fold lower tacrolimus clearance compared with those who received a whole liver from a child donor. The authors postulated that the transplanted organ retains the metabolic characteristics of the donor and that an adult donor liver has lower drug clearance than a child donor liver. If the age of the donor had been included in the model by Staatz *et al*., the type of transplant may have not been identified as a significant covariate.

The concomitant use of steroids has the potential to increase elimination of tacrolimus by inducing CYP3A4 [31]. In this sense, the results reported in the literature are contradictory. While some authors found that steroids may induce CYP3A4, other studies have suggested decreased tacrolimus metabolism [32]. The effect of concomitant administration of steroids could not be demonstrated in the present study, which is in agreement with findings from other paediatric studies [15, 33]. Haematocrit and albumin concentrations were also tested as potential covariates because tacrolimus accumulates in erythrocytes and is highly bound to plasma proteins. As such, low haematocrit and albumin concentrations are expected to result in a reduction in total drug concentration in whole blood and an increase in total clearance. Similar to Garcia Sanchez *et al*. [15], haematocrit and albumin were not found to be influential covariates. This is in opposition to results found by Zhao *et al*., where clearance of tacrolimus was significantly higher in patients with low levels of haematocrit (<33%) [33]. Albumin was not tested in the latter study.

The estimated PK parameter values using the final model are close to those previously reported in paediatric liver transplant recipients [12, 13, 15, 16]. A typical paediatric patient (WT = 20 kg) would have CL/F and total volume of distribution (V_d/F), which is the sum of V_1/F and V_2/F, of 12.1 l h^{-1} and 321.3 l, respectively. These values are similar to those previously reported for CL/F (4.6–13.1 l h^{-1}) [12, 15, 18, 19] and V_d/F (196.1–617 l) [12, 13, 16, 19].

Between-subject variabilities were 55.6 and 126.1% for CL/F and V_1/F, respectively, and were in the range of previously reported variabilities for CL (24.3–110%) and V_1/F (27.4–297%) [12–18]. Several reasons could potentially explain the remaining BSV. It is now well recognized that *CYP3A5* genetic polymorphism is influential on tacrolimus PK [34]. Indeed, *CYP3A5* expression was identified as a significant covariate on tacrolimus apparent clearance in both paediatric kidney and liver transplant recipients. Kidney transplant patients expressing *CYP3A5* [with a least one functioning allele (*CYP3A5*1* allele)] and liver transplant recipients with a *CYP3A5*1*-carrying graft liver were found to have a significantly higher apparent clearance than *CYP3A5* non-expresser kidney recipients and liver recipients with a non-*CYP3A5*1*-carrying graft, respectively [12, 33]. More recently, the *CYP3A5* genotype of paediatric liver recipients was also identified as a significant covariate for apparent clearance of tacrolimus [18]. Unfortunately, the impact of this genetic variation could not be tested in the present study because *CYP3A5* genotypes of neither donors nor recipients were available. To date, no paediatric study has simultaneously evaluated the impact of recipient and donor *CYP3A5* expression on tacrolimus metabolism in liver transplant recipients.

Another explanation for the remaining between-subject variability in CL/F is the extremely variable tacrolimus bioavailability (3–77%) [2]. Furthermore, half of the patients received the drug as an extemporaneously compounded oral suspension prepared by CHU Ste-Justine hospital [35] or community drugstores (possibly using a different reconditioning method), for which bioavailability is unknown.

As recommended by the US Food and Drug Administration [36], the stability and precision of the PopPK model were evaluated using a nonparametric bootstrap. The results of this analysis demonstrated the model stability (93.9% successful convergence) as well as the accuracy of the estimated parameters (bias <9%).

In a final step, a maximum *a posteriori* Bayesian estimator was developed and validated using the jackknife technique for the prediction of tacrolimus exposure in paediatric liver transplant recipients. Optimal sampling strategies involved a limited number of blood samples within a short period of time (4 h) after drug administration, allowing for transferability to routine patient care. Given that C_{trough} checks for adherence, helps to identify patients with high tacrolimus clearance and is a commonly used marker by clinicians, it was included in all tested schedules [26, 37]. Using the design with three sampling times (0, 0.5 and 2 h) proposed by WinPOPT®, $AUC_{(0-12)}$ bias and precision obtained during the validation procedure were below 15% with respect to the reference $AUC_{(0-12)}$ values. However, the 95% confidence intervals for bias and precision exceeded the acceptable 20%, limit and this is not surprising given that the method of D-optimal design minimizes total variance for all parameter estimates.

As the best marker for drug exposure is AUC, which is related to clearance, different OSSs were evaluated to optimize the Bayesian estimation of individual *CL/F*. In this context, the sampling combinations previously reported based on MRA (C_{trough}–C_1–C_4, C_{trough}–$C_{0.5}$–C_2–C_4 and C_{trough}–C_1–C_2–C_4) for the estimation of tacrolimus AUC in paediatric liver transplant recipients were tested with MAP-BE [24]. The limited sampling strategy using MAP-BE based on predose, 1 and 4 h postdose estimated tacrolimus AUC with ±20% prediction error limit. The mean bias and precision (95% confidence interval) of this design were –1.93% (–14.90 to 15.34%) and 8.44% (0.60 to 17.59%), respectively, which is clinically acceptable predictive performance. Inclusion of a fourth sample resulted in further improvement of both bias and precision but impairs practical application.

The comparison of the predictive performance of both MAP-BE and MRA for the same sampling times shows that both approaches can accurately estimate tacrolimus exposure in paediatric liver transplant recipients. However, even though precision was similar, MRA had a tendency to underestimate $AUC_{(0–12)}$, with a 95% confidence interval for bias excluding zero.

Although MRA results in a simple equation, it can only be used for the estimation of a single PK parameter with an almost identical dosing schedule and requires precise sampling times. In contrast, MAP-BE allows the description of the PK profile and the estimation of PK parameters, considering the patient's characteristics, with flexibility in the timing and number blood samples, making this method compatible with real-life situations [38]. Moreover, Bayesian predictions can improve as more patient-specific data are added to the population model.

The optimal sampling strategies developed in this study were based on data from paediatric liver transplant recipients and, as such, can be applied only to the same population. Transferability of these models to other transplant types (kidney, heart etc.) could be envisioned following proper validation in these groups. Furthermore, these models should be applied with caution in the following situations: (i) in young infants (<1 year old), because there were only four patients in this age group among the study population; and (ii) in the early post-transplantation period, because no included patients were studied during the first 2 weeks after transplantation.

In conclusion, PopPK of tacrolimus and empirical Bayesian estimates represent an accurate and convenient method to predict tacrolimus $AUC_{(0–12)}$ in paediatric liver transplant recipients, despite high between-subject variability in PK and patient demographics. The developed OSS will allow the undertaking of prospective trials to define the tacrolimus AUC-based therapeutic window and dosing guidelines in this population and to evaluate the efficacy of alternative strategies to C_{trough}-based monitoring in order to prevent graft rejection while minimizing toxicity events.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

L. Labbé was the recipient of an Rx&D/CIHR Health Research Foundation Career Award in health sciences. We thank Carole Viau for her technical assistance. We also thank the nursing staff of the Multispeciality Clinic and medical technologists at CHU Ste-Justine for their help with sample collection and analysis, respectively.

REFERENCES

1 Wallemacq P, Armstrong VW, Brunet M, Haufroid V, Holt DW, Johnston A, Kuypers D, Le Meur Y, Marquet P, Oellerich M, Thervet E, Toenshoff B, Undre N, Weber LT, Westley IS, Mourad M. Opportunities to optimize tacrolimus therapy in solid organ transplantation: report of the European consensus conference. Ther Drug Monit 2009; 31: 139–52.

2 Wallemacq PE, Furlan V, Moller A, Schafer A, Stadler P, Firdaous I, Taburet AM, Reding R, Clement De Clety S, De Ville De Goyet J, Sokal E, Lykavieris L, Van Leeuw V, Bernard O, Otte JB, Undre NA. Pharmacokinetics of tacrolimus (FK506) in paediatric liver transplant recipients. Eur J Drug Metab Pharmacokinet 1998; 23: 367–70.

3 Wallemacq PE, Verbeeck RK. Comparative clinical pharmacokinetics of tacrolimus in paediatric and adult patients. Clin Pharmacokinet 2001; 40: 283–95.

4 Claeys T, Van Dyck M, Van Damme-Lombaerts R. Pharmacokinetics of tacrolimus in stable paediatric renal transplant recipients. Pediatr Nephrol 2010; 25: 335–42.

5 Filler G, Feber J, Lepage N, Weiler G, Mai I. Universal approach to pharmacokinetic monitoring of immunosuppressive agents in children. Pediatr Transplant 2002; 6: 411–8.

6 Montini G, Ujka F, Varagnolo C, Ghio L, Ginevri F, Murer L, Thafam BS, Carasi C, Zacchello G, Plebani M. The pharmacokinetics and immunosuppressive response of tacrolimus in paediatric renal transplant recipients. Pediatr Nephrol. 2006; 21: 719–24.

7 Ting LS, Villeneuve E, Ensom MH. Beyond cyclosporine: a systematic review of limited sampling strategies for other immunosuppressants. Ther Drug Monit 2006; 28: 419–30.

8 Barrett JS, Mondick JT, Narayan M, Vijayakumar K, Vijayakumar S. Integration of modeling and simulation into hospital-based decision support systems guiding pediatric pharmacotherapy. BMC Med Inform Decis Mak 2008; 8: 6.

9 Benkali K, Premaud A, Picard N, Rerolle JP, Toupance O, Hoizey G, Turcant A, Villemain F, Le Meur Y, Marquet P, Rousseau A. Tacrolimus population pharmacokinetic-pharmacogenetic analysis and Bayesian estimation in renal transplant recipients. Clin Pharmacokinet 2009; 48: 805–16.

10 Barraclough KA, Isbel NM, Kirkpatrick CM, Lee KJ, Taylor PJ, Johnson DW, Campbell SB, Leary DR, Staatz CE. Evaluation of limited sampling methods for estimation of tacrolimus exposure in adult kidney transplant recipients. Br J Clin Pharmacol 2011; 71: 207–23.

11 Monchaud C, de Winter BC, Knoop C, Estenne M, Reynaud-Gaubert M, Pison C, Stern M, Kessler R, Guillemain R, Marquet P, Rousseau A. Population pharmacokinetic modelling and design of a Bayesian estimator for therapeutic drug monitoring of tacrolimus in lung transplantation. Clin Pharmacokinet 2012; 51: 175–86.

12 Fukudo M, Yano I, Masuda S, Goto M, Uesugi M, Katsura T, Ogura Y, Oike F, Takada Y, Egawa H, Uemoto S, Inui K. Population pharmacokinetic and pharmacogenomic analysis of tacrolimus in pediatric living-donor liver transplant recipients. Clin Pharmacol Ther 2006; 80: 331–45.

13 Staatz CE, Taylor PJ, Lynch SV, Willis C, Charles BG, Tett SE. Population pharmacokinetics of tacrolimus in children who receive cut-down or full liver transplants. Transplantation 2001; 72: 1056–61.

14 Sam WJ, Aw M, Quak SH, Lim SM, Charles BG, Chan SY, Ho PC. Population pharmacokinetics of tacrolimus in Asian paediatric liver transplant patients. Br J Clin Pharmacol 2000; 50: 531–41.

15 Garcia Sanchez MJ, Manzanares C, Santos-Buelga D, Blazquez A, Manzanares J, Urruzuno P, Medina E. Covariate effects on the apparent clearance of tacrolimus in paediatric liver transplant patients undergoing conversion therapy. Clin Pharmacokinet 2001; 40: 63–71.

16 Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V, McMichael J, Lever J, Burckart G, Starzl T. Clinical pharmacokinetics of tacrolimus. Clin Pharmacokinet 1995; 29: 404–30.

17 Wallin JE, Bergstrand M, Wilczek HE, Nydert PS, Karlsson MO, Staatz CE. Population pharmacokinetics of tacrolimus in pediatric liver transplantation: early posttransplantation clearance. Ther Drug Monit 2011; 33: 663–72.

18 Abdel Jalil MH, Hawwa AF, McKiernan PJ, Shields MD, McElnay JC. Population pharmacokinetic and pharmacogenetic analysis of tacrolimus in paediatric liver transplant patients. Br J Clin Pharmacol 2014; 77: 130–40.

19 Guy-Viterbo V, Scohy A, Verbeeck RK, Reding R, Wallemacq P, Musuamba FT. Population pharmacokinetic analysis of tacrolimus in the first year after pediatric liver transplantation. Eur J Clin Pharmacol 2013; 69: 1533–42.

20 Anderson BJ, Allegaert K, Holford NH. Population clinical pharmacology of children: general principles. Eur J Pediatr 2006; 165: 741–6.

21 Anderson BJ, Allegaert K, Holford NH. Population clinical pharmacology of children: modelling covariate effects. Eur J Pediatr 2006; 165: 819–29.

22 Karlsson MO, Savic RM. Diagnosing model diagnostics. Clin Pharmacol Ther 2007; 82: 17–20.

23 Henderson AR. The bootstrap: a technique for data-driven statistics. Using computer-intensive analyses to explore experimental data. Clin Chim Acta 2005; 359: 1–26.

24 Delaloye JR, Kassir N, Lapeyraque AL, Alvarez F, Lallier M, Beaunoyer M, Labbe L, Theoret Y, Litalien C. Limited sampling strategies for monitoring tacrolimus in pediatric liver transplant recipients. Ther Drug Monit 2011; 33: 380–6.

25 Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. J Pharmacokinet Biopharm 1981; 9: 503–12.

26 David OJ, Johnston A. Limited sampling strategies for estimating cyclosporin area under the concentration-time curve: review of current algorithms. Ther Drug Monit 2001; 23: 100–14.

27 Holford NH. A size standard for pharmacokinetics. Clin Pharmacokinet 1996; 30: 329–32.

28 Harihara Y, Sano K, Makuuchi M, Kawarasaki H, Takayama T, Kubota K, Ito M, Mizuta K, Yoshino H, Hirata M, Kita Y, Hisatomi S, Kusaka K, Miura Y, Hashizume K. Correlation between graft size and necessary tacrolimus dose after living-related liver transplantation. Transplant Proc 2000; 32: 2166–7.

29 Sugawara Y, Makuuchi M, Kaneko J, Ohkubo T, Imamura H, Kawarasaki H. Correlation between optimal tacrolimus doses and the graft weight in living donor liver transplantation. Clin Transplant 2002; 16: 102–6.

30 Yasuhara M, Hashida T, Toraguchi M, Hashimoto Y, Kimura M, Inui K, Hori R, Inomata Y, Tanaka K, Yamaoka Y. Pharmacokinetics and pharmacodynamics of FK 506 in pediatric patients receiving living-related donor liver transplantations. Transplant Proc 1995; 27: 1108–10.

31 Kim JS, Aviles DH, Silverstein DM, Leblanc PL, Matti Vehaskari V. Effect of age, ethnicity, and glucocorticoid use on tacrolimus pharmacokinetics in pediatric renal transplant patients. Pediatr Transplant 2005; 9: 162–9.

32 Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. Clin Pharmacokinet 2004; 43: 623–53.

33 Zhao W, Elie V, Roussey G, Brochard K, Niaudet P, Leroy V, Loirat C, Cochat P, Cloarec S, Andre JL, Garaix F, Bensman A, Fakhoury M, Jacqz-Aigrain E. Population pharmacokinetics and pharmacogenetics of tacrolimus in de novo pediatric kidney transplant recipients. Clin Pharmacol Ther 2009; 86: 609–18.

34 Staatz CE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: part I. Clin Pharmacokinet 2010; 49: 141–75.

35 Jacobson PA, Johnson CE, West NJ, Foster JA. Stability of tacrolimus in an extemporaneously compounded oral liquid. Am J Health Syst Pharm 1997; 54: 178–80.

36 U.S. Department of Health Services, Food and drug Administration. Guidance for Industry Population Pharmacokinetics. February 1999 [updated 31 January 2012]; Available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072137.pdf (last accessed 4 October 2013).

37 Hoyer PF. Therapeutic drug monitoring of cyclosporin A: should we use the area under the concentration-time curve and forget trough levels? Pediatr Transplant 2000; 4: 2–5.

38 van der Meer AF, Marcus MA, Touw DJ, Proost JH, Neef C. Optimal sampling strategy development methodology using maximum a posteriori Bayesian estimation. Ther Drug Monit 2011; 33: 133–46.