Importance of hematocrit for a tacrolimus target concentration strategy

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Abstract

Purpose To identify patient characteristics that influence tacrolimus individual dose requirement in kidney transplant recipients.

Methods Data on forty-four 12-h pharmacokinetic profiles from 29 patients and trough concentrations in 44 patients measured during the first 70 days after transplantation (1,546 tacrolimus whole blood concentrations) were analyzed. Population pharmacokinetic modeling was performed using NONMEM 7.2®.

Results Standardization of tacrolimus whole blood concentrations to a hematocrit value of 45 % improved the model fit significantly ($p < 0.001$). Fat-free mass was the best body size metric to predict tacrolimus clearance and volume of distribution.

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Bioavailability was 49 % lower in expressers of cytochrome P450 3A5 (CYP3A5) than in CYP3A5 nonexpressers. Younger females (<40 years) showed a 35 % lower bioavailability than younger males. Bioavailability increased with age for both males and females towards a common value at age >55 years that was 47 % higher than the male value at age <40 years. Bioavailability was highest immediately after transplantation, decreasing steeply thereafter to reach its nadir at day 5, following which it increased during the next 55 days towards an asymptotic value that was 28 % higher than that on day 5.

Conclusions Hematocrit predicts variability in tacrolimus whole blood concentrations but is not expected to influence unbound (therapeutically active) concentrations. Fat-free mass, *CYP3A5* genotype, sex, age and time after transplant influence the tacrolimus individual dose requirement. Because hematocrit is highly variable in kidney transplant patients and increases substantially after kidney transplantation, hematocrit is a key factor in the interpretation of tacrolimus whole blood concentrations.

Keywords Tacrolimus · Hematocrit · Fat-free mass · Population pharmacokinetics · Target concentration intervention · Kidney transplantation

Introduction

Tacrolimus is an immunosuppressive drug widely used after kidney transplantation. Applying a target concentration strategy is recommended to ensure adequate immunosuppression [1]. The initial dose of tacrolimus is typically calculated in proportion to total body weight, with subsequent doses adjusted on the basis of whole blood trough concentration measurements. The target concentration for initial and subsequent dosing varies from center to center with no detectable relationship between achieved whole blood concentrations and the risk of acute rejection [2]. Predicting the individual dose requirement

to achieve and maintain the desired target whole blood concentration of tacrolimus is challenging due to considerable variability in apparent whole blood clearance (CL/F) between patients [coefficient of variance (CV) 30–42 %] as well as between dosing occasions within patients (CV 40–71 %) [3]. In addition, dose-normalized whole blood concentrations of tacrolimus tend to increase during the first months after kidney transplantation, which has led to the suggestion of time-dependent pharmacokinetics [4, 5]. Both under- and overdosing frequently occur during this period, and several dose adjustments are typically performed [6].

Tacrolimus distributes into and binds to erythrocytes, showing a blood-to-plasma ratio of approximately 50 with a hematocrit of 45 % [7], and the unbound fraction in the blood is <1 % [8]. The drug is routinely measured as total concentrations in whole blood, although the unbound concentration is expected to mediate the pharmacological effect. Hebert et al. [9] have recently reviewed the pharmacological principles underlying the interpretation of whole blood concentrations and pointed out the potential dangers of adjusting tacrolimus doses based on whole blood concentration alone. Since tacrolimus is a low-extraction-ratio drug [10], whole blood concentrations are expected to increase in proportion to erythrocyte binding (proportional to hematocrit), while the unbound, therapeutically active concentration remains unchanged. At the time of kidney transplant, hematocrit is generally low, and it usually increases during the first months after transplantation as the recipients recover from kidney failure and erythrocyte production normalizes [11]. Because changes in hematocrit are expected to change the concentration of bound tacrolimus without modifying the unbound concentration [9], this trend should not be interpreted as the need for dose adjustment. It is currently unclear how much of the apparent time-dependent pharmacokinetics of tacrolimus during the first post-transplant months are explained by changes in hematocrit.

In this study, a population pharmacokinetic model for tacrolimus was developed using data from adult kidney transplant recipients. The purpose was to identify patient characteristics that influence tacrolimus individual dose requirement. Measured whole blood concentrations of tacrolimus were standardized to a normal hematocrit value (45 %). This led to the development of a pharmacokinetic model that can be used to individualize tacrolimus dosing to achieve a target concentration that better reflects the unbound pharmacologically active drug.

Materials and methods

Patients and data

Data on a total of 1,546 tacrolimus whole blood concentrations were obtained from 69 adult patients who underwent kidney transplantation at Oslo University Hospital, Rikshospitalet and

who received tacrolimus as part of their immunosuppressive therapy. Patients who received multiple organs or who received drugs that were known to pharmacokinetically interact with tacrolimus were excluded. However, if such drugs were used by >5 % of the patients, the patients were allowed in the analysis, and the drug was analyzed as a covariate. The patients contributed data to an Intensive Group or a TDM (therapeutic drug monitoring) Group. Four patients contributed data to both groups. The Intensive Group consisted of 12-h concentration–time profiles from 29 patients in a stable phase after transplantation (range 26 days–18 years). The data were collected on a total of 44 dosing occasions as described in three previously published studies [12–14]. The TDM Group consisted of routine trough concentrations (C_0) measured in 44 patients during the first 70 days following kidney transplantation. Dosing at 12-h intervals and full compliance were presumed unless missed doses were recorded in the patient chart. Patients were told to be fasting at the time of drug intake.

The following data were collected for covariate analysis: cytochrome P450 3A5 (*CYP3A5*) genotype, hematocrit, sex, age, total body weight, height, serum albumin, serum creatinine, C-reactive protein, aspartate aminotransferase, alanine aminotransferase, total serum bilirubin, alkaline phosphatase, acute rejection episodes, oral prednisolone dose, intravenous methylprednisolone dose, and use of other potentially interactive drugs. Covariates with time-dependent values were updated for each recorded tacrolimus measurement. Covariates missing on the day of transplantation were imputed by carrying back the first known value. Covariates missing at any later time point were imputed by carrying forward the last known value. However, if more than two hematocrit values were missing in sequence and the hemoglobin level was known, hematocrit values were derived by linear regression between all known hematocrit and hemoglobin values within the patient. A summary of the study designs is listed in Table 1.

The study was approved by the Regional Committee for Medical Research Ethics and by the Norwegian Medicines Agency. All patients gave written informed consent.

Immunosuppressive therapy

All patients received oral tacrolimus (Prograf® capsules; Astellas Pharma US Inc., Northbrook, IL). According to the center transplant protocol, initial dosage for the TDM Group was 0.04 mg/kg total body weight (0.05 mg/kg for high-risk patients) twice daily, adjusted to the nearest 0.5 mg dose. The dosage was subsequently adapted by measuring whole blood trough concentrations to maintain concentrations within the range of 3–7 μg/L in standard risk patients or 8–12 μg/L in high-risk patients. High-risk patients were defined by panel reactive antibody of >20 % and/or presence of donor-specific antibodies. Trough concentrations of tacrolimus were initially measured three to four times per week. Depending on clinical

Table 1 Characteristics of tacrolimus pharmacokinetic studies

Study and reference	Number of tacrolimus whole blood concentrations for analysis	Patients[a] (n)	Assay type	LLOQ (μg/L)	Assay CV (%)	Sampling times
1. [12]	134	5[b]	MEIA[c]	3.0	13 at 5 μg/L 7 at 23 μg/L	0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 12 h after administration
2. [13]	164	7[b]	LC-MS/MS[d]	1.1	≤5.2	0.25, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 h after administration
3. [14]	216	19[b]	CMIA[e]	1.0	<6 at 2.3 μg/L <9 at 7.0 μg/L	0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 23 and 24 h after administration
4. NPP	1,032	44	CMIA[e]	1.0	<6 at 2.3 μg/L <9 at 7.0 μg/L	Predose (trough concentrations)

CMIA, Chemiluminescent microparticle immunoassay; CV, between-series coefficient of variation; LC-MS/MS, liquid chromatography/tandem mass spectrometry; LLOQ, lower limit of quantification; MEIA, microparticle enzyme immunoassay; NPP, not previously published data

[a] Six patients contributed data to more than one study. These were identified and their data were associated with the same ID in the data set, with an increase in the number of occasions. The total number of individuals was 69

[b] Number of patients is different from number in original study due to exclusion criteria of the present study

[c] Analyzed on the IMx® instrument (Abbott Laboratories, Abbott Park, IL)

[d] Converted to corresponding immunoassay concentrations: CM=(LC−0.19)/0.80; see main text for details

[e] Analyzed on the Architect® instrument (Abbott Laboratories)

status and graft function, this frequency decreased in parallel with increasing time since transplantation.

In addition to tacrolimus, the immunosuppressive regimen for the TDM Group consisted of 0.75 g mycophenolate mofetil twice daily (without target concentration intervention), induction therapy with 20 mg of basiliximab on the day of transplant (day 1) and on day 5, an intravenous bolus of 250 mg methylprednisolone on day 1 and oral prednisolone once daily according to the following tapering regimen: 20 mg on days 2–15, 15 mg on days 16–29, 10 mg on days 30–61, 7.5 mg on days 62–180 and 5 mg from day 181 onwards. High-risk patients received induction therapy with 500 mg/kg intravenous human immune globulins daily on days 1–6 and 375 mg/m^2 rituximab on day 1, intravenous boluses of 500 mg and 80 mg methylprednisolone on day 1 and 2, respectively, and oral prednisolone at a total dose of 80 mg on day 3, followed by tapering during days 4–7 to 30 mg daily, continued with once-daily doses of 20 mg on days 8–29, 15 mg on days 30–61, 10 mg from day 62 and in selected patients tapered to 5 mg from day 181 onwards. For the Intensive Group, prednisolone dose was the only recorded concomitant immunosuppressive drug on which information was provided (range 0–25 mg daily).

Analytical assays

Table 1 shows the analytical assays used to measure tacrolimus concentrations in each sub-study. Chemiluminescent microparticle immunoassay (CMIA) is currently used as a standard analytical method at our center. Of the concentrations available for analysis, 80 % were determined by CMIA, 9 % by microparticle enzyme immunoassay (MEIA) and 11 % by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Tacrolimus concentrations determined by CMIA and MEIA correlate well at concentrations of >5 μg/L [15]. As only 1 % of the concentrations in the present analysis determined by MEIA were <5 μg/L, no conversion was performed between immunoassay measurements. On the contrary, tacrolimus concentrations determined by immunoassay tend to be higher than those determined by mass spectrometry, mainly due to cross-reactivity with tacrolimus metabolites [1]. In order to use tacrolimus concentrations determined by LC-MS/MS in the present analysis, we converted LC-MS/MS measurements into CMIA-equivalent measurements by the means of a conversion equation (Eq. 1) derived from linear regression between 43 tacrolimus whole blood concentrations determined by both LC-MS/MS (range 2.7–13.0 μg/L) and CMIA (range 3.6–14.4 μg/L) (R^2=0.94). The regression analysis was available from routine procedures performed at our laboratory during transition between analytical assays.

$$CM = \frac{(LC-0.19)}{0.80} \qquad (1)$$

In Eq. 1, CM is the CMIA tacrolimus concentration in micrograms per liter and LC is the LC-MS/MS tacrolimus concentration in micrograms per liter.

CYP3A5 genotyping

DNA was extracted from EDTA anti-coagulated whole blood using the MagNA Pure instrument (Roche Applied Science,

Penzberg, Germany). *CYP3A5* genotyping (rs776746; N G _ 0 0 7 9 3 8 . 1 : g . 1 2 0 8 3 G > A , A = *C Y P 3 A 5 * 1* a n d G=*CYP3A5*3*) was performed by real-time PCR and melt curve analysis with hybridization probes on the LightCycler® 480 instrument (Roche Applied Science) or by a previously reported PCR-restriction fragment length polymorphism assay [16]. Primers for the melt curve assay were designed using LightCycler Probe Design software version 2 (Roche Applied Science), and hybridization probe sequences were derived from Cheung et al. [17].

Population pharmacokinetic modeling

Structural model

Models with one or two compartments, zero or first order absorption and with or without an absorption lag time were investigated. Pharmacokinetic parameters were estimated in terms of clearance (CL), intercompartmental clearance (Q) and volumes of distribution (V_1, V_2). The population value of bioavailability (F) was defined as 1, and subgroup values and individual values of F were estimated relative to the population value. Because only data following oral administration were available, apparent disposition parameter estimates were obtained (e.g. CL/F, V_1/F). Random effects in the pharmacokinetic parameters were modeled in terms of between subject variability (BSV) and between occasion variability (BOV). BSV and BOV were initially estimated for all pharmacokinetic parameters except lag time. Random effects were modeled exponentially (Eq. 2):

$$P_{ik} = P_{GRP} \times e^{\eta_i + \eta_k} \tag{2}$$

where P_{ik} is the value of parameter P for the i-th individual at the k-th occasion, P_{GRP} is the group value of P in the population, e^{η_i} is the deviation between P_{GRP} and the individual value P_i and e^{η_k} is the deviance between the individual value P_i and the occasion-specific value P_{ik} [18]. For the Intensive Group, one hospital visit was defined as one occasion. For the TDM Group, a new occasion was defined when a dose change took place. Equation 3 shows the residual error model used throughout the analysis:

$$Y_{ij} = Conc_{ij} + \theta_{study_i} \times (\varepsilon_1 \times Conc_{ij} + \varepsilon_2) \times e^{\eta_{err_i}} \tag{3}$$

where Y_{ij} and $Conc_{ij}$ are the model-predicted drug concentrations with and without residual error, respectively, for the i-th individual at the j-th measurement, θ_{study_i} is a study-specific fixed effect, ε_1 is a residual error term proportional to the measured concentrations, ε_2 is an additive error term and $e^{\eta_{err_i}}$ is the individual random difference in residual error for the i-th individual [19]. The random variables η_i, η_k, ε_1, ε_2 and η_{err_i}

were assumed to be normally distributed with means of zero and variances ω^2_P, π^2_P, σ_1^2, σ_2^2 and ω_{err}^2, respectively.

Covariate model

Tacrolimus total concentrations (C_{tot}) in whole blood are the sum of the unbound (C_u), plasma bound (C_p) and erythrocyte bound (C_b) concentrations. Assuming a single binding site, C_{tot} can be described as a function of the unbound concentration (Eq. 4):

$$C_{tot} = C_u \times \left(1 + \frac{Cb_{max}}{C_u + Cu_{50}} \right) \tag{4}$$

where Cb_{max} is the maximum concentration bound to erythrocytes and Cu_{50} is the unbound concentration leading to half maximum binding. With a blood:plasma ratio of approximately 50 [7], C_{tot} will mainly reflect C_b. By assuming that C_u is small relative to Cu_{50} and by standardizing Cb_{max} to a hematocrit value of 45 %, Eq. 4 can be written as Eq. 5:

$$C_{tot} \cong C_b = C_{std} \times R \times \frac{Hct}{45\%} \tag{5}$$

where C_{std} is the standardized concentration proportional to C_u, and R is the ratio between Cb_{max} and Cu_{50} at a hematocrit of 45 %. Equation 5 was included in the model by relating the pharmacokinetic parameters and predictions to C_{std} rather than to C_{tot}. Potential concentration-dependent binding of tacrolimus to erythrocytes was investigated using Eq. 6:

$$C_{tot} = C_{std} \times \frac{Cstd_{max}}{C_{std} + Cstd_{50}} \times \frac{Hct}{45\%} \tag{6}$$

where $Cstd_{max}$ and $Cstd_{50}$ are estimable parameters reflecting maximum binding capacity of tacrolimus to erythrocytes and the standardized concentration associated with half maximum binding, respectively. Similar principles to those described for hematocrit were used to investigate the standardization of plasma protein binding using serum albumin concentrations.

The pharmacokinetic disposition parameters were related to fat-free mass using theory-based allometric coefficients of 3/4 for clearances and 1 for volumes of distribution [20]. Fat-free mass was predicted from total body weight, height and sex (Eqs. 20 and 21 in [21]). Potential additional effects of fat mass (i.e. total body weight—fat-free mass) to the effect of fat-free mass on the pharmacokinetic parameters were estimated as described by Anderson and Holford [20].

All available patient characteristics were evaluated as covariates on parameters where an effect was biologically plausible or if an effect was found in previous studies. Regarding covariates for which little is known about the mechanism of

influence on tacrolimus pharmacokinetics (age, time after transplantation, liver function test values), continuous covariate effects were initially investigated by binning observed concentrations by covariate value (e.g. age bins of 20–29 years, 30–39 years and so on). The early post-transplant period was binned by dividing it into 12 time intervals with similar number of observations in each interval: day 2, 3, 4–5, 6–7, 8–10, 11–14, 15–21, 22–28, 29–42, 43–56, 57–70 and >70 days. A mean pharmacokinetic parameter (CL or F) was estimated within each bin relative to a reference bin. Empirical models (linear, power, exponential or sigmoid functions) were subsequently used to match the discrete distribution of parameter values as a function of the continuous covariate and of time. This method helps to identify and obtain initial estimates of continuous functions that describe the data when there is little prior information on what relationship to expect [22]. Binary covariate effects (e.g. sex) were investigated by estimating the fractional change in one group compared to the other.

Model evaluation and statistical analysis

Model selection was guided by biological plausibility and the likelihood ratio test [difference in objective function value (ΔOFV)]. A decrease in OFV of >6.6 was considered to be statistically significant for the addition of one parameter ($p < 0.01$) [23]. Prediction-corrected visual predictive checks (pcVPCs) were generated to compare observed data with 90 % prediction intervals constructed from 100 simulated subsets of the original dataset [24]. Time after dose, time after transplantation and other continuous covariates were used as independent variables in pcVPCs. Covariates were included stepwise and subsequently independently deleted from the full model (backward elimination) to evaluate their final statistical contribution [25]. Confidence intervals (CIs) of the parameter estimates were generated from 1,000 nonparametric bootstrap replicates [26]. These were used to guide model simplification. Random effects were retained if the lower 2.5th percentile did not approach zero. Covariates were retained if the 95 % CI of the covariate coefficient estimate did not include the value equivalent to no effect. To describe variability in hematocrit between and within patients, hematocrit was modeled by BSV and BOV (considering each hematocrit measurement as a single occasion). The time course of hematocrit after transplantation was described by a sigmoid Emax function.

Modeling was performed in NONMEM® v. 7.2 [23] using the first order conditional estimation method with interaction. Models and bootstrap replicates were run using Wings for NONMEM® [27]. Statistical and graphical analyses were performed using the statistical package R® (v. 2.15.0; R Foundation for Statistical Computing, Vienna, Austria). Means for normally distributed covariates were compared

using two-tailed t tests with assumption of equal variance. If not stated otherwise, descriptive statistics are expressed as the mean ± standard deviation.

Results

Patients and data

Demographic and clinical characteristics, details about tacrolimus treatment and the number of missing covariate values are presented in Table 2. Patients using carbamazepine ($n = 2$) were excluded because of potential drug interaction with tacrolimus. Patients using nifedipine ($n = 26$), lansoprazole ($n = 12$) and/or cinacalcet ($n = 5$) were retained. Figure 1a shows the evolution of the dose-normalized tacrolimus trough whole blood concentrations after transplantation. In total, 41 % of the measured trough concentrations in the TDM group were outside the target concentration ranges defined by the hospital protocol. The percentage of measured concentrations outside these ranges for week 1, 2, 3, 4, 5–6, 7–8 and 9–10 after transplantation were 42, 41, 47, 42, 43, 37 and 36 %, respectively. It was however not known if the clinician voluntarily had deviated from the protocol.

Hematocrit increased during the first 70 days after transplantation (Fig. 1b). The population baseline hematocrit on day 1 was estimated to be 29.7 %, increasing towards an asymptotic value of 37.4 % with half maximum increase on day 19 post-transplant. BSV and BOV of the hematocrit were estimated to be 8 and 11 %, respectively. Average hematocrit did not vary by sex ($p = 0.28$) or CYP3A5 genotype ($p = 0.51$). Average total body weight was 19 kg higher in males (86± 21 kg) than in females (67±15 kg) ($p < 0.001$). Average fat-free mass was 18 kg higher in males (64±10 kg) than in females (46±5 kg) ($p < 0.001$).

Population pharmacokinetic modeling

Structural model

Tacrolimus pharmacokinetics were best described by a two-compartment model and first order absorption with a lag time. A study-specific absorption rate and lag time improved the description of substudy 2. When the BOV was estimated for F, the lower 2.5th percentile of the bootstrap distribution of BOV in the four disposition parameters (CL/F, V_1/F, Q/F and V_2/F) approached zero. BOV in these parameters were therefore removed (ΔOFV+7.3 for four parameters, $p = 0.12$). For the absorption rate constant (k_a), BSV was removed and BOV was retained (ΔOFV+3.4 by removing BSV, $p = 0.07$; ΔOFV+28.7 by removing BOV, $p < 0.001$), indicating that the variability in absorption rate is mainly dependent on dosing occasion rather than being a subject-specific process.

Table 2 Demographic and clinical data

Data	Number	Mean[a]	95 % observation interval	Change in TDM Group after transplantation				Number of missing data points
				Day 1		Day 70		
				Mean	95 % observation interval	Mean	95 % observation interval	
Demographic data								
Patients	69							
Sex (male /female)	50/19							0/69
CYP3A5 genotype (*1/*1, *1/*3, *3/*3)	0/10/59							0/69
Age (years)		43	22–73					0/69
Height (cm)		176	151–197					0/69
Total body weight (kg)		81	51–131	83	53–140	80	49–128	142/1,546
Predicted fat-free mass (kg)		59	40–82	60	41–84	55	39–82	142/1,546
Hematocrit (%)		36	29–43	31	25–39	38	32–42	109/1,546
Serum creatinine (μmol/L)		147	73–347	409	123–1,079	120	78–208	2/1,546
C-reactive protein (mg/L)		7	<0.6–24	12	<0.6–34	3	<0.6–10	49/1,546
Serum albumin (g/L)		42	33–47	37	27–45	45	37–50	498/1,546
Total serum bilirubin (μmol/L)		7	3–13	5	1–14	6	3–15	500/1,546
Aspartate aminotransferase (IU/L)		23	15–40	35	13–111	20	14–29	498/1,546
Alanine aminotransferase (IU/L)		31	10–91	50	13–170	22	11–43	498/1,546
Alkaline phosphatase (IU/L)		66	30–106	60	34–95	70	41–105	499/1,546
Pharmacokinetic data (TDM Group)								
Samples	1,032							
Samples per patient		23	13–29					
Tacrolimus trough concentration (μg/L)		7.0	2.7–12.4					
Pharmacokinetic data (Intensive Group)								
Samples	514							
Samples per patient		18	9–39					
Tacrolimus concentration (μg/L)		11.3	3.4–26.7					

CYP3A5, Cytochrome P450 3A5; TDM, therapeutic drug monitoring

[a] Calculated from mean value across all observation times in each patient

We chose to estimate a full correlation matrix between CL/F, V_1/F, Q/F and V_2/F (ΔOFV=−59.0 for six parameters, $p<0.001$) rather than to estimate BSV in F (ΔOFV=−32.4 for one parameter, $p<0.001$). The pcVPC over the range of hematocrit revealed a systematic prediction error at this stage (Fig. 2b).

Covariate model

The model fit improved significantly by linearly standardizing whole blood concentrations to a hematocrit of 45 % (ΔOFV= −78.3, $p<0.001$). The estimated value for the parameter R in Eq. 5 was 0.99, and this parameter was removed without worsening the model fit (ΔOFV=+1.2, $p=0.27$). By standardizing for hematocrit, the systematic prediction error over the range of hematocrit values was no longer present (Fig. 2c). Accounting for the possibility of saturated erythrocyte proteins

(Eq. 6) or including similar standardization of the concentrations using serum albumin did not improve the model fit.

The parameters describing the effects of fat mass in addition to the effect of fat-free mass on the disposition parameters were estimated to be close to zero [bootstrap means of 0.1 (95 % CI −0.7 to 1.9) for clearances and 0.3 (95 % CI −0.7 to 2.4) for volumes of distribution]. This indicated that fat-free mass is a more predictive body size metric for tacrolimus pharmacokinetics than total body weight. The OFV did not increase by fixing these parameters to zero. *CYP3A5* genotype significantly influenced both CL and F ($p<0.001$).

After including body size in the model, it was found that F was significantly lower in females (ΔOFV=−9.8, $p=0.002$). When an age-specific value of F was estimated in each of the five age categories, it became apparent that F changed between age 40 and 50 years and was essentially constant at a lower relative value in patients aged <40 years and constant
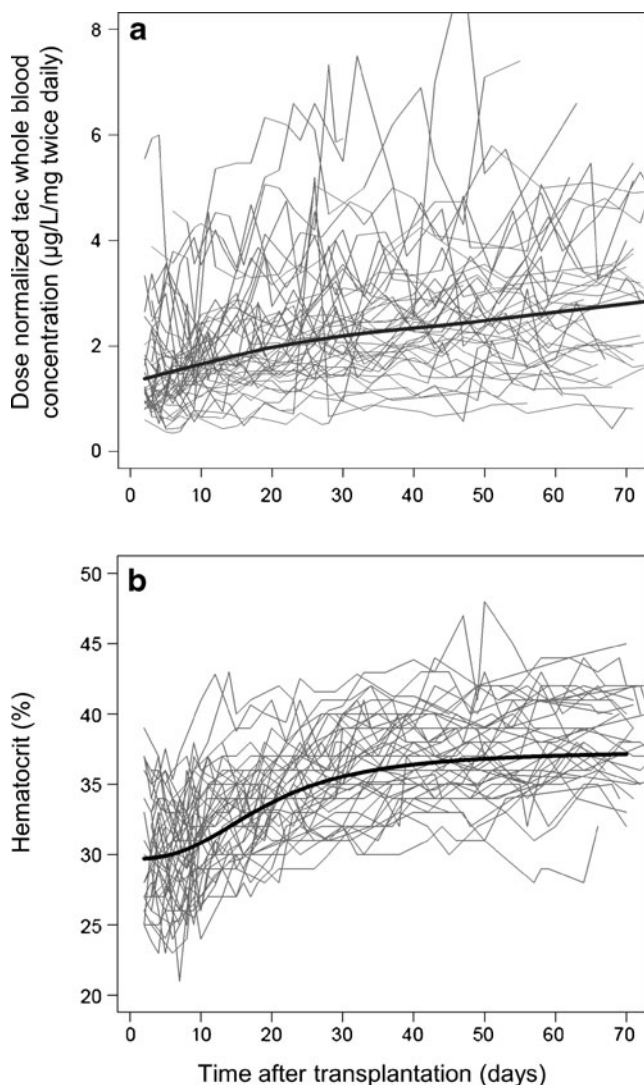
Fig. 1 **a** Dose-normalized whole blood trough concentrations of tacrolimus (*tac*) observed in the TDM Group during the first 70 days after transplantation. *Thin lines* 44 individual patients, *thick line* a smoother to show the trend with time. **b** Time course of hematocrit (%) during the first 70 days after transplantation. *Thin lines* 44 individual patients that were followed during this period, *thick line* sigmoid Emax model fit to the data to describe changes in hematocrit as a function of time

at a higher relative value in patients aged >55 years. A sigmoid function of age was useful to describe these changes and improved the model further (ΔOFV=−18.1 for three parameters, p <0.001) (Eq. 7):

$$F_{GRP} = Fmin_{age} + \frac{1-Fmin_{age}}{1 + \left(\frac{Age}{Fage_{50}}\right)^{-Hill_{Fage}}} \qquad (7)$$

where $Fmin_{age}$ is the asymptotic value of F at a low age relative to the highest value of F with increasing age, and $Fage_{50}$ is the age of the half maximum change in F with the associated shape coefficient $Hill_{Fage}$. The estimates of $Fage_{50}$ and $Hill_{Fage}$ were similar for males and females, whereas $Fmin_{age}$ was different between males and females. These

results indicate that the sex difference in F is greatest in younger patients (<40 years) and that the difference diminishes at age >55 years (Fig. 3a).

The time interval-specific value of F changed systematically with time after transplantation. These changes could be described using two distinct sigmoid functions of time after transplant (Fig. 4a and Eq. 8). A random effect was included to describe BSV in the extent of change at a late time point:

$$F_i = \left(Fmax_{early} + \frac{1-Fmax_{early}}{1 + \left(\frac{Txt}{F_{early50}}\right)^{-Hill_{F_{early}}}}\right) \times \left(1 + \frac{Fmax_{late} \times e^{\eta_i}}{1 + \left(\frac{Txt}{F_{late50}}\right)^{-Hill_{F_{late}}}}\right)$$
$$(8)$$

where F_i is the individual value of F, $Fmax_{early}$ is the maximum value of F immediately after transplantation, $F_{early50}$ is the day with half maximum early effect on F with the associated shape coefficient $Hill_{Fearly}$ describing the steepness of this change, F_{late50} is the day with half maximum later effect on F with the associated steepness coefficient $Hill_{Flate}$, $Fmax_{late}$ is the asymptotic maximum value of F with increasing posttransplant time and e^{η_i} is the difference between the individual asymptote and the population asymptote, where η_i's are assumed to be normally distributed with mean zero and variance ω_{Flate}^2. The day of transplant was defined as day 1. The six sigmoid model parameters replaced the 12 time interval parameters without loss in model goodness of fit (ΔOFV= −64.8 vs. −69.5, respectively, compared to a model without time after transplantation as a covariate). Estimation of BSV in $Fmax_{late}$ resulted in a further decrease (ΔOFV=−40.7 for one parameter, p <0.001). The data did not support estimation of BSV in any of the remaining parameters in Eq. 8. No time-varying covariate, including prednisolone dose and liver function test values, replaced the empirical time model for F without a loss in model goodness of fit.

Model reduction and evaluation

The study-specific residual errors for substudies 1 and 4 were similar and joined (ΔOFV+0.9 for one parameter, p =0.34). The lower 2.5th percentile of the bootstrap distribution of the additive residual error term and BSV in the residual error approached zero. Therefore, only the proportional error term was retained. All random effects describing correlations between disposition parameters except from between CL/F and Q/F were removed because their 95 % CIs included zero. The effect of *CYP3A5* genotype on CL was removed because the 95 % CI of 0.81–1.46 did not support its inclusion in addition to an effect on F, which had a 95 % CI not including 1 (0.39–0.83) (ΔOFV +0.01 for one parameter, p =0.91). Removing the remaining covariates significantly worsened the goodness of fit.

Expressed as bootstrap averages of the final model (Table 3), we found that F was 49 % lower in CYP3A5 expressers than in
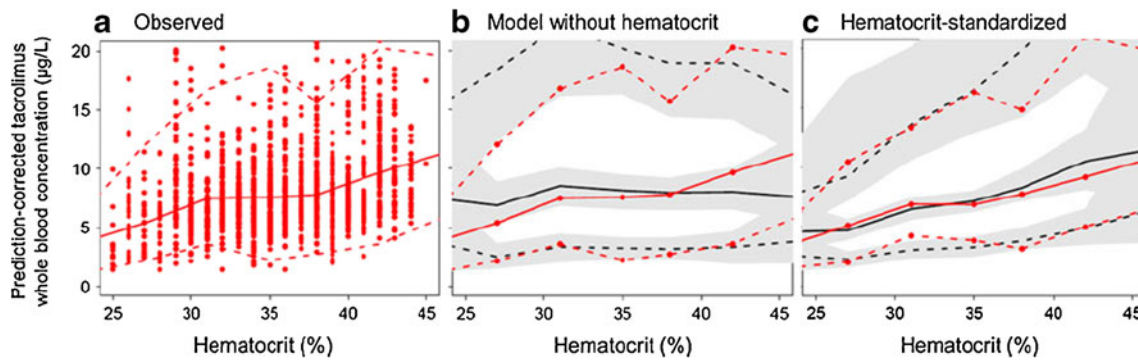
Fig. 2 a Prediction-corrected tacrolimus whole blood concentrations over the range of hematocrit, **b** prediction-corrected visual predictive check (pcVPC) over the range of hematocrit using the base model without covariates, **c** pcVPC over the range of hematocrit using the base model with hematocrit standardized concentrations. *Solid red line* Median observed tacrolimus concentration (prediction-corrected), *dashed red lines* 90 % observation interval (5th to 95th percentile), *solid black line* median model-predicted concentrations, *dashed black lines* 90 % prediction interval. *Gray-shaded areas* 95 % confidence intervals (CIs) for each model-predicted percentile
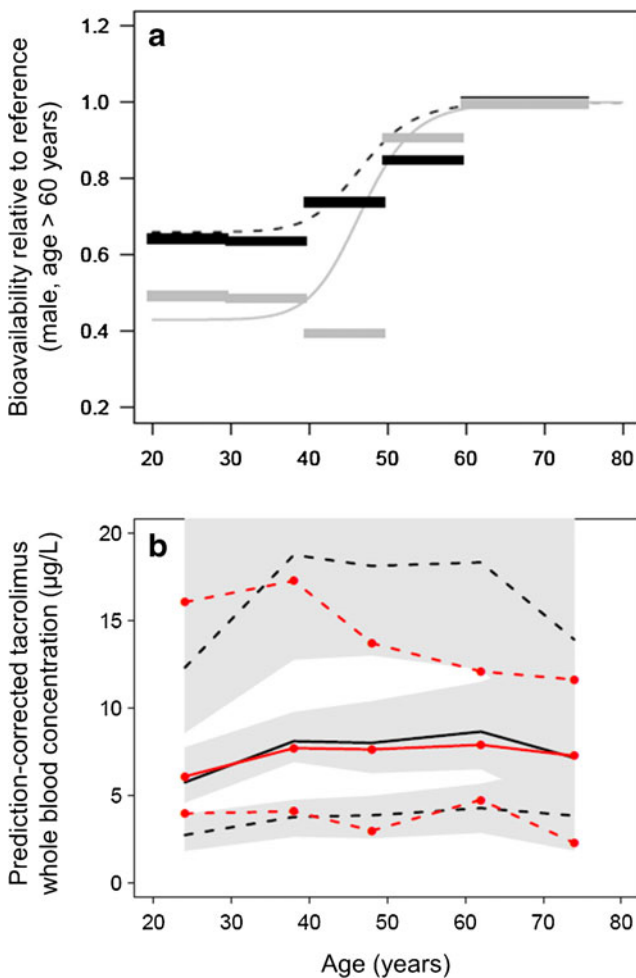

Fig. 3 a *Thick horizontal lines* Age interval-specific average values of bioavailability (*F*) estimated in males (*black*) and females (*gray*) relative to males aged >60 years (defined as *F*=1). *Lines* Model-estimated functions for changes in bioavailability with age in males (*black dashed*) and females (*gray solid*). **b** pcVPC using the final model as a function of age. *Solid red line* Median observed tacrolimus concentration (prediction-corrected), *dashed red lines* 90 % observation interval (5th to 95th percentile), *solid black line* median model-predicted concentrations, *dashed black lines* 90 % prediction interval. *Gray-shaded areas* 95 % CI for each model-predicted percentile
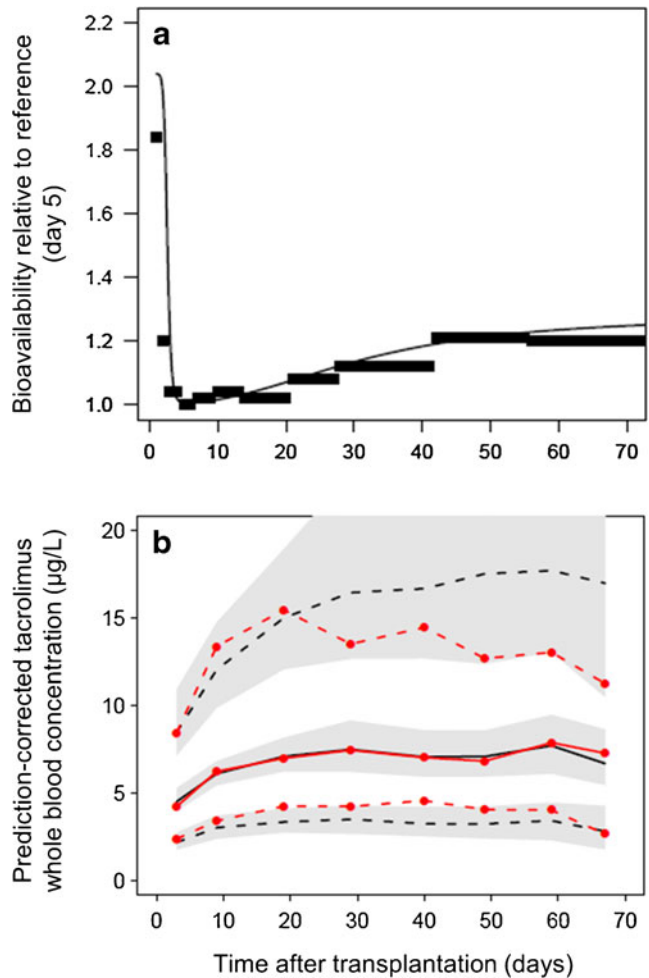
Fig. 4 a *Thick horizontal lines* Time interval-specific average values of bioavailability after transplantation, estimated relative to the value at day 5 (defined as *F*=1). *Thin continuous line* Model-estimated time course of relative bioavailability during the first 70 days after transplantation. **b** pcVPC using the final model as a function of the first 70 days after transplantation. *Solid red line* Median observed tacrolimus concentration (prediction-corrected), *dashed red lines* 90 % observation interval (5th to 95th percentile), *solid black line* median model predicted concentrations, *dashed black lines* 90 % prediction interval. *Gray-shaded areas* 95 % CI for each model-predicted percentile

Table 3 Original data estimates and bootstrap average parameter estimates with 95 % confidence intervals

Parameter	Unit	Original data estimate[a]	Bootstrap average	Bootstrap 95 % CI[b]
CL/Fn	L/h	20.5	20.5	17.3–24.6
V_1/Fn	L	105	107	87–136
Q/Fn	L/h	35.8	37.3	21.5–48.5
V_2/Fn	L	450	424	297–583
k_a	h^{-1}	1.14	1.18	0.80–1.78
$k_{a, study 2}$	h^{-1}	0.37	0.38	0.31–0.48
Lag time	h	0.21	0.22	0.20–0.24
Lag time$_{study 2}$	h	0.82	0.81	0.65–0.90
Covariates on F				
CYP3A5 *1/*3	factor	0.51	0.51	0.42–0.62
Age and sex				
$Fmin_{age, females}$		0.44	0.43	0.23–0.60
$Fmin_{age, males}$		0.68	0.66	0.36–0.89
$Fage_{50}$	years	44	47	39–70
$Hill_{Fage}$		12.2	14.0	3.5–24.9
Time, early				
$Fmax_{early}$		1.87	2.04	1.60–3.9
$F_{early50}$	days	2.6	2.5	1.7–3.0
$Hill_{Fearly}$		10.0[c]	9.4	3.7–10.0[c]
Time, late				
$Fmax_{late}$		0.27	0.28	0.14–0.49
F_{late50}	days	30	31	24–40
$Hill_{Flate}$		2.3	2.5	1.6–4.7
BSV				
CL/Fn	%	33	31	21–40
V_1/Fn	%	14	14	0.2–37
Q/Fn	%	91	86	51–114
V_2/Fn	%	52	52	32–74
$Hill_{Flate}$	%	117	113	78–146
Correlation				
CL/Fn ~ Q/Fn		0.75	0.74	0.41–0.92
BOV				
Fn	%	16	16	13–18
k_a	%	63	60	41–84
Residual variability				
Proportional error	%	16.7	16.7	15.2–18.4
Study 2	factor	0.57	0.56	0.41–0.72
Study 3	factor	0.73	0.72	0.57–0.90

CI, Confidence interval, Fn, bioavailability at baseline nadir (5 days after transplantation); CL/Fn, apparent clearance; V_1/Fn, apparent central volume of distribution; Q/Fn, apparent intercompartmental clearance; V_2/Fn, apparent peripheral volume of distribution; k_a, absorption rate constant; FFM, fat-free mass; $Fmin_{age}$, the minimum value of F at decreasing age relative to reference (male > 60 years); $Fage_{50}$, the age with half maximum effect on F; $Hill_{Fage}$, shape coefficient for the change in F with age; $Fmax_{early}$, the maximum increase in F immediately after transplantation relative to Fn; $F_{early50}$, the day with half maximum early effect on F; $Hill_{Fearly}$, shape coefficient for the change in F at early time; $Fmax_{late}$, maximum increase in F at later time point, relative to Fn; F_{late50}, the day with half maximum later effect on F; $Hill_{Flate}$, shape coefficient for the change in F at later time; BSV, between subject variability; BOV, between occasion variability

The final model was parameterized as follows: CL/Fn=$20.5 \times (FFM/60)^{3/4}$ L/h; V_1/Fn=$107 \times (FFM/60)$ L; Q/Fn=$37.3 \times (FFM/60)^{3/4}$ L/h; V_2/Fn=$424 \times (FFM/60)$ L

F=$[2.04+(1-2.04)/(1+(TXT/2.5)^{-9.4})] \times [1+0.28 / (1+(TXT/31)^{-2.5})] \times [Fmin_{age}+(1-Fmin_{age})/(1+(AGE/47)^{-14})] \times F_{CYP}$ where $Fmin_{age}$=0.43 in females and 0.66 in males, F_{CYP} is 0.51 in CYP3A5 expressers and 1 in CYP3A5 nonexpressers and TXT is the time after transplantation in days

[a] Standardized to 40-year-old male, CYP3A5 nonexpresser with fat-free mass of 60 kg and hematocrit of 45 %, at a time point with the lowest estimated bioavailability (Fn, day 5 post-transplant)

[b] 2.5–97.5 percentile obtained from 500 bootstrap replicates

[c] Upper bound

CYP3A5 nonexpressers. Furthermore, F was reduced to 0.43 and 0.66 for females and males, respectively, at the lowest ages compared to the plateau value set to 1 at highest age in males. The plateau value for females with increasing age was estimated as 1 (not different from males). The steepest change in F with age occurred around the age of 47 years for both sexes. Moreover, F was 104 % higher immediately after transplantation relative to its lowest value, followed by a steep decrease (Hill$_{Fearly}$=9.4) to a nadir (the reference baseline where F is defined as 1), with half of the change at day 2.5 after transplantation. F then increased more slowly (Hill$_{Flate}$=2.5) to an asymptotic value 28 % higher than the nadir (BSV=113 %) with half of the change after 31 days. PcVPCs indicated that the model was able to predict tacrolimus concentrations over the range of all the included covariates (shown for age in Fig. 3b and for time after transplantation in Fig. 4b).

Discussion

In this study, a population pharmacokinetic model was developed for tacrolimus by combining trough concentration data from the early post-transplant phase with rich sampling from patients in a stable phase after kidney transplantation. Hematocrit influenced the whole blood concentrations, while fat-free mass, *CYP3A5* genotype, sex, age and time post-transplant were identified as factors influencing tacrolimus individual dose requirement to achieve a target concentration proportional to the unbound active drug. Although the importance of hematocrit has been highlighted in several population pharmacokinetic analyses of tacrolimus in kidney transplant patients [28–32], these analyses used hematocrit as an empirical predictor only of clearance or volume of distribution, but not both simultaneously. A novel approach of our study was the modeling of hematocrit as a covariate to adjust measured whole blood concentrations based on the theory that all whole blood-based pharmacokinetic disposition parameters are equally influenced by hematocrit for a low extraction ratio drug such as tacrolimus. When not accounting for differences in hematocrit, the pharmacokinetic model showed a clear systematic prediction error across the range of observed hematocrit values. The systematic prediction error was, however, no longer present after correction for the predictions to account for the actual hematocrit value (Fig. 2). This can be understood in terms of pharmacokinetic theory for a low-extraction-ratio drug, with elimination and distribution processes being independent of unbound drug concentration. Of note, the same theory described for hematocrit also applies to the binding of tacrolimus to albumin, α_1-glycoprotein and lipoproteins in plasma [8, 9]. Although we did not identify any influence of albumin, it might have been obscured by the correlation between increases in albumin and hematocrit following kidney transplantation.

The challenge of reaching and maintaining a target concentration of tacrolimus during the first months after kidney transplantation has been described previously by Ekberg et al. who observed that approximately 50 % of tacrolimus trough concentrations during the first 8 post-transplant weeks were outside the intended range (3–7 µg/L) [6]. In our study, the percentage outside the target range was somewhat lower, but it was not reduced during the first 6 weeks after transplantation (42 % week 1, 43 % week 5–6). These challenges are, at least in part, related to the substantial increase in hematocrit following kidney transplantation (Fig. 1b) and high hematocrit within-subject variability (CV 11 %). Based on pharmacological theory and confirmation that hematocrit standardization is consistent with unbound concentration-independent pharmacokinetics, we recommend that tacrolimus whole blood concentrations be standardized to a hematocrit (Hct) of 45 % (C_{std}=C_{tot}×45 %/Hct) to reflect the unbound (active) drug more closely. This recommendation is based on the same well-known concepts that have guided the interpretation of phenytoin plasma concentration measurements when plasma proteins differ from normal [33, 34].

There is no consistent appreciation of the role of body size as a determinant of tacrolimus distribution and elimination [35], despite its biological basis and clinical use for predicting initial doses. This may be partly explained by small ranges of size, but also by not recognizing the role of body composition (fat-free mass and fat mass) as a determinant of functional body size. We investigated a large range of weights and identified fat-free mass as a predictor of tacrolimus pharmacokinetics, which to our knowledge is a novel finding. It is reasonable to accept fat-free mass as a predictor of tacrolimus clearance because fat mass is not expected to influence metabolic capacity directly [20]. The fact that volume of distribution of tacrolimus appeared to be closer related to fat-free mass than to total body weight indicates that the extent of tissue distribution of tacrolimus may be determined mainly by binding to proteins in lean tissues. For the patients in the current dataset with the lowest and highest total body weights (males of 43 kg and 160 kg with predicted fat-free masses of 39 and 95 kg, respectively), the model predicts apparent clearances of 16 and 27 L/h, respectively (given otherwise equal conditions; see Table 3). These calculations show that although total body weight may differ by fourfold in this patient group, the required dose differs by less than twofold and that the currently applied initial dose algorithm increases risk of overtreatment in obese patients. Indeed, previous studies have reported that high tacrolimus concentrations were more frequently observed in patients with higher weights [36, 37]. The proposed model provides a more rational use of body size for tacrolimus dose selection.

After appropriately accounting for body size differences in the model, we then identified sex differences in the pharmacokinetics of tacrolimus. The model fit was slightly better

using sex as a covariate on bioavailability rather than on clearance, and there was little evidence for an additional effect on clearance. This result is somewhat supported by previously reported results [37, 38], although most population studies on tacrolimus did not establish a relationship with sex [29, 35, 39]. The difference between males and females was most apparent in younger patients, and as age increased, bioavailability increased for both sexes while the sex difference diminished. It has been previously described that sex differences in the pharmacokinetics of CYP3A substrates seem to be more pronounced at younger ages compared with in the elderly [40]. The effect may alternatively be caused by sex- and/or age-related differences in intestinal P-glycoprotein (ABCB1) activity. Previous reports have also described higher tacrolimus concentrations in elderly patients [37, 41, 42], which is consistent with our findings. It should be noted that only four females in our study were aged >50 years. Further studies are needed to recommend appropriate initial dosing of tacrolimus based on sex and age.

A major limitation of our study was the use of a high proportion (67 %) of trough concentrations. Concentrations measured at trough provide only modest pharmacokinetic information. Another limitation was that seven patients who received simultaneous pancreas–kidney transplantation and two patients who used carbamazepine were excluded from the analysis. This may have led to underprediction of the variability seen across patients in a true clinical setting. Furthermore, conversion between multiple analytical assay techniques for tacrolimus measurements may have introduced additional overall variability to the analysis. Hematocrit-related bias in concentration determinations by analytical immunoassays should however not be a relevant limitation because CMIA is not known to be affected by hematocrit [1]. All samples analyzed with MEIA had corresponding hematocrit values not associated with bias (25–45 %) [43]. Finally, the 95 % interval of the ordered observed hematocrit values was 29–43 %. In general, extrapolation of results to patients with covariate values outside of the investigated range should be done with caution. Yet, because the hematocrit model has a mechanistic basis, extrapolation is more likely to be reliable.

The whole blood concentration–effect relationship of tacrolimus is poorly characterized, and there is no consensus among transplant centers on an optimal tacrolimus concentration [1, 2]. Although a trough concentration range of 3–7 µg/L has been proposed [44] and is used at our center, ranges of 5–10 or 10–15 µg/L are targeted at other centers [45]. An increasing hematocrit leads to a decrease in therapeutically active unbound concentrations with time after transplantation if doses are adjusted to maintain unchanged total whole blood concentrations [9]. Differences in active unbound concentrations not reflected by whole blood concentrations will be a confounding factor in studies attempting to characterize the whole blood concentration-effect relationship. In a study in liver transplant patients, the estimated unbound concentrations of tacrolimus were significantly lower in patients experiencing acute rejection than in stable patients ($p < 0.001$), without a difference in whole blood concentrations ($p = 0.5$) [8]. Standardizing whole blood concentrations to hematocrit in future studies should improve our understanding of the concentration–effect relationship for tacrolimus and allow a more consistent target concentration to be achieved.

In conclusion, a population pharmacokinetic model was developed for tacrolimus in kidney transplant patients. Fat-free mass, CYP3A5 genotype, sex, age and time post-transplant were identified as predictors of individual tacrolimus dose requirement. Allometric scaling to fat-free mass provides a more rational use of body size than linear scaling to total body weight. Hematocrit is a predictor of variability in tacrolimus whole blood concentrations, but it is not expected to influence unbound (therapeutically active) concentrations. Because hematocrit is highly variable in kidney transplant patients and increases substantially after kidney transplantation, hematocrit is a key factor for interpretation of tacrolimus whole blood concentrations.

References

1. Wallemacq P, Armstrong VW, Brunet M et al (2009) Opportunities to optimize tacrolimus therapy in solid organ transplantation: report of the European consensus conference. Ther Drug Monit 31(2):139–152
2. Bouamar R, Shuker N, Hesselink DA et al (2013) Tacrolimus predose concentrations do not predict the risk of acute rejection after renal transplantation: a pooled analysis from three randomized-controlled clinical trials. Am J Transplant 13:1253–1261. doi:10.1111/ajt.12191
3. Barraclough KA, Isbel NM, Kirkpatrick CM et al (2011) Evaluation of limited sampling methods for estimation of tacrolimus exposure in adult kidney transplant recipients. Br J Clin Pharmacol 71:207–223. doi:10.1111/j.1365-2125.2010.03815.x
4. Scholten EM, Cremers SC, Schoemaker RC et al (2005) AUC-guided dosing of tacrolimus prevents progressive systemic overexposure in renal transplant recipients. Kidney Int 67:2440–2447. doi:10.1111/j.1523-1755.2005.00352.x

5. Saint-Marcoux F, Woillard J-B, Jurado C, Marquet P (2013) Lessons from routine dose adjustment of tacrolimus in renal transplant patients based on global exposure. Ther Drug Monit 35:322–327. doi: 10.1097/FTD.0b013e318285e779

6. Ekberg H, Mamelok RD, Pearson TC et al (2009) The challenge of achieving target drug concentrations in clinical trials: experience from the Symphony Study. Transplantation 87:1360–1366. doi:10. 1097/TP.0b013e3181a23cb2

7. Jusko WJ, Piekoszewski W, Klintmalm GB et al (1995) Pharmacokinetics of tacrolimus in liver transplant patients. Clin Pharmacol Ther 57:281–290. doi:10.1016/0009-9236(95)90153-1

8. Zahir H, McCaughan G, Gleeson M et al (2004) Factors affecting variability in distribution of tacrolimus in liver transplant recipients. Br J Clin Pharmacol 57:298–309

9. Hebert MF, Zheng S, Hays K et al (2013) Interpreting tacrolimus concentrations during pregnancy and postpartum. Transplantation 95:908–915. doi:10.1097/TP.0b013e318278d367

10. Möller A, Iwasaki K, Kawamura A et al (1999) The disposition of 14C-labeled tacrolimus after intravenous and oral administration in healthy human subjects. Drug Metab Dispos 27:633–636

11. Malyszko J, Oberbauer R, Watschinger B (2012) Anemia and Erythrocytosis in patients after kidney transplantation. Transpl Int 25:1013–1023. doi:10.1111/j.1432-2277.2012.01513.x

12. Falck P, Vethe NT, Åsberg A et al (2008) Cinacalcet's effect on the pharmacokinetics of tacrolimus, cyclosporine and mycophenolate in renal transplant recipients. Nephrol Dial Transplant 23:1048–1053. doi:10.1093/ndt/gfm632

13. Amundsen R, Åsberg A, Robertsen I et al (2009) Rimonabant affects cyclosporine A, but not tacrolimus pharmacokinetics in renal transplant recipients. Transplantation 87:1221–1224. doi:10.1097/TP. 0b013e31819f1001

14. Midtvedt K, Jenssen T, Hartmann A et al (2011) No change in insulin sensitivity in renal transplant recipients converted from standard to once-daily prolonged release tacrolimus. Nephrol Dial Transplant 26: 3767–3772. doi:10.1093/ndt/gfr153

15. Marubashi S, Nagano H, Kobayashi S et al (2010) Evaluation of a new immunoassay for therapeutic drug monitoring of tacrolimus in adult liver transplant recipients. J Clin Pharmacol 50:705–709. doi: 10.1177/0091270009352188

16. Falck P, Åsberg A, Byberg KT et al (2008) Reduced elimination of cyclosporine A in elderly (>65 years) kidney transplant recipients. Transplantation 86:1379–1383. doi:10.1097/TP.0b013e31818aa4b6

17. Cheung CY, Op den Buijsch RA, Wong KM et al (2006) Influence of different allelic variants of the CYP3A and ABCB1 genes on the tacrolimus pharmacokinetic profile of Chinese renal transplant recipients. Pharmacogenomics 7:563–574. doi:10.2217/14622416.7.4.563

18. Karlsson MO, Sheiner LB (1993) The importance of modeling interoccasion variability in population pharmacokinetic analyses. J Pharmacokinet Biopharm 21:735–750

19. Kerbusch T, Wahlby U, Milligan PA, Karlsson MO (2003) Population pharmacokinetic modelling of darifenacin and its hydroxylated metabolite using pooled data, incorporating saturable first-pass metabolism, CYP2D6 genotype and formulation-dependent bioavailability. Br J Clin Pharmacol 56:639–652

20. Anderson BJ, Holford NH (2009) Mechanistic basis of using body size and maturation to predict clearance in humans. Drug Metab Pharmacokinet 24:25–36

21. Janmahasatian S, Duffull SB, Ash S et al (2005) Quantification of lean bodyweight. Clin Pharmacokinet 44:1051–1065

22. Størset E, Staatz C, Hennig S, et al (2013) Identification of continuous covariate relationships. Presented at the annual meeting of Population Approach Group of Australia and New Zealand (PAGANZ). Available at: http://www.paganz.org/abstracts/identification-of-continuous-covariate-relationships

23. Beal SL, Sheiner LB, Boeckmann AJ (1989–2006) NONMEM user's guides. Icon Development Solutions, Ellicott City, MD

24. Bergstrand M, Hooker AC, Wallin JE, Karlsson MO (2011) Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. AAPS J 13:143–151. doi:10.1208/s12248-011-9255-z

25. Wahlby U, Jonsson EN, Karlsson MO (2002) Comparison of stepwise covariate model building strategies in population pharmacokinetic-pharmacodynamic analysis. AAPS PharmSci 4:E27. doi:10.1208/ps040427

26. Efron B (1979) Bootstrap methods: another look at the jackknife. Ann Stat 7:1–26

27. Holford NHG (2011) Wings for NONMEM, v. 720 for NONMEM 7.2. Available at: http://wfn.sourceforge.net

28. Han N, Yun HY, Hong JY et al (2012) Prediction of the tacrolimus population pharmacokinetic parameters according to CYP3A5 genotype and clinical factors using NONMEM in adult kidney transplant recipients. Eur J Clin Pharmacol. doi:10.1007/s00228-012-1296-4

29. Woillard JB, de Winter BC, Kamar N et al (2011) Population pharmacokinetic model and Bayesian estimator for two tacrolimus formulations–twice daily Prograf and once daily Advagraf. Br J Clin Pharmacol 71:391–402. doi:10.1111/j.1365-2125.2010.03837.x

30. Benkali K, Premaud A, Picard N et al (2009) Tacrolimus population pharmacokinetic-pharmacogenetic analysis and Bayesian estimation in renal transplant recipients. Clin Pharmacokinet 48:805–816. doi: 10.2165/11318080-000000000-00000

31. de Jonge H, de Loor H, Verbeke K et al (2012) In vivo CYP3A4 activity, CYP3A5 genotype, and hematocrit predict tacrolimus dose requirements and clearance in renal transplant patients. Clin Pharmacol Ther 92:366–375. doi:10.1038/clpt.2012.109

32. Musuamba FTF, Mourad MM, Haufroid VV et al (2012) A simultaneous d-optimal designed study for population pharmacokinetic analyses of mycophenolic acid and tacrolimus early after renal transplantation. J Clin Pharmacol 52:1833–1843. doi:10. 1177/0091270011423661

33. Reidenberg MM, Odar-Cederlöf I, von Bahr C et al (1971) Protein binding of diphenylhydantoin and desmethylimipramine in plasma from patients with poor renal function. N Engl J Med 285:264–267. doi:10.1056/NEJM197107292850506

34. Musteata FM (2012) Calculation of normalized drug concentrations in the presence of altered plasma protein binding. Clin Pharmacokinet 51:55–68. doi:10.2165/11595650-000000000-00000

35. Press RR, Ploeger BA, den Hartigh J et al (2009) Explaining variability in tacrolimus pharmacokinetics to optimize early exposure in adult kidney transplant recipients. Ther Drug Monit 31:187–197. doi: 10.1097/FTD.0b013e31819c3d6d

36. Rodrigo E, de Cos MA, Sanchez B et al (2005) High initial blood levels of tacrolimus in overweight renal transplant recipients. Transplant Proc 37:1453–1454. doi:10.1016/j.transproceed.2005.02.055

37. Stratta P, Quaglia M, Cena T et al (2012) The interactions of age, sex, body mass index, genetics, and steroid weight-based doses on tacrolimus dosing requirement after adult kidney transplantation. Eur J Clin Pharmacol 68:671–680. doi:10.1007/s00228-011-1150-0

38. Kuypers DR, Claes K, Evenepoel P et al (2004) Time-related clinical determinants of long-term tacrolimus pharmacokinetics in combination therapy with mycophenolic acid and corticosteroids: a prospective study in one hundred de novo renal transplant recipients. Clin Pharmacokinet 43:741–762

39. Antignac M, Barrou B, Farinotti R et al (2007) Population pharmacokinetics and bioavailability of tacrolimus in kidney transplant patients. Br J Clin Pharmacol 64:750–757. doi:10.1111/j.1365-2125.2007.02895.x

40. Cotreau MM, von Moltke LL, Greenblatt DJ (2005) The influence of age and sex on the clearance of cytochrome P450 3A substrates. Clin Pharmacokinet 44:33–60

41. Jacobson PA, Schladt D, Oetting WS et al (2012) Lower calcineurin inhibitor doses in older compared to younger kidney transplant recipients yield similar troughs. Am J Transplant 12:3326–3336. doi:10.1111/j.1600-6143.2012.04232.x

42. Passey C, Birnbaum AK, Brundage RC et al (2011) Dosing equation for tacrolimus using genetic variants and clinical factors. Br J Clin Pharmacol 72:948–957. doi:10.1111/j.1365-2125.2011.04039.x

43. Tomita T, Homma M, Hasegawa Y et al (2008) Use of sample hematocrit value to correct blood tacrolimus concentration derived by microparticle enzyme immunoassay. Biol Pharm Bull 31:1250–1253

44. Ekberg H, Tedesco-Silva H, Demirbas A et al (2007) Reduced exposure to calcineurin inhibitors in renal transplantation. N Engl J Med 357:2562–2575. doi:10.1056/NEJMoa067411

45. Schiff J, Cole E, Cantarovich M (2007) Therapeutic monitoring of calcineurin inhibitors for the nephrologist. Clin J Am Soc Nephrol 2:374–384. doi:10.2215/CJN.03791106