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ORIGINAL ARTICLE

A population pharmacokinetic model to predict the individual starting dose of tacrolimus in adult renal transplant recipients

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AIMS

The aims of this study were to describe the pharmacokinetics of tacrolimus immediately after kidney transplantation, and to develop a clinical tool for selecting the best starting dose for each patient.

METHODS

Data on tacrolimus exposure were collected for the first 3 months following renal transplantation. A population pharmacokinetic analysis was conducted using nonlinear mixed-effects modelling. Demographic, clinical and genetic parameters were evaluated as covariates.

RESULTS

A total of 4527 tacrolimus blood samples collected from 337 kidney transplant recipients were available. Data were best described using a two-compartment model. The mean absorption rate was 3.6 h^{-1}, clearance was 23.0 l h^{-1} (39% interindividual variability, IIV), central volume of distribution was 692 l (49% IIV) and the peripheral volume of distribution 5340 l (53% IIV). Interoccasion variability was added to clearance (14%). Higher body surface area (BSA), lower serum creatinine, younger age, higher albumin and lower haematocrit levels were identified as covariates enhancing tacrolimus clearance. Cytochrome P450 (CYP) 3A5 expressers had a significantly higher tacrolimus clearance (160%), whereas *CYP3A4*22* carriers had a significantly lower clearance (80%). From these significant covariates, age, BSA, *CYP3A4* and *CYP3A5* genotype were incorporated in a second model to individualize the tacrolimus starting dose:

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$$\text{Dose (mg)} = 222\ \text{ng h ml}^{-1} * 22.5\ \text{l h}^{-1} * [(1.0, \text{if CYP3A5}*3/*3) \text{ or } (1.62, \text{if CYP3A5}*1/*3 \text{ or CYP3A5}*1/*1)] * [(1.0, \text{if CYP3A4}*1 \text{ or unknown}) \text{ or } (0.814, \text{if CYP3A4}*22)] * \left(\frac{\text{Age}}{56}\right)^{-0.50} * \left(\frac{\text{BSA}}{1.93}\right)^{0.72} / 1000$$

Both models were successfully internally and externally validated. A clinical trial was simulated to demonstrate the added value of the starting dose model.

CONCLUSIONS

For a good prediction of tacrolimus pharmacokinetics, age, BSA, *CYP3A4* and *CYP3A5* genotype are important covariates. These covariates explained 30% of the variability in *CL/F*. The model proved effective in calculating the optimal tacrolimus dose based on these parameters and can be used to individualize the tacrolimus dose in the early period after transplantation.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Patients with low tacrolimus predose concentrations are at an increased risk for rejection while those with high predose concentrations are at a higher risk of toxicity. Achieving the therapeutic range is important, but it can take up to two weeks.
- In clinical practice only 37% of patients are directly within the target range at first steady state.
- Two externally validated models to predict the starting dose of tacrolimus have been published. One of these was prospectively tested and could not predict the tacrolimus exposure.

WHAT THIS STUDY ADDS

- In the first 3 months post-transplantation, age, albumin, body surface area, serum creatinine, *CYP3A5* genotype, *CYP3A4* genotype, haematocrit and lean bodyweight significantly influence the pharmacokinetics of tacrolimus in adult renal transplant recipients.
- A separate model for the starting dose was developed:

$$\textit{Dose (mg)} = 222\ \text{ng h ml}^{-1} * 22.5\ \text{l h}^{-1} * [(1.0, \textit{if CYP3A5}*3/*3) \textit{ or } (1.62, \text{if CYP3A5}*1/*3 \text{ or CYP3A5}*1/*1)] * [(1.0, \textit{if CYP3A4}*1 \text{ or unknown}) \textit{ or } (0.814, \text{if CYP3A4}*22)] * \left(\frac{\textit{Age}}{56}\right)^{-0.50} * \left(\frac{\textit{BSA}}{1.93}\right)^{0.72} / 1000$$

- The tacrolimus starting dose should be higher in *CYP3A5* expressers, younger patients and those with a higher body surface area (BSA). It should be lower in patients carrying the *CYP3A4*22* allele.
- The starting dose model can be used to individualize the tacrolimus starting dose following kidney transplantation.

Introduction

Tacrolimus is the most used immunosuppressive drug to prevent acute rejection following renal transplantation [1]. Short-term kidney allograft survival has greatly improved with the use of immunosuppressive drug combination therapy [2, 3]. However, prolonged use of immunosuppressive drugs leads to substantial toxicity, including increased rates of infections, hypertension, post-transplant diabetes mellitus, neurotoxicity and nephrotoxicity [4–7]. These adverse events augment the limited long-term renal allograft survival and the high cardiovascular morbidity and mortality of transplant recipients [8, 9]. Rejection rates and most of the adverse events seem to be concentration related, with higher tacrolimus concentrations being related to toxicity and lower concentrations to an increased risk of acute rejection [10, 11].

The use of tacrolimus is hampered by its narrow therapeutic index with large intra- and interpatient variability in its pharmacokinetics that requires therapeutic drug monitoring (TDM) to individualize the dose to prevent toxicity and rejection [11]. Multiple factors influence the clearance (CL) of tacrolimus, including cytochrome P450 (CYP) 3A genotype [12, 13], haematocrit [14], age [10, 15], bodyweight, ethnicity [16, 17] and drug–drug interactions [18]. In routine clinical practice, the tacrolimus starting dose is based solely on bodyweight, even though the available evidence is scarce [19]. Pharmacokinetic models have conflicting results demonstrating that bodyweight does [20–24] or does not [10, 25–27] have a statistically significant influence on the clearance of tacrolimus. Subsequent doses are adjusted by means of TDM, which limits the time a patient is exposed to concentrations outside the target range, although it may take up to 14 days to reach the target exposure [24]. Therefore, patients are at an increased risk of sub- or supratherapeutic tacrolimus exposure during these first weeks after transplantation, and may have an increased risk of developing adverse events [28].

A population pharmacokinetic model with clinically relevant covariates may help predict an individual's tacrolimus pharmacokinetics and can be applied prior to the start of therapy to reach target exposure as soon as possible [29]. To date, several models to predict the tacrolimus starting dose have been developed for adult [10, 12, 14, 20–23, 26, 27, 30] and paediatric renal transplant recipients [31]. Of these adult models, only two were successfully externally validated in an independent dataset [10, 12]. One of these models was subsequently prospectively tested by another research group in a completely new population. Unfortunately, this model was unable to successfully predict tacrolimus exposure [32]. The other externally validated model had several shortcomings, including flip-flop kinetics, where the absorption constant is much slower than the elimination constant. Besides this, the external validation cohort had its limitations as only patients 1 month post-transplant were included and only five were *CYP3A4*22* carriers [12]. The algorithm by Chen *et al*. was not externally validated but was prospectively tested in a randomized clinical trial in Chinese patients [22]. Unfortunately, an algorithm designed for Asian patients cannot be extrapolated to Caucasian transplant populations.

The aim of the current study was to describe the population pharmacokinetics of twice-daily, immediate-release tacrolimus in the first 3 months following renal transplantation, and to develop a dosing algorithm for the starting dose. In contrast to previous studies, many covariates were tested (including *CYP3A* genotype, haematocrit and age), a rich database was used [for 100 patients a full area under the concentration *vs*. time curve (AUC) was available], and the model was extensively validated, both internally and externally. A separate starting dose model was developed.

Methods

Study design

The model building cohort consisted of a total of 337 patients. Of these patients, 237 were transplanted in the Erasmus MC and participated in a randomized–controlled clinical trial (RCT; Rotterdam cohort) [33]. For these patients, additional pharmacokinetic data were retrospectively retrieved from the medical records. The Ethics Review Board of the Erasmus MC provided a waiver for the Medical Research Involving Human Subjects Act, for this study (Medical Ethical Review Board number 2017–1029).

The remaining 100 patients were transplanted in the Leiden University Medical Center (LUMC, Leiden cohort) [34]. The inclusion criteria and patient demographics of these two cohorts have been described previously [33, 34]. All clinical data were collected from 24 h before transplantation until 3 months post-transplantation.

External validation of the pharmacokinetic model was performed on an independent dataset consisting of 304 adult renal transplant recipients (validation cohort). This cohort has been described previously [12]. These patients were not included in the initial model building cohort.

Immunosuppression

All patients were treated with oral twice-daily tacrolimus (Prograft, Astellas Pharma, Leiden, The Netherlands) in combination with mycophenolic acid. Tacrolimus doses were tailored using TDM. The tacrolimus predose concentration (C_0) was measured for the first time on day 3 following transplantation in the Rotterdam cohort [33]. In the Leiden cohort, blood samples were drawn before tacrolimus ingestion, and 1, 2, 3, 4, 5 and 6 h postingestion. This is routine clinical care in Leiden. In the Leiden cohort, tacrolimus concentrations for the pharmacokinetic curve were obtained at steady state, with a median of 2 weeks after transplantation. The validation cohort consisted of 304 patients, of whom seven participated in the Symphony-Elite study. In this study, blood samples were drawn before tacrolimus ingestion, and 0.3, 0.7, 1.3, 2, 3, 4, 6, 8, 10 and 12 h postingestion [35]. For the remaining 297 patients, only C_0 was available [12].

In the Rotterdam cohort, the target tacrolimus C_0 range was 10.0–15.0 ng ml^{-1} in week 1–2, 8.0–12.0 ng ml^{-1} in weeks 3–4, and 5.0–10.0 ng ml^{-1} after week 4 post transplantation. In the Leiden cohort, the target AUC was 210 ng h ml^{-1} with a corresponding C_0 range of 10.0–15.0 ng ml^{-1} the first 6 weeks following transplantation. After week 6 post transplantation, the target AUC was 125 ng h ml^{-1} with a corresponding C_0 range of 4.0–9.0 ng ml^{-1}.

Laboratory analysis

Genotyping for *CYP3A5*3* and *CYP3A4*22* was performed as described previously [33, 34]. Deviations from Hardy–Weinberg equilibrium were tested using the χ^2 goodness-of-fit (GOF) test. Tacrolimus concentrations in the Rotterdam cohort were analysed in whole-blood samples using two different immunoassays: the antibody-conjugated magnetic immunoassay (ACMIA) and the enzyme multiplied technique (EMIT), as described previously [33]. The lower limits of quantification were 1.5 ng ml^{-1} (ACMIA) and 2.0 ng ml^{-1} (EMIT). The upper limit of quantification was 30.0 ng ml^{-1}. Tacrolimus concentrations in the Leiden cohort were measured using a validated liquid chromatography–tandem mass spectrometry (LC–MS/MS) method [34]. The lower limit of quantification was 1.0 ng ml^{-1} and the upper limit of quantification 50.0 ng ml^{-1}. In the validation cohort, samples were measured using a validated LC–MS/MS [12].

Population pharmacokinetic modelling

Pharmacokinetic analysis was conducted by nonlinear mixed-effects modelling using NONMEM version 7.2 (FOCE+I, ICON Development Solutions, Ellicott City, MD, USA) and PsN version 4.6.0. Pirana software was used as an interface between NONMEM, R (version 3.2.2) and Xpose (version 4).

Base model development. One- and two-compartment models were considered based on visual inspection of the data and a review of the literature. Typical values for lag-time (t_{lag}), absorption rate constant (k_a), central volume of distribution (V_1), peripheral volume of distribution (V_2), CL and intercompartmental clearance (Q) were estimated. As bioavailability (F) could not be estimated, F was fixed to 1 and certain values were estimated as ratios: *CL/F*, *Q/F*, V_1/F

and V_2/F. Interindividual variability (IIV) and interoccasion variability (IOV) were modelled for each pharmacokinetic parameter using an exponential model. An occasion was defined as the measurement of a C_0. Residual variability was incorporated as an additive and proportional error for immunoassay, and as a proportional error for LC–MS/MS. For all model parameters for which IIV was estimated, shrinkage was calculated. A shrinkage value below 25% was considered acceptable [36]. Minimum objective function values (OFVs, $P < 0.01$), parameter precision, error estimates, shrinkage value and visual inspection of the GOF plots were considered for model selection.

Covariate model development. Covariates were selected based on their known or theoretical relationships with tacrolimus pharmacokinetics and theoretical plausibility. The following demographic, clinical and genetic characteristics were evaluated as potential model covariates: weight, height, time post-transplant, sex, age, ethnicity, haematocrit, creatinine, estimated glomerular filtration rate (Cockcroft–Gault and Modification of Diet in Renal Disease), aspartate aminotransferase, albumin, C-reactive protein, total protein, bilirubin, *CYP3A4* genotype, *CYP3A5* genotype, combination of *CYP3A4* and *CYP3A5* (as described previously [12]), *ABCB1* (previously known as multidrug resistance-1) genotype 3435C > T polymorphism, *P-450 oxidoreductase*28* (*POR*) genotype, comedication known to interact with tacrolimus (calcium channel blockers, glucocorticoids), glucocorticoid dose, primary kidney disease, number of previous kidney transplantations, renal replacement therapy prior to transplantation (pre-emptive, haemodialysis or peritoneal dialysis), delayed graft function, human leucocyte antigen mismatches, panel reactive antibodies, body mass index, lean body weight (LBW), ideal bodyweight, fat mass and body surface area.

First, the relationship between IIV and covariates was investigated graphically. Covariates with a visually apparent relationship and a clinically plausible relationship with the pharmacokinetic parameter were univariately added to the model. Covariates included in previously published population pharmacokinetic models were also univariately added to the model, regardless of the visually apparent relationship. A univariate analysis was performed to determine which covariates improved the model ($P < 0.05$). The stepwise covariate modelling with forward inclusion-backward elimination method was used [37]. Covariates that significantly improved the model ($P < 0.05$, i.e. decrease in OFV of 3.84) were added to the full model. A backward elimination process with a stricter statistical criterion was then performed ($P < 0.01$, i.e. increase in OFV of 6.64). A shark plot was generated for each covariate for case-deletion diagnostics.

Internal model evaluation. The model was validated using a prediction corrected visual predictive check (VPC) by simulating 500 datasets, and a normalized prediction distribution errors (NPDE) analysis (1000 simulations). The VPC was stratified for the covariates included in the final model.

Simulations were performed with the final model with different values of the covariate to evaluate the effect of significant covariates. All simulated patients received 0.2 mg kg^{-1} divided into two equal doses. Concentration–time profiles were simulated for 1000 patients for each included covariate. All other parameters were fixed to the median.

External model evaluation. An independent dataset consisting of 340 adults treated with the same immunosuppressive regimen was used for external validation using a VPC. The VPC was prediction corrected and stratified for the covariates included in the final model.

Statistical analyses other than those mentioned above, were performed using SPSS version 23 (SPSS Inc., Chicago, IL, USA). Data on patients' baseline characteristics are presented as median value and range for continuous variables.

Starting dose model. The final model was used to develop a model for the starting dose of tacrolimus after kidney transplantation. Each significant covariate in the final model was evaluated if it was clinically relevant, feasible to use, and if it significantly influenced the starting dose of tacrolimus. The starting dose model was then validated using the techniques mentioned in sections Internal Model Evaluation and External Model Evaluation.

Simulation trial. To demonstrate the added value of the starting dose model, a clinical trial was simulated using the patient characteristics of those included in the model building cohort. Each patient was given the standard bodyweight dose and a dose based on the starting dose model calculated using equation (3). For each patient, the C_0 and AUC were simulated 1000 times at day 10 post transplantation.

Results

A total of 337 patients were included in the model building group. Patient characteristics are presented in Table 1. From these patients, 4527 blood samples were collected and analysed for tacrolimus concentrations (range 1.6–96.0 ng ml^{-1}). A quarter of the blood samples in the model building groups was drawn the first week following transplantation. In total, three samples (0.07%) in the Rotterdam cohort were below the lower limit of quantification of the immunoassay and were discarded. A total of 40 samples (0.88%) in the Rotterdam cohort were above the upper limit of quantification. These samples were estimated by NONMEM and after every critical model building step checked if the estimate was plausible. The allele frequencies of the tested single-nucleotide polymorphisms are depicted in Table 1. There was no deviation from the Hardy–Weinberg equilibrium.

Base model

The data were best described by a two-compartment model with first order absorption. Including IIV on CL/F, V_1/F, V_2/F and Q/F significantly improved the model fit. The OFV decreased further, and parameter precision, error estimates and GOF plots improved after introduction of IOV on CL/F. Building the different analytical techniques for tacrolimus into the

Table 1

Patient characteristics

	Model building group 1 (n = 237)	Model building group 2 (n = 100)	Model validation group (n = 304)
Recipient sex			
Male	148 (62.4%)	56 (56.0%)	200 (65.8%)
Age of recipient (years)	58.5 (19.4–79.4)	54.0 (15.0–77.0)	52.0 (17.0–83.0)
Ethnicity			
Caucasian	186 (78.4%)	78 (78.0%)	304 (100%)
Asian	23 (9.7%)	8 (8.0%)	0 (0%)
African descent	23 (9.7%)	1 (1.0%)	0 (0%)
Other	5 (2.1%)	13 (13.0%)	0 (0%)
Bodyweight (kg)*	79.4 (37.6–132.0)	74.0 (40.0–114.0)	68.0 (40.0–106.0)
Height (cm)*	183 (145–203)	172 (141–195)	166 (145–190)
Body mass index (kg m^{-2})	25.8 (17.2–42.2)	24.8 (15.6–38.2)	24.7 (16.3–44.1)
Body surface area (m^2)	2.03 (1.24–2.66)	1.90 (1.33–2.48)	1.78 (1.18–2.36)
Ideal bodyweight (kg)	68.3 (46.8–89.8)	65.3 (41.7–83.9)	60.9 (45.7–80.2)
Lean bodyweight (kg)	64.0 (33.1–85.3)	55.9 (33.6–81.7)	51.4 (34.9–76.3)
Fat mass (kg)	21.7 (12.0–44.0)	26.0 (14.1–49.5)	25.5 (11.3–50.2)
Laboratory measurements*			
Haematocrit (l l^{-1})	0.34 (0.15–0.80)	0.34 (0.24–0.45)	0.33 (0.18–0.59)
Creatinine (μmol l^{-1})	137 (38–1885)	124 (62–920)	139 (47–1284)
ASAT (U l^{-1})	21 (<5–662)	Unknown	Unknown
Albumin (g l^{-1})	42 (12–57)	Unknown	Unknown
Bilirubin (μmol l^{-1})	6 (<2–305)	Unknown	Unknown
Total protein (g l^{-1})	64 (23–86)	Unknown	Unknown
CRP (mg l^{-1})	11 (<0.3–320)	Unknown	Unknown
Genotype			
CYP3A4			
****1***	205 (86.5%)	91 (91.0%)	275 (90.5%)
****22***	22 (9.3%)	9 (9.0%)	29 (9.5%)
Unknown	10 (4.2%)	0 (0%)	0 (0%)
CYP3A5			
****1/*1***	9 (3.8%)	4 (4.0%)	0 (0%)
****1/*3***	56 (23.6%)	17 (17.0%)	49 (16.1%)
****3/*3***	172 (72.6%)	76 (76.0%)	255 (83.9%)
****3/*6***	0 (0%)	3 (3.0%)	0 (0%)
***ABCB1* 3435C > T**			
CC	55 (24.3%)	Unknown	Unknown
CT	111 (49.1%)	Unknown	Unknown
TT	60 (26.5%)	Unknown	Unknown

(continues)

Table 1

(Continued)

	Model building group 1 (*n* = 237)	Model building group 2 (*n* = 100)	Model validation group (*n* = 304)
POR*28			
CC	128 (56.4%)	Unknown	Unknown
CT	78 (34.4%)	Unknown	Unknown
TT	21 (9.3%)	Unknown	Unknown
Primary diagnosis			
Diabetic nephropathy	48 (20.3%)	21 (21.0%)	16 (5%)
Polycystic kidney disease	39 (16.5%)	15 (15.0%)	36 (12%)
Glomerulonephritis	44 (18.6%)	15 (15.0%)	97 (32%)
Hypertensive nephropathy	42 (17.7%)	15 (15.0%)	16 (5%)
Reflux/chronic pyelonephritis	23 (9.7%)	3 (3.0%)	0 (0%)
Other	20 (8.4%)	26 (26.0%)	51 (17%)
Unknown	21 (8.9%)	5 (5.0%)	88 (29%)
Number of kidney transplantations			
1	218 (92.0%)	Unknown	243 (80%)
2	16 (6.8%)	Unknown	49 (16%)
3 or more	3 (1.3%)	Unknown	12 (4%)
RRT prior to transplantation			
Haemodialysis	90 (38.0%)	Unknown	Unknown
Peritoneal dialysis	44 (18.6%)	Unknown	Unknown
Pre-emptive	102 (43.0%)	Unknown	Unknown
Delayed graft function			
Yes	11 (4.6%)	Unknown	Unknown
No	224 (94.5%)	Unknown	Unknown
Unknown	2 (0.8%)	Unknown	Unknown
Co-medication			
Calcium channel blockers		Unknown	Unknown
Amlodipine	25 (10.5%)		
Nifedipine	44 (18.6%)		
Barnidipine	2 (0.8%)		
Time of tacrolimus concentration measurement (days after transplantation)	23.7 (0.7–99.9)	7.2 (3–100)	30 (6–97.5)
Distribution of tacrolimus samples			
Total samples	3661	866	1334
0–7 days post-transplantation	734 (20.0%)	359 (41.5%)	287 (21.5%)
8–14 days post-transplantation	642 (17.5%)	244 (28.2%)	60 (4.5%)
15–30 days post-transplantation	722 (19.7%)	113 (13.0%)	604 (45.3%)
31–100 days post-transplantation	1563 (42.7%)	150 (17.3%)	383 (28.7%)

Model building group 1 consists of patients transplanted in the Erasmus MC (Rotterdam cohort). Model building group 2 consists of patients transplanted in the LUMC (Leiden cohort)

ASAT, aspartate aminotransferase; CRP, C-reactive protein; CYP, cytochrome P450; POR*28, P-450 oxidoreductase*28; RRT, renal replacement therapy

*Presented as median and range over a 3-month period for continuous variables

residual error model improved the base model. The residual error was described with a combined additive and proportional error model for the immunoassay measured concentrations, and with a separate proportional error model for the LC–MS/MS measured concentrations. Parameter estimates of the base model, final model and simulation model are presented in Table 2.

Covariate analysis

The base two-compartment model with IOV on *CL/F* was used as a reference for the covariate analysis. After graphical analysis, the univariate analysis resulted in seven significant covariates correlated with *CL/F*. The covariates were added in the following order: haematocrit (dOFV 94.0), *CYP3A5* genotype (dOFV 74.0), albumin (dOFV 71.9), creatinine (dOFV 36.0), age (dOFV 32.7), *CYP3A4* genotype (dOFV 8.2) and body surface area (BSA; dOFV 19.2). Based on graphical analysis there was no difference in the effect on *CL/F* between CYP3A5 expressers (*CYP3A5*1/*1* and *CYP3A5*1/**3). LBW significantly influenced V_1/F (dOFV 24.3). After forward inclusion-backward elimination (stepwise covariate modelling method) [37], the covariates remained in the final model.

Table 2

Parameter estimates of the base model, final model and bootstrap analysis

Parameter	Base model (RSE %) [shrinkage]	Final model (RSE %) [shrinkage]	Starting dose model (RSE %) [shrinkage]
t_{lag} (h)	0.29 (17)	0.38 (49)	0.39 (12)
k_a ($l\ h^{-1}$)	3.26 (19)	3.58 (40)	3.70 (13)
CL/F ($l\ h^{-1}$)	25.9 (3)	23.0 (3)	22.5 (3)
V_1/F (l)	655 (7)	692 (8)	685 (5)
Q/F ($l\ h^{-1}$)	10.5 (7)	11.6 (10)	10.6 (6)
V_2/F (l)	6320 (14)	5340 (22)	6590 (14)
Covariate effect on CL			
*CYP3A5*1*	-	1.63 (15)	1.62 (14)
*CYP3A4*22*	-	0.80 (32)	0.81 (36)
Haematocrit ($l\ l^{-1}$)	-	−0.76 (11)	-
Creatinine ($\mu mol\ l^{-1}$)	-	−0.14 (26)	-
Albumin ($g\ l^{-1}$)	-	0.43 (30)	-
Age (years)	-	−0.43 (19)	−0.50 (15)
BSA (m^2)	-	0.88 (24)	0.72 (29)
Covariate effect on V_1			
Lean bodyweight (kg)	-	1.52 (20)	-
IIV (%)			
CL/F	46.3 (5) [10]	38.6 (6) [8]	39.4 (6) [10]
V_1/F	50.2 (11) [19]	49.2 (7) [25]	54.0 (11) [19]
V_2/F	52.3 (14) [38]	53.0 (16) [39]	53.7 (13) [40]
Q/F	79.6 (12) [29]	78.7 (11) [28]	79.6 (11) [29]
IOV (%)			
CL/F	15.1 (9)	13.6 (10)	14.6 (9)
Residual variability			
Proportional (%)			
Immunoassay	16.6 (6) [26]	17.7 (7) [22]	16.9 (6) [25]
LC–MS/MS	24.7 (5) [12]	24.5 (5) [12]	24.4 (5) [12]
Additive Immunoassay ($\mu g\ l^{-1}$)	1.02 (9) [26]	0.88 (13) [22]	1.02 (10) [25]

CL, clearance; *CYP*, cytochrome P450; F, bioavailability of oral tacrolimus; IIV, interindividual variability; IOV, interoccasion variability; K_a, absorption rate constant; LC–MS/MS, liquid chromatography–tandem mass spectrometry; OFV, objective function value; Q, intercompartmental clearance of tacrolimus; RSE, residual standard error; t_{lag}, lag time; V_1, central compartment for tacrolimus; V_2, peripheral compartment for tacrolimus

Equation (1) describes the final model for estimation of tacrolimus *CL/F* (l h^{-1}) in the first 3 months post-transplant:

$$
\begin{aligned}
{}^{CL}/_{F} = {} & 23*[(1.0, \text{if CYP3A5}*3/*3)\ or \\
& (1.631, \text{if CYP3A5}*1/*3 \text{ or CYP3A5}*1/*1)] \\
& *[(1.0, \text{if CYP3A4}*1 \text{ or unknown})\ or \\
& (0.8, \text{if CYP3A4}*22)]*\left(\frac{Age}{56}\right)^{-0.43}*\left(\frac{Albumin}{42}\right)^{0.43} \\
& *\left(\frac{BSA}{1.93}\right)^{0.88}*\left(\frac{Creatinine}{135}\right)^{-0.14}*\left(\frac{Hematocrit}{0.34}\right)^{-0.76}
\end{aligned}
\tag{1}
$$

The NONMEM control stream for the analysis has been included in the Supporting Information Data S1.

Evaluation of the final model

All estimates were within the limits, given the criteria as defined in the Methods section, with the exception of shrinkage for V_2 and Q. The population and individual predictions were evenly distributed around the line of unity. The conditional weighted residuals were normally distributed. (Figure 1). The median and variability of the C_0 fell mostly within the corresponding simulations as shown in the VPCs, with concentrations in simulations slightly lower than the measured concentrations approximately 2.5–4 h after dose (Figure 2A). NPDE analysis showed adequate predictive ability with distribution of the NPDEs within an acceptable deviation from a normal distribution (Supporting Information Data S2). Evaluation of the individual's influence on a change in OFV by shark plot showed that 73% of patients had a decrease in OFV with the final model compared with the base model. In the external validation, the median of the observed data was close to the lower bound of the simulated data in the second half of the curve. However, for an external validation in clinical data, the median was acceptably described (Figure 2B). Unfortunately, we had no albumin levels at our disposal in the external validation cohort and therefore we fixed the albumin concentration to the population albumin median in the external validation.

Simulations

The effects of the significant covariates on *CL/F* and are shown in Figure 3. Based on the final model, *CYP3A5* expressers had a 1.6 times higher CL/*F*. Patients carrying the *CYP3A4*22* allele had a 0.8 times lower CL/*F*. An increase in age from 25 to 65 years resulted in a 34% lower CL/*F*, whereas a decrease in BSA from 2.25 to 1.5 resulted in a 43% lower CL/*F*. In total, these covariates explained 30% of the variability in *CL/F* of tacrolimus.

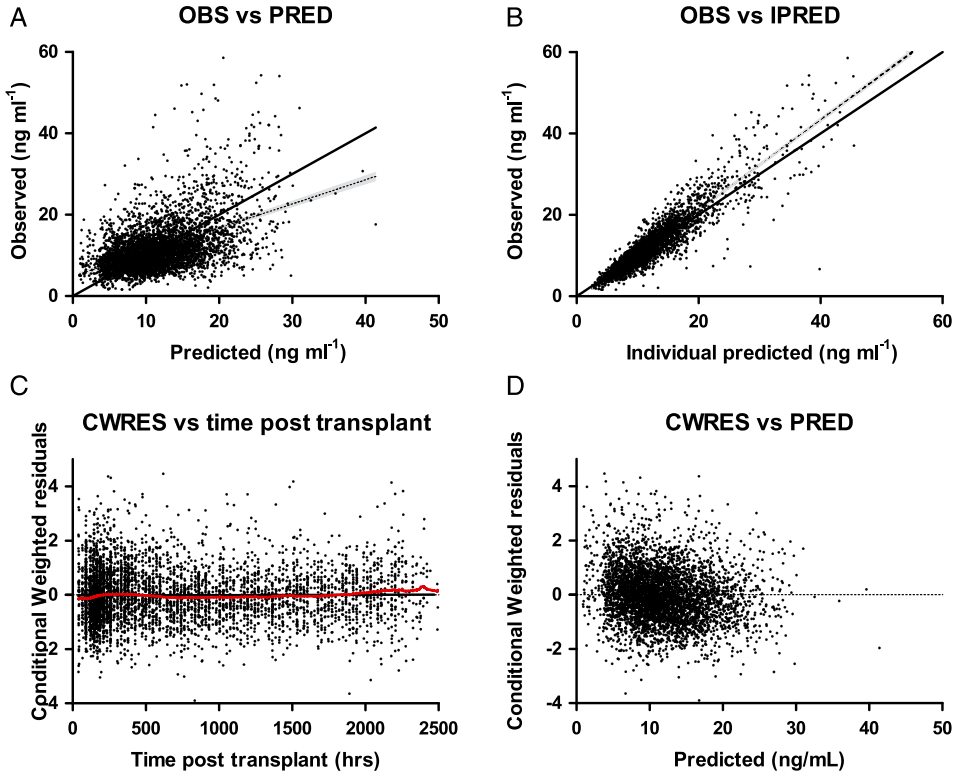

Figure 1

Goodness-of-fit plots of the final model. (A) DV plotted against PRED. (B) DV plotted against IPRED. (C) The correlation of CWRES with the time after the tacrolimus dose. (D) The correlation of CWRES with PRED. The line represents the line of identity. CWRES, conditional weighted residuals; DV, observed concentrations; IPRED, individual predicted concentration; OBS, observed concentration; PRED, predicted concentration

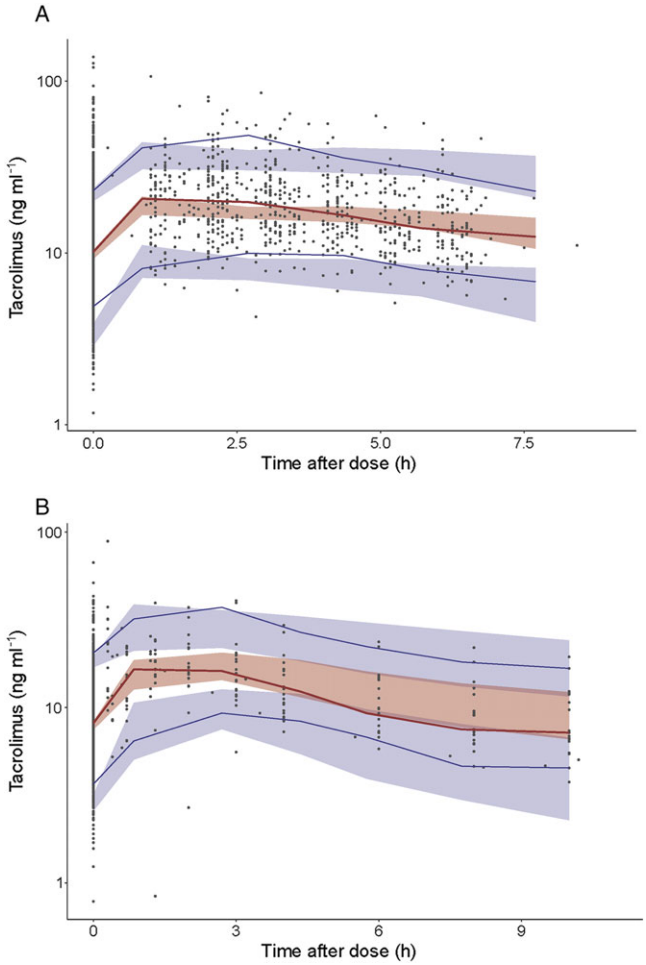

Figure 2

Prediction-corrected visual predictive check (VPC) showing how well the average trend of the observations (red line) and how well the variability of the observed data (blue lines) fall within the model simulated (n = 500) average trend (red shaded area) and the model simulated variability (blue shaded areas) represented as 95% confidence interval. The average and the variability of the observed data both fall within the corresponding simulations. (A) Prediction-corrected VPC of the final model (internal dataset). (B) Prediction-corrected VPC of the final model (external dataset)

Starting dose model

The final model was used to develop a model for the starting dose of tacrolimus after kidney transplantation. Time after transplantation was not a significant covariate, therefore the starting dose model was based on the same data as the final model. As in clinical practice C_0 is commonly used, and CL is the main parameter that influences C_0, only those covariates significantly influencing *CL/F* were included in the starting dose model. The last measured albumin, serum creatinine and haematocrit before transplantation did not significantly influence the *CL/F*, and because these parameters change substantially after transplantation, they were also not incorporated in the starting dose model. Equation (2) describes the estimation of tacrolimus *CL/F* (l h^{-1}) right after transplantation:

$$
\begin{aligned}
{}^{CL}/_{F} = {} & 22.5*[(1.0, \text{if CYP3A5}*3/*3)\ \textit{or} \\
& (1.62, \text{if CYP3A5}*1/*3 \text{ or CYP3A5}*1/*1)] \\
& *[(1.0, \text{if CYP3A4}*1 \text{ or unknown})\ \textit{or} \\
& (0.814, \text{if CYP3A4}*22)]*\left(\frac{Age}{56}\right)^{-0.50}*\left(\frac{BSA}{1.93}\right)^{0.72}
\end{aligned}
\tag{2}
$$

The median and variability of the C_0 fell mostly within the corresponding simulations as shown in the VPCs, demonstrating the good predictive performance in the internal validation (Figure 4A). In the external validation, both the median and variability were adequately described (Figure 4B).

The required dose can be calculated using the equation: Dose = *CL/F* * AUC. In our study, a tacrolimus C_0 of 10 ng ml^{-1} corresponded with an $AUC_{0\text{-}12h}$ of 222 ng h ml^{-1}, 12.5 ng ml^{-1} with 277 ng h ml^{-1}, and 15 ng ml^{-1} with 332 ng h ml^{-1}. This leads to equation (3) for a target C_0 of 10 ng ml^{-1} based on a twice daily dose:

$$
\begin{aligned}
Dose\ (mg) = {} & 222 \text{ ng h ml}^{-1} * 22.5 \text{ l h}^{-1} *[(1.0, \textit{if CYP3A5}*3/*3) \\
& \textit{or } (1.62, \text{if CYP3A5}*1/*3 \text{ or CYP3A5}*1/*1)] \\
& *[(1.0, \textit{if CYP3A4}*1 \text{ or unknown})\ \textit{or} \\
& (0.814, \text{if CYP3A4}*22)]*\left(\frac{Age}{56}\right)^{-0.50} \\
& *\left(\frac{BSA}{1.93}\right)^{0.72}/1000
\end{aligned}
\tag{3}
$$

The NONMEM control stream for the analysis is shown in the Supporting Information Data S1.

Simulation trial

The results are shown in Figure 5. In the standard bodyweight-based group, 26.1% were on target (10–15 ng ml^{-1}) [41] *vs.* 33.0% in the model-based group. In the bodyweight-based group, 44.5% were above target compared with 36.8% in the model-based group. The median tacrolimus C_0 in the bodyweight-based dose group was 13.9 ng ml^{-1}, and in the model-based dose group 12.9 ng ml^{-1}. There were fewer extreme concentrations in the model-based dose group, with 5.2% markedly subtherapeutic (<5 ng ml^{-1}) compared to 7.2% in the bodyweight-based group. In the model-based dose group, 15.6% were markedly supratherapeutic (>20 ng ml^{-1}) compared to 24.6% in the bodyweight-based group.

Discussion

This study demonstrates that multiple clinical (albumin, creatinine, haematocrit), demographic (age, BSA, LBW), and genetic (*CYP3A4* and *CYP3A5* genotype) factors significantly influence the pharmacokinetics of tacrolimus in the first 3 months following renal transplantation. Together, these covariates explained 30% of the total variability in tacrolimus *CL/F*. A model for the starting dose was developed incorporating *CYP3A5* genotype, *CYP3A4* genotype, age and BSA. A simulation showed that more patients were on target

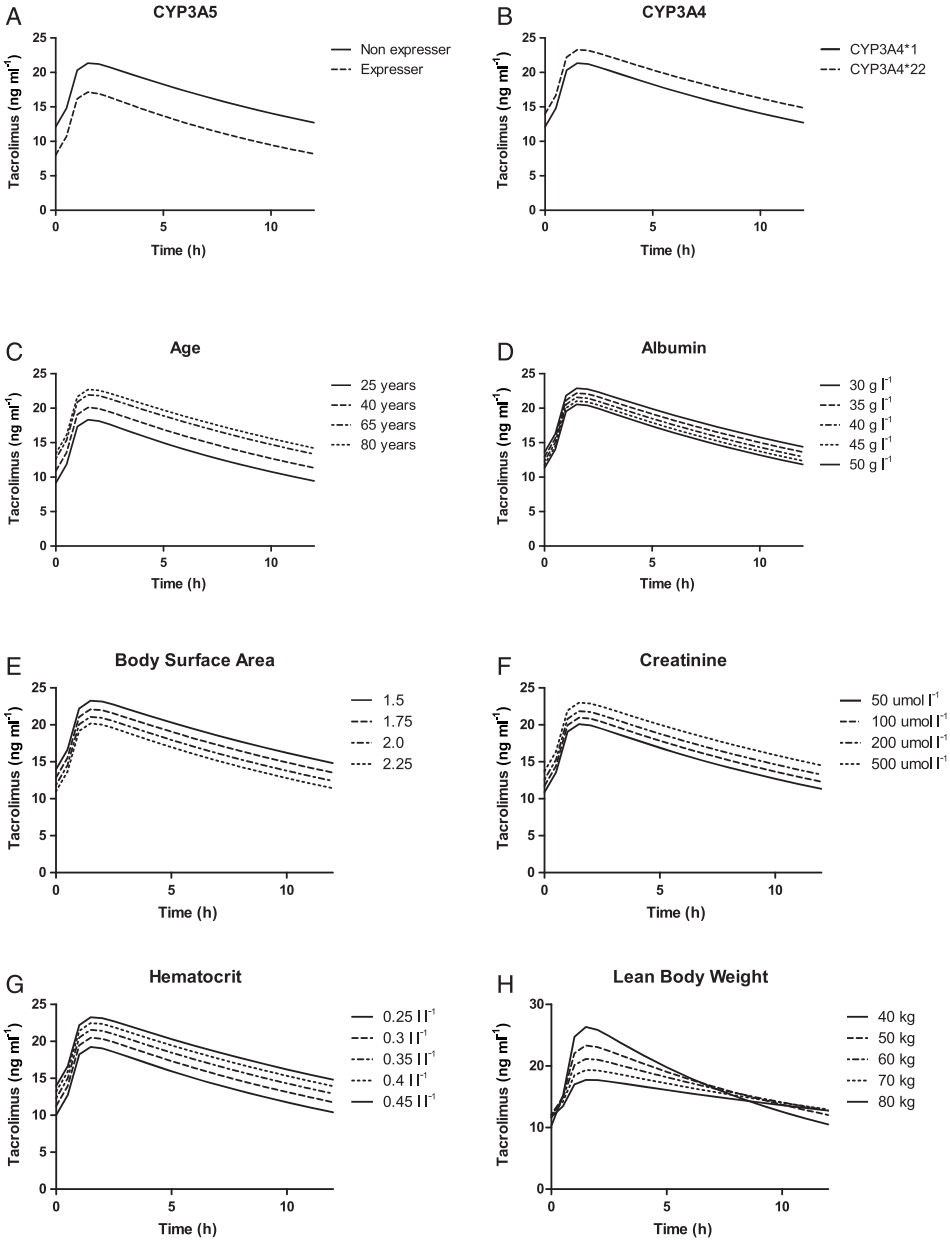

Figure 3

Simulated plasma profiles of tacrolimus at first steady state after transplantation. (A) Simulated plasma profiles of tacrolimus for CYP3A5 nonexpressers (*CYP3A5*3/*3)* and CYP3A5 expressers (*CYP3A5*1/*1* or *CYP3A5*1/*3*). (B) Simulated plasma profiles of tacrolimus for patients carrying the *CYP3A4*1* allele and the *CYP3A4*22* allele. (C) Simulated plasma profiles of tacrolimus for patients aged 25, 40, 65 and 80 years. (D) Simulated plasma profiles of tacrolimus for patients with albumin levels of 30, 35, 40, 45 and 50 g l^{-1}. (E) Simulated plasma profiles of tacrolimus for patients with a BSA of 1.5, 1.75, 2 and 2.25 m^2. (F) Simulated plasma profiles of tacrolimus for patients with creatinine concentrations of 50, 100, 200 and 500 μmol l^{-1}. (G) Simulated plasma profiles of tacrolimus for patients with haematocrit levels of 0.25, 0.3, 0.35, 0.4 and 0.45 l l^{-1}. (H) Simulated plasma profiles of tacrolimus for patients with an LBW of 40, 50, 60, 70 and 80 kg. BSA, body surface area; CYP, cytochrome P450; LBW, lean body weight

when the starting dose proposed by the model was used compared with the standard bodyweight-based dose group (33.0% *vs*. 26.1%).

In this study, *CYP3A5* expressers required a 1.6-fold higher tacrolimus dose than *CYP3A5* nonexpressers. This is in line with previous research [42–46]. Patients carrying the *CYP3A4*22* allele required 20% less tacrolimus than the *CYP3A4*1* carriers independent of *CYP3A5* genotype status, confirming findings from previous research [7, 12, 47–49]. Given the wide availability of TDM, genotyping patients for *CYP3A* is most useful prior to initiation of tacrolimus therapy. Two RCTs demonstrated that optimization of the initial tacrolimus dose using *CYP3A5* genetic testing does not improve clinical outcomes when TDM is performed [24, 33].

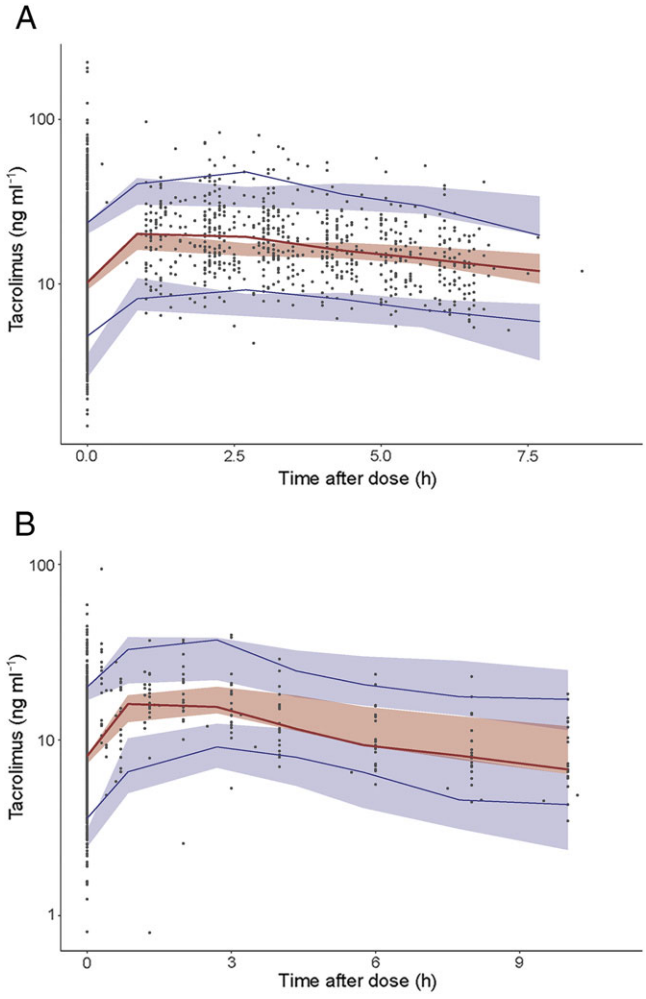

Figure 4

Prediction-corrected visual predictive check (VPC) of the starting dose model. (A) Prediction-corrected VPC of the starting dose model (internal dataset). (B) Prediction-corrected VPC of the starting dose model (external dataset)

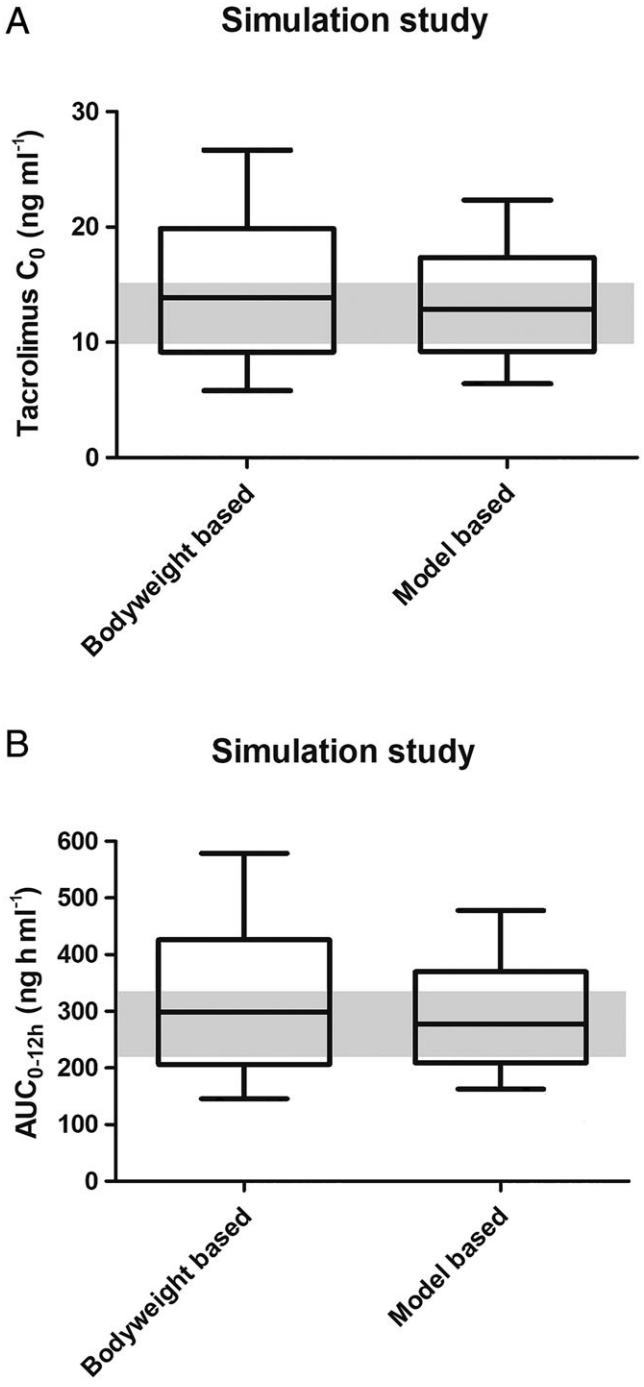

Figure 5

Boxplot with 10–90 percentile whiskers comparing simulations of the standard bodyweight-based dose and a dose based on the starting dose model. (A) Simulated predose concentrations. The median tacrolimus C_0 in the bodyweight-based dose group was 13.9 ng ml^{-1}, and in the model-based dose group 12.9 ng ml^{-1}. (B) Simulated AUCs. The median tacrolimus AUC in the bodyweight-based dose group was 298.5 ng h ml^{-1}, and in the model-based dose group 277.9 ng h ml^{-1}. AUC, area under the curve

However, this model is more sophisticated than basing the dose solely on bodyweight and *CYP3A5* genotype. For example, it has been suggested that the *CYP3A4*22* allele should be included in the Clinical Pharmacogenetics Consortium guidelines when considering a Caucasian population [42, 50].

As approximately 70–80% of tacrolimus is distributed in erythrocytes, low haematocrit will reduce the whole-blood concentrations of tacrolimus [51]. We found in our study that patients with a lower haematocrit had higher *CL/F*. This underlines previous findings [12, 14, 23, 26, 27, 52, 53]. The unbound concentration of tacrolimus is pharmacologically active. Haematocrit does not influence the unbound fraction. However, low albumin concentrations will increase the unbound fraction [52]. In contrast to what we expected, patients with hypoalbuminaemia had a lower tacrolimus *CL/F*. We did not find a similar effect on V_1, which one would expect if the correlation were due to protein binding. A possible explanation could be that the reduced CL/F is caused by an underlying inflammatory response, as described previously [54]. Hypoalbuminaemia can be an expression of inflammation, which can result in reduced CYP3A activity [55–57].

Unfortunately, no C-reactive protein levels were available to test this hypothesis. Patients with lower serum creatinine concentrations had an increased CL/*F*. Tacrolimus undergoes almost no renal elimination and therefore the explanation for the observed association remains unclear. Some studies have reported a significant correlation between serum creatinine and tacrolimus CL [58, 59], whereas others found no such effect [39, 60, 61]. Research has shown that *CYP3A5* expresser genotype is associated with a greater extent of renal tacrolimus metabolism and a lower apparent urinary tacrolimus CL compared with subjects having the *CYP3A5*3/*3* genotype. This is indicative of substantial intrarenal CYP3A5-dependent tacrolimus metabolism. Patients with poor renal function, and especially patients with delayed graft function, may therefore have a lower tacrolimus CL [62]. It is unclear whether this is caused by decreased intrinsic metabolic capacity of the kidney or is an indirect effect of uraemic toxins on hepatic metabolism [40].

Younger patients had an increased tacrolimus CL compared with older patients. A few years ago, Jacobson *et al*. examined age-related changes in the metabolism of tacrolimus and nicely demonstrated that older patients (>65 years) had significantly higher weight-normalized tacrolimus C_0 than younger patients (<34 years) [38]. Other developed pharmacokinetic models have found a similar effect [10, 12, 63]. Research has shown that basing the tacrolimus starting dose solely on bodyweight, will result in overexposure in a considerable proportion of patients [19]. BSA is a better indicator of metabolic mass than bodyweight because it is less affected by abnormal adipose mass. In both cohorts of the model building group this correlation between CL and BSA was seen. To the best of our knowledge this is the first pharmacokinetic model to incorporate BSA as a covariate.

In the prediction-corrected VPCs, the median and variability of the observations fell for the biggest part within the corresponding 95% prediction intervals of the simulations. However, approximately 2.5–4 h postingestion the simulations were slightly lower than the observations. This is explained by the relatively small proportion of patients with an AUC at our disposal (19%). Furthermore, the aim of this study was to develop a pharmacokinetic model for the starting dose of tacrolimus. Therefore, we chose to not describe the absorption with an over-parametrized transit compartment model.

The main strength of this study is the extensive validation of both models. The models were validated both internally and externally with clinical data using VPCs, and an NPDE was performed. Another strength of the study is the large number of patients included, and the high proportion of patients for whom an AUC was available. Furthermore, the Rotterdam data were of high quality as they were collected in a large RCT, rather than routinely collected clinical data. Another strength is the usage of data collected in four different centres. The final strength of the study is that a separate model was developed to predict the starting dose of tacrolimus.

The main limitation of the current study is that in the model building cohort, three different analytical techniques were used (ACMIA and EMIT in model building group 1, and LC–MS/MS in model building group 2). However, to solve this issue, the residual error model was coded in such a way that it calculates separate residuals errors for the two different bioanalytical techniques. Furthermore, albumin concentrations were not available in the external validation cohort and therefore we could not validate the model for this parameter. The final limitation is the relatively large proportion of C_0 (81%) in the model building group. However, in clinical practice tacrolimus is usually dosed based on C_0, rather than AUC. Furthermore, population pharmacokinetics using nonlinear mixed effect modelling is the optimal method to handle unevenly distributed data.

The next step is to prospectively test the starting dose model in a pilot study. We have received approval from the ERB and have started dosing patients based on the starting dose algorithm presented in this manuscript [64]. If this is successful, the final step to show the additional value of a model based starting dose would be to prospectively test the developed models in an RCT. The starting dose in the experimental arm of such a trial should be adjusted using the starting dose model, with subsequent dose adjustments based on the final model which includes all significant covariates.

Conclusion

The population pharmacokinetics of tacrolimus during the first 3 months following renal transplantation was adequately described using the models presented in this article. *CYP3A5* expressers and *CYP3A4*1* homozygotes had a higher tacrolimus *CL/F*. Higher BSA, lower creatinine, younger age, higher albumin and lower haematocrit also resulted in higher tacrolimus *CL/F*. In total, these covariates explained 30% of the variability in *CL/F*. By combining these clinical, demographic and genetic parameters, an individualized model has been developed that accurately estimates the tacrolimus CL and which can be used clinically to calculate the starting dose and posterior dose adjustments. The tacrolimus starting dose should be increased to 160% in individuals carrying the *CYP3A5*1* allele, whereas it should be reduced to 80% in patients carrying the *CYP3A4*22* allele.

Competing Interests

D.A.H. has received lecture and consulting fees from Astellas Pharma and Chiesi Farmaceutici Spa, as well as grant support (paid to his institution) from Astellas Pharma, Chiesi Farmaceutici Spa, and Bristol Myers-Squibb. T.v.G. has received lecture and consulting fees from Astellas Pharma, Roche Diagnostics and Chiesi Farmaceutici Spa, as well as grant support (paid to his institution) from Astellas Pharma and Chiesi Farmaceutici Spa. D.J.A.R.M. has received lecture fees from Astellas Pharma and Chiesi Farmaceutici, as well as grant support (paid to his institution) from Astellas Pharma and Chiesi Farmaceutici. B.C.M.d.W. has received grant support from Astellas Pharma. The other authors have no competing interests to declare.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

http://onlinelibrary.wiley.com/doi/10.1111/bcp.13838/suppinfo

Data S1 Example NONMEM control stream
Data S2 Normalized prediction distribution error (NPDE) plot for the starting dose model showing NPDE quantiles