


A Population Pharmacokinetic Model to Predict the Individual Starting Dose of Tacrolimus Following Pediatric Renal Transplantation

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Published online: 5 July 2017

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Abstract

Background Multiple clinical, demographic, and genetic factors affect the pharmacokinetics of tacrolimus in children, yet in daily practice, a uniform body-weight based starting dose is used. It can take weeks to reach the target tacrolimus pre-dose concentration.

Objectives The objectives of this study were to determine the pharmacokinetics of tacrolimus immediately after kidney transplantation and to find relevant parameters for dose individualization using a population pharmacokinetic analysis.

Methods A total of 722 blood samples were collected from 46 children treated with tacrolimus over the first 6 weeks

after renal transplantation. Non-linear mixed-effects modeling (NONMEM[®]) was used to develop a population pharmacokinetic model and perform a covariate analysis. Simulations were performed to determine the optimal starting dose and to develop dosing guidelines.

Results The data were accurately described by a two-compartment model with allometric scaling for body-weight. Mean tacrolimus apparent clearance was 50.5 L/h, with an inter-patient variability of 25%. Higher body-weight, lower estimated glomerular filtration rate, and higher hematocrit levels resulted in lower total tacrolimus clearance. Cytochrome P450 3A5 expressers and recipients who received a kidney from a deceased donor had a significantly higher tacrolimus clearance. The model was successfully externally validated. In total, these covariates explained 41% of the variability in clearance. From the significant covariates, the cytochrome P450 3A5 genotype, bodyweight, and donor type were useful to adjust the starting dose to reach the target pre-dose concentration. Dosing guidelines range from 0.27 to 1.33 mg/kg/day.

Conclusion During the first 6 weeks after transplantation, the tacrolimus weight-normalized starting dose should be higher in pediatric kidney transplant recipients with a lower bodyweight, those who express the cytochrome P450 3A5 genotype, and those who receive a kidney from a deceased donor.

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Key Points

In the first 6 weeks post-transplantation bodyweight, the cytochrome P450 3A5 genotype, donor type (deceased vs. living), estimated glomerular filtration rate, and hematocrit significantly influence the clearance of tacrolimus in children receiving a kidney transplant

The tacrolimus weight-normalized starting dose in pediatric kidney transplant recipients should be higher in children with a lower bodyweight, those who express the cytochrome P450 3A5 genotype, and in patients who receive a kidney from a deceased donor

1 Introduction

The use of tacrolimus-based immunosuppressive therapy following pediatric renal transplantation has drastically improved patient and graft survival. There still is, however, a long way to go regarding the reduction in tacrolimus treatment-related co-morbidities [1, 2]. Adverse events associated with the use of tacrolimus include nephrotoxicity, neurotoxicity, alopecia, gastrointestinal disturbances, hypertension, and post-transplantation diabetes mellitus [3–5]. These side effects contribute to patient non-adherence, limited long-term kidney allograft survival, and high cardiovascular morbidity and mortality of transplant recipients [6, 7]. High tacrolimus concentrations are associated with toxicity and lower concentrations seem to be related to an increased risk of acute rejection episodes [8, 9]. Glucocorticoid-sparing protocols are becoming more common, making it even more crucial to reach the tacrolimus target concentration as soon as possible to reduce the risk of acute rejection [8].

Tacrolimus is a critical dose drug with a narrow therapeutic index for which therapeutic drug monitoring (TDM) is routinely performed. Multiple factors, including bodyweight [10, 11], age [12, 13], drug interactions [14], hematocrit [10, 15], ethnicity [16], treatment with glucocorticoids [17], and cytochrome P450 (CYP) 3A genotype [10] affect the pharmacokinetics of tacrolimus. This has been extensively investigated in adults; however, data in children are limited. Children aged younger than 5 years appear to need higher weight-normalized doses of tacrolimus than older children to reach the target range [12]. The reason for this age-related increased clearance (CL) is unknown. It has been demonstrated that *CYP3A5* expressers (those with the *CYP3A5**1/1 or *CYP3A5**1/*3

genotype) require at least a 1.5-fold higher tacrolimus dose compared with *CYP3A5* non-expressers (*CYP3A5**3/*3 genotype) [10, 12, 18–21]. *CYP3A4**22 is associated with lower tacrolimus dose requirements post-renal transplantation [22–24]. The *CYP3A4**1G allele is associated with a higher tacrolimus dose requirement; however, its contribution to tacrolimus exposure is less than half of that of the *CYP3A5* genotype [25]. This effect is thought to be independent of *CYP3A5* status [16]. In routine clinical practice, these factors are not taken into consideration. The starting dose of tacrolimus is usually based on bodyweight and then adjusted by means of TDM. Therapeutic drug monitoring limits the time a patient is exposed to sub- and supra-therapeutic tacrolimus concentrations, but it can take up to 2 weeks to reach the target exposure range [26].

The use of a population pharmacokinetic model may help in predicting an individual's response to tacrolimus and can be applied before the start of therapy. To date, four models have been developed for pediatric renal transplant recipients [20, 27–30]. Of these models, only one was developed using transplant recipients in the immediate post-transplant phase and could therefore be used to determine the starting tacrolimus dose [10]. However, this model was developed with children treated in nine hospitals with different immunosuppressive regimens, had sparse tacrolimus sampling, important covariates such as ethnicity were not included, and the model was not externally validated.

The aim of the current study was to describe the population pharmacokinetics of twice-daily immediate-release tacrolimus in the first weeks after pediatric renal transplantation and to develop a dosing guideline for the starting dose. In contrast to previous studies, all children were treated with the same immunosuppressive regimen, had an abbreviated time profile measured with additional extensive tacrolimus sampling, important covariates such as ethnicity and *CYP3A5* genotype were included, and the model was externally validated.

2 Materials and Methods

2.1 Study Design

A retrospective analysis of pediatric transplant recipients who received a donor kidney between November 2009 and April 2016 was performed. Clinical and demographic data were retrieved from the medical records for the first 6 weeks after pediatric renal transplantation. Data were collected in the pediatric nephrology department at the Erasmus Medical Center, Sophia Children's Hospital, Rotterdam, the Netherlands. Eligible for enrolment were patients aged younger than 18 years, who received a

kidney from an ABO compatible living or a deceased donor, and were treated with tacrolimus as part of their initial immune suppressive regimen. All clinical values were collected from 24 h before transplantation until 6 weeks post-transplantation.

External validation of the pharmacokinetic model was performed on an independent dataset consisting of 23 children who underwent transplantation between March 2012 and July 2015 in the Radboud University Medical Center, Amalia Children's Hospital, Nijmegen, The Netherlands. These children were not included in the initial model building dataset, and were selected using the same inclusion criteria.

The study was designed in accordance with the Declaration of Helsinki of 1975. For the model building dataset, all laboratory analyses were performed for routine clinical practice. The Ethics Review Board of the Erasmus Medical Center decided that the rules laid down in the Medical Research Involving Human Subjects Act do not apply to this study (Medical Ethical Review Board No. 2017-092). The extra genotyping was approved by the Ethics Review Board of the Erasmus Medical Center (No. 2010-219). For the validation dataset, the Ethics Review Board of the Radboud University Medical Center approved the genotyping for *CYP3A5* in leftover material (No. 2014-1282). The parents or legal caregivers of all participants signed an informed consent prior to DNA collection.

2.2 Immunosuppression

All patients were treated according to the TWIST protocol with basiliximab, tacrolimus, mycophenolic acid, and a 5-day course of glucocorticoids [31]. Both the twice-daily formulations Prograf® (Astellas Pharma, Leiden, The Netherlands) and Modigraf® (Astellas Pharma, Leiden, The Netherlands) granules for suspension were used. An extemporaneously prepared suspension from the Prograf® capsules in an oral suspending vehicle from our Good Manufacturing Practice-certified pharmacy was used by some children. All children received an initial tacrolimus dose of 0.3 mg/kg/day divided into two doses every 12 h. The subsequent doses were adjusted using TDM.

In our hospital, it is common to measure the tacrolimus pre-dose concentration (C_0) for the first time after four to five dosages of tacrolimus, i.e., approximately 3 days after transplantation. As this is a study with data obtained in routine clinical practice, not all patients had their first tacrolimus C_0 measurement on day 3. This depended on multiple factors, including clinical factors (e.g., signs of tacrolimus toxicity or rejection), as well as logistic factors (tacrolimus concentrations are not routinely measured during the weekend in our hospital). In the first 3 weeks post-transplantation, the target C_0 was 10–15 ng/mL, from

then onwards the target C_0 was 7–12 ng/mL. Approximately 2 weeks after transplantation, an abbreviated 4-h tacrolimus concentration vs. time profile was obtained. For these profiles, blood samples were taken before tacrolimus administration, and 10, 30, 90, 120, and 240 min post-ingestion. The dose of immunosuppression and other co-medication was recorded in the electronic prescribing system during the entire study period. Tacrolimus sample collection times and time of latest ingestion were also recorded.

The following clinical data were collected retrospectively from medical records: weight, height, time post-transplant, sex, age, ethnicity, hematocrit, creatinine, aspartate aminotransferase, albumin, C-reactive protein, total protein, *CYP3A4* genotype, *CYP3A5* genotype, co-medication, glucocorticoid dose, primary diagnosis, previous transplantations, renal replacement therapy prior to transplantation (pre-emptive, hemodialysis or peritoneal dialysis), donor (living or post-mortal), human leukocyte antigen mismatches. The estimated glomerular filtration rate (eGFR) was calculated using the adapted Schwartz formula ($K * \text{height (cm)}/\text{serum creatinine } (\mu\text{mol/L})$) with a K value of 36.5 and the body surface area using the formula according to Mosteller [32, 33].

2.3 Laboratory Analysis

Genotyping for *CYP3A5* and *CYP3A4* was performed as described previously [34]. For *CYP3A4*, the *1G and *22 polymorphisms were tested, and for *CYP3A5* we tested for the *3 and *7 alleles. Deviations from the Hardy–Weinberg equilibrium were tested using the Chi squared goodness-of-fit test. Most tacrolimus concentrations were analyzed in whole-blood samples using a validated liquid chromatography-tandem mass spectrometry (LC–MS/MS) method, with a lower limit of quantification of 1.0 ng/mL. The remaining tacrolimus concentrations of the model building dataset (9%) were measured before the introduction of the LC–MS/MS method using the immunoassay with a lower limit of quantification of 1.5 ng/mL. The accuracy of the quality-control samples was between 85 and 115% and the intra- and inter-assay imprecision was less than 15% during the study period. As it is known that there is a difference between tacrolimus concentrations measured using an LC–MS/MS and an immunoassay, this was built into the residual error model.

2.4 Population Pharmacokinetic Modeling

Pharmacokinetic analysis was conducted by non-linear mixed-effects modeling 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).

2.4.1 Base Model Development

Based on visual inspection of the data and a review of the literature, one- and two-compartment models were considered to describe the concentration–time data. Typical values for lag-time, absorption rate constant (k_a), volume of distribution, CL, and inter-compartmental clearance (Q) were estimated. Bioavailability could not be quantified; therefore, certain parameters were estimated as ratios: apparent oral clearance (CL/F), Q/F , and apparent volume of distribution. Inter-individual variability (IIV) and inter-occasion variability (IOV) were modeled for each pharmacokinetic parameter using an exponential model, and residual variability was incorporated as an additive and proportional error for each analytical method. Allometric scaling was used to account for variability in pharmacokinetic parameters owing to differences in bodyweight. Shrinkage was calculated for all model parameters for which IIV was estimated. A shrinkage value below 20% was considered acceptable [35]. Model selection was based on minimum objective function values (OFVs), parameter precision, error estimates, shrinkage values, and visual inspection of the goodness-of-fit plots.

2.4.2 Covariate Model Development

Covariates were selected on the basis of their known or theoretical relationships with tacrolimus pharmacokinetics. Demographic, clinical, and genetic characteristics were evaluated as potential model covariates, all selected covariates are shown in Table 1. Co-medications known to interact with the tacrolimus concentration were included. The differences between the capsules and suspension (Modigraf[®] and the extemporaneously prepared suspension) were also tested as a covariate.

The relationship between covariates and IIV was first investigated graphically and covariates with a visually apparent relationship were singly added to the model. A univariate analysis was performed to determine if they improved the model. The forward inclusion-backward elimination method was used [36]. Covariates that significantly improved the model ($p < 0.05$) were added to the full model. A backward elimination process was then performed with a stricter statistical significance of $p < 0.001$ ($OFV > 10.83$). To be accepted in the model, the covariate also needed to reduce the IIV of the pharmacokinetic parameter involved. A shark plot was generated for each covariate for case-deletion diagnostics.

2.4.3 Model Evaluation

Multiple procedures were used to validate the final model. First of all, a bootstrap resampling method was applied [37]. Five hundred bootstrap datasets were generated by sampling randomly from the original dataset with replacement. The validity of the model was evaluated by comparing the median values and their corresponding 95% confidence intervals of the bootstrap replicates with the estimates of the original dataset. The model was further validated using the visual predictive check (VPC) by simulating 500 datasets [38], and a normalized prediction distribution errors (NPDE) analysis [39]. To investigate how the OFV differences between the covariate and base model is distributed across individuals, a shark plot was generated. Finally, an independent dataset containing 23 children treated with the same immunosuppressive regimen was used for external validation using a VPC. The VPCs were prediction corrected and stratified for the covariates included in the final model.

To evaluate the effect of the significant covariates, simulations were performed using the final population pharmacokinetic model with varying parameters for the covariate. For each covariate, concentration–time profiles were simulated for 1000 patients. All other parameters were fixed to the median. To develop the dosing guidelines for the starting dose of tacrolimus, simulations were also performed using a simulation model, which only included the covariates that significantly influence the starting dose. For each combination of these covariates, concentration–time profiles were simulated for 1000 patients, and a new starting dose was calculated to reach a target level of 12.5 ng/mL.

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

3 Results

A total of 46 children were included in the model building group. Patient characteristics are presented in Table 1. From these patients, 722 blood samples were collected and analyzed for tacrolimus concentrations (range 2–109 ng/mL). Each patient had at least one pharmacokinetic profile over 4 h approximately 2 weeks (range 8–42 days) post-transplantation. The median number of tacrolimus concentrations per patient was 16 (range 9–23). None of the samples were below the lower limit of quantification. There was no deviation from the Hardy–Weinberg equilibrium.

Table 1 Patient characteristics the first 6 weeks after transplantation

Characteristics	Model building group (<i>n</i> = 46)	Model validation group (<i>n</i> = 23)
Recipient sex, <i>n</i> (%)		
Male	26 (56.5)	14 (61.0)
Age of recipient (years)	9.1 (2.4–17.9)	8.2 (1.6–17.1)
Ethnicity, <i>n</i> (%)		
Caucasian	34 (73.9)	18 (78.3)
Asian	2 (4.3)	0 (0)
Black	6 (13.0)	1 (4.3)
Other	4 (8.7)	4 (17.4)
Bodyweight (kg) ^a	28.4 (11.6–83.7)	21.0 (10.4–83.0)
Height (cm) ^a	128.5 (83.5–186)	120.0 (73.0–176.0)
Laboratory measurements ^a		
Hematocrit (L/L)	0.29 (0.16–0.43)	0.30 (0.20–0.43)
Creatinine (μmol/L)	72 (12–1454)	65 (12–1148)
eGFR (mL/min/1.73 m ²)	69 (3–262)	71 (4–274)
ASAT (U/L)	29 (8–217)	Unknown
Albumin (g/L)	33 (11–52)	Unknown
Genotype, <i>n</i> (%)		
<i>CYP3A4</i>		
*1/*1	10 (21.7)	0 (0)
*1/*1G	4 (8.7)	0 (0)
*1G/*1G	2 (4.3)	0 (0)
Unknown	30 (65.2)	23 (100)
<i>CYP3A5</i>		
*1/*1	2 (4.3)	0 (0)
*1/*3	5 (10.9)	1 (4.4)
*3/*3	18 (39.1)	9 (39.1)
*3/*7	2 (4.3)	0 (0)
Unknown	19 (41.3)	13 (56.5)
Primary diagnosis, <i>n</i> (%)		
CAKUT	23 (50.0)	11 (47.8)
Glomerular kidney disease	13 (28.3)	3 (13.0)
Cystic kidney disease/Nephronophthisis	5 (10.8)	4 (17.4)
Other/unknown	5 (10.8)	5 (21.7)
Number of kidney transplantations, <i>n</i> (%)		
First	43 (93.5)	22 (96)
Second	3 (6.5)	1 (4.0)
RRT prior to kidney transplantation, <i>n</i> (%)		
Hemodialysis	10 (21.7)	10 (43.5)
Peritoneal dialysis	16 (34.8)	3 (13.0)
Pre-emptive	20 (43.5)	10 (43.5)
Donor type, <i>n</i> (%)		
Living	36 (78.3)	19 (82.6)
Deceased	10 (21.7)	4 (17.4)
Mismatch, <i>n</i> (%)		
0	3 (6.5)	Unknown
1	8 (17.4)	Unknown
2	12 (26.1)	Unknown
3	23 (50.0)	Unknown

Table 1 continued

Characteristics	Model building group ($n = 46$)	Model validation group ($n = 23$)
Tacrolimus initial daily dose (mg) ^a	0.29 (0.23–0.39)	0.31 (0.24–0.54)
First available tacrolimus C_0 (ng/mL)	8.7 (2.0–51.4)	8.8 (1.7–29.6)
Route of administration, n (%)		
Suspension	13 (28.3)	Unknown
Capsule	45 (97.8)	Unknown
Co-medication		
Calcium channel blockers, n (%)		
Amlodipine	15 (32.6)	6 (24.0)
Nifedipine	13 (28.3)	0 (0)
Antibiotics, n (%)		
Erythromycin	1 (2.2)	0 (0)
Antimycotics, n (%)		
Fluconazole	2 (4.3)	Unknown
Voriconazole	1 (2.2)	0 (0)
Glucocorticoids (prednisolone equivalents) dose/day (mg/kg)	0.8 (0.1–17.8)	Unknown

ASAT aspartate aminotransferase, C_0 pre-dose concentration, *CAKUT* congenital anomalies of the kidney and the urinary tract, *CYP* cytochrome P450, *eGFR* estimated glomerular filtration rate, *RRT* renal replacement therapy

^a Presented as median and range for continuous variables

3.1 Base Model

The data were best described by a two-compartment model. The residual error was described with a combined additive and proportional error model. Allometric scaling with fixed exponents [0.75 (CL/F and Q/F) and 1 (V_1/F and V_2/F)] significantly improved the model ($p < 0.001$). Estimation of the exponents did not improve the model and resulted in values not significantly different than the fixed values. Including IIV on CL/F , V_1/F , V_2/F , and k_a significantly improved the model fit. The OFV decreased further after introduction of IOV on CL/F and V_2/F . Parameter estimates of the base model, final model, and simulation model are presented in Table 2.

3.2 Covariate Analysis

The base two-compartment model with allometric scaling was used as a reference for the covariate analysis. After graphical analysis, the univariate analysis resulted in ten significant covariates, as shown in Table 3. After backward elimination, only *CYP3A5* genotype, donor, hematocrit, and *eGFR* were found to correlate significantly with CL/F , and remained in the final model. The following equation described the final model for estimation of tacrolimus CL/F (L/h) in the first 6 weeks post-transplant:

$$\begin{aligned}
 CL/F = & 50.5 * \left(\frac{\text{weight}}{70} \right)^{0.75} \\
 & * [(1.0, \text{ if } CYP3A5^*3/^*3 \text{ or unknown}) \\
 & \text{ or } (2.0, \text{ if } CYP3A5^*1/^*3 \text{ or } CYP3A5^*1/^*1)] \\
 & * (0.74, \text{ if living donor}) * \left(\frac{eGFR}{69} \right)^{0.19} \\
 & * \left[\left(\frac{\text{hematocrit}}{0.3} \right)^{-0.44}, \text{ if hematocrit } < ; 0.3 \right].
 \end{aligned}$$

3.3 Evaluation of the Final Model

All estimates were within the limits, given the criteria as defined under Sect. 2, except for shrinkage on V_2 , which was 27%. Goodness-of-fit plots of the final model showed the population predictions and individual predictions were evenly distributed around the line of unity, and the conditional weighted residuals were normally distributed (Fig. 1).

A bootstrap analysis with 500 bootstrap replicates was performed to obtain 95% confidence intervals for all pharmacokinetic parameters. Owing to minimization and boundary errors, the bootstrap results were recomputed without filtering these samples. Results of the bootstrap are shown in Table 2. Visual predictive checks showed that the median and the variability fell within the

Table 2 Parameter estimates of the base model, final model, and bootstrap analysis

Parameter	Base model	Final model	Simulation model	Bootstrap of the final model	
				Estimate	95% CI
OFV	-411.7	-450.7	-429.4		
t_{lag} (h)	0.42	0.37	0.43	0.40	0.30–0.45
k_a (L/h)	0.52	0.56	0.43	0.58	0.40–1.25
CL/ F (L/h)	48.0	50.5	54.9	54.0	43.5–68.1
V_1/F (L)	161	206	119	211	122–363
Q/F (L/h)	147	114	147	116	82–187
V_2/F (L)	1950	1520	1900	1544	1052–2140
Covariate effect on CL					
<i>CYP3A5</i> *3/*3		1.04	1.00	1.02	0.85–1.21
<i>CYP3A5</i> *1/*1 or *1/*3		1.98	1.82	1.91	1.56–2.43
eGFR (mL/min/1.73 m ²)		0.19		0.18	0.04–0.32
Donor living		0.74	0.74	0.70	0.55–0.86
Hematocrit < 0.3 (L/L)		-0.44		-0.42	-0.87 to 0.24
IIV (%)					
k_a	116	188	119	195	139–256
CL/ F	42	25	30	24	17–34
V_1/F	115	69	115	82	20–122
V_2/F	62	62	89	59	31–87
IOV (%)					
CL/ F	19	18	19	18	11–23
V_2/F	32	35	26	35	22–49
Residual variability					
Additional					
Immunoassay	0.83	1.01	0.81	0.88	0.01–2.56
LC-MS/MS	0.71	0.28	0.73	0.70	0.01–1.26
Proportional					
Immunoassay	0.13	0.13	0.13	0.11	0.001–0.29
LC-MS/MS	0.20	0.21	0.21	0.20	0.15–0.24

CI confidence interval, CL clearance, CYP cytochrome P450, eGFR estimated glomerular filtration rate, F bioavailability of oral tacrolimus, IIV inter-individual variability, IOV inter-occasion variability, K_a absorption rate constant, LC-MS/MS liquid chromatography-mass spectrometry, OFV objective function value, Q inter-compartmental clearance of tacrolimus, t_{lag} lag time, V_1 central compartment for tacrolimus, V_2 peripheral compartment for tacrolimus

corresponding simulations (Fig. 2a). This demonstrates the good predictive performance of the final model in the internal validation. Evaluation of the predictive performance by NPDE analysis showed adequate predictive ability, with distribution of the NPDEs not significantly deviating from a normal distribution and the majority of the values were between -2 and 2. Evaluation of the individual's influence on a change in OFV by the shark plot showed 70% of the included children had a decrease in OFV with the final model vs. the base model. In the external validation, the median and variability were adequately described, confirming the validation of the model (Fig. 2b). There was an insufficient number of

CYP3A5 expressers in this validation cohort to validate the algorithm for this subgroup.

3.4 Simulations

Based on the final model, *CYP3A5* expressers had a two times higher CL/ F . An increase in eGFR from 30 to 90 mL/min resulted in 19% higher CL/ F , whereas a decrease in hematocrit levels from 0.3 to 0.25 L/L corresponded with a 20% higher tacrolimus CL/ F . Deceased donor was associated with a 35% higher tacrolimus CL/ F than living donor. The effects of *CYP3A5* genotype, hematocrit, eGFR, donor, and bodyweight on CL/ F are

Table 3 Covariate effects in the univariate analysis compared with the base model

Covariate	Δ OFV	Covariate effect	Included after backward elimination
Covariates on CL/F			
Ethnicity	5.3	0.84, 0.95, and 1.15 ^a	No
<i>CYP3A5</i>	4.3	0.99 and 1.88 ^b	Yes
Donor	6.6	0.73 ^c	Yes
Hematocrit < 0.3 (L/L)	4.6	-0.41	Yes
eGFR (mL/min/1.73 m ²)	8.4	0.154	Yes
Age >7 (years) ^d	3.9	-0.32	No
Covariates on k_a			
Ethnicity	4.1	1.06, 0.19, and 1.01 ^a	No
Hematocrit (L/L)	7.4	2.91	No
Mismatches ^e	4.7	2.3	No
Covariates on V_2			
<i>CYP3A4</i>	5.0	1.36, 1.99, and 0.49 ^f	No

CYP cytochrome P450, *CL/F* apparent oral clearance, *eGFR* estimated glomerular filtration, k_a absorption rate constant, *OFV* objective function value, V_2 peripheral compartment for tacrolimus

^a Caucasian, Asian, and Black ethnicity, respectively, compared with subjects from other ethnicities

^b *CYP3A5* non-expressor and expresser, respectively, compared with unknown genotype

^c Living donor compared with a deceased donor

^d Age >7 years was chosen after visual inspection of the data. Age as a continuous variable was not a significant covariate

^e No mismatches compared with one or more mismatches

^f *CYP3A4**1/*1, *CYP3A4**1/*1G, and *CYP3A4**1G/*1G, respectively, compared with unknown genotype

Fig. 1 Goodness-of-fit plots of the final model. **a** Observed concentration (DV) plotted against predicted concentration (PRED). **b** DV plotted against individual predicted concentration (IPRED). **c** The correlation of conditional weighted residuals (CWRES) with the time after the tacrolimus dose. **d** The correlation of CWRES with PRED. The line represents the line of identity. *OBS* observed concentration

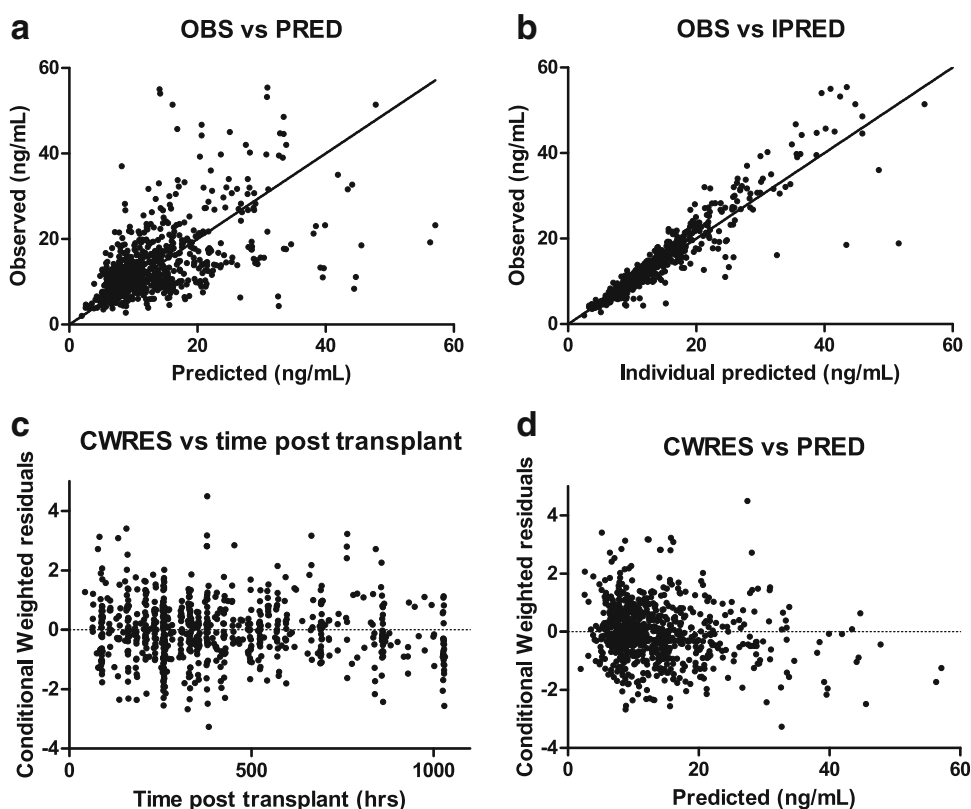
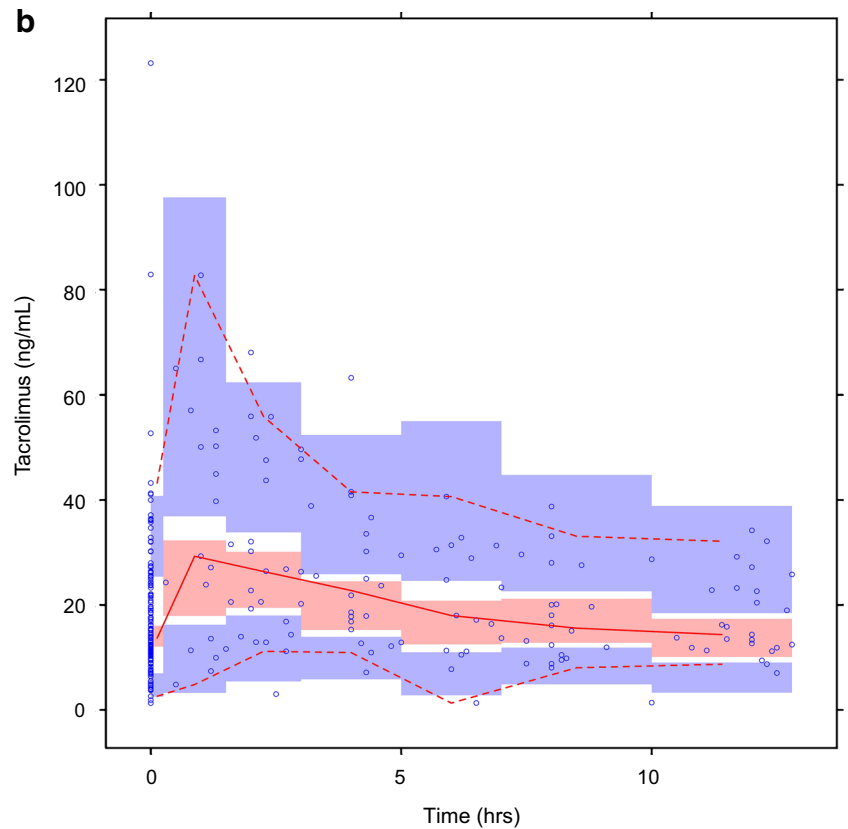
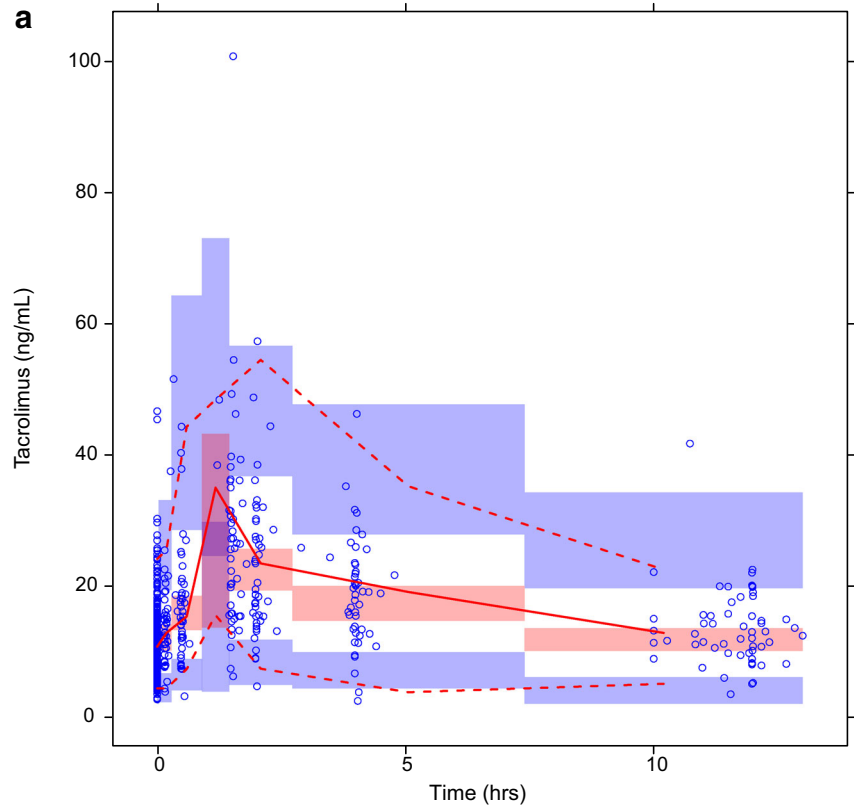


Fig. 2 Prediction-corrected visual predictive check (VPC) showing how well the average trend of the observations (*solid line*) and how well the variability of the observed data (*two dashed lines*) fall within the model simulated average trend (*red shaded area*) and the model-simulated variability (*blue shaded areas*) represented as a 95% confidence interval (CI). The average and the variability of the observed data both fall within the corresponding simulations.
a Internal VPC of the final model. **b** External VPC of the final model



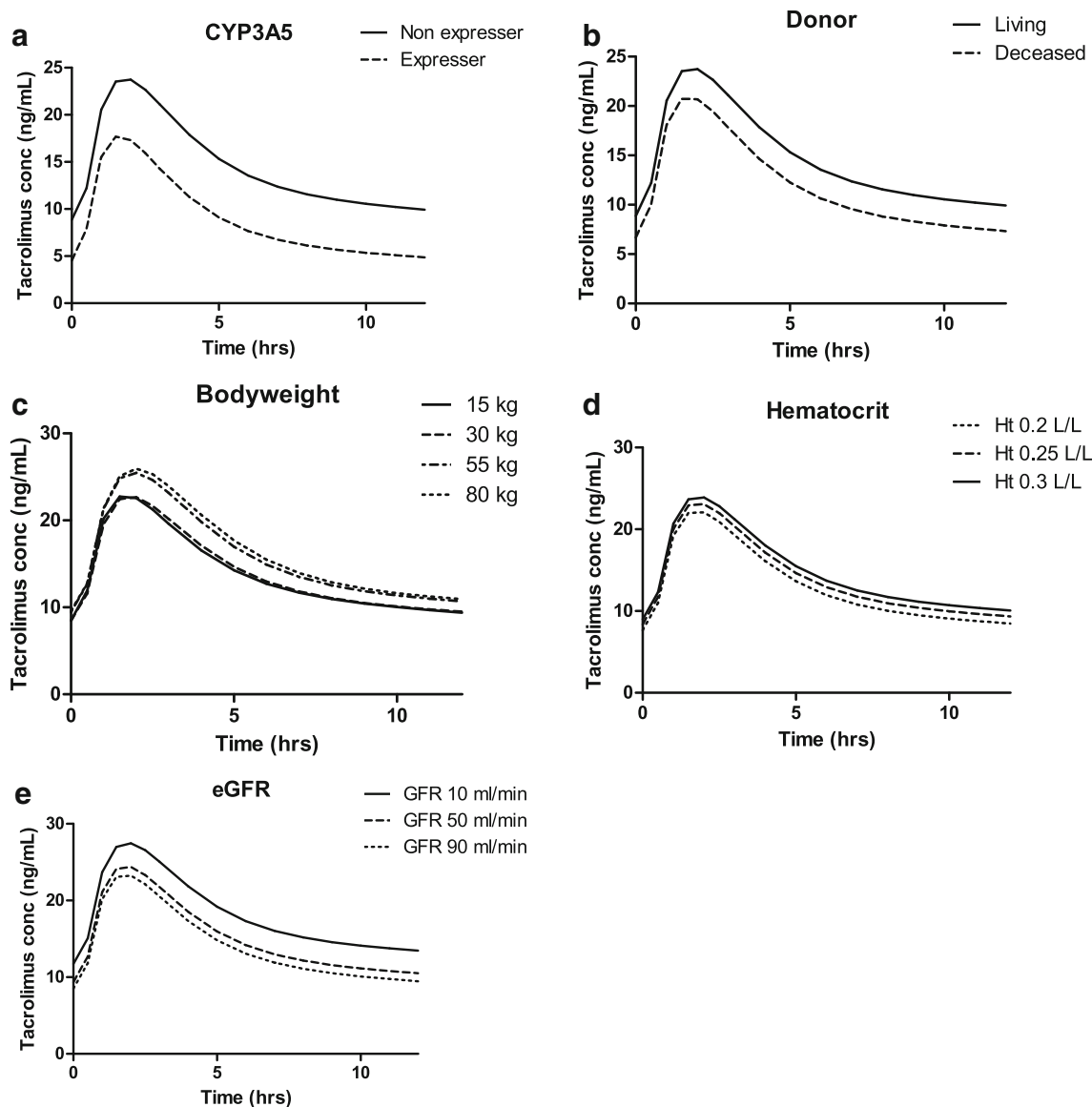


Fig. 3 Simulated plasma profiles of tacrolimus at first steady state after transplantation. **a** Simulated plasma profiles of tacrolimus for cytochrome P450 (CYP) 3A5 non-expressers (*CYP3A5*3/*3*) and *CYP3A5* expressers (*CYP3A5*1/*1* or *CYP3A5*1/*3*). **b** Simulated plasma profiles of tacrolimus for patients receiving a kidney from a living or deceased donor. **c** Simulated plasma profiles of tacrolimus

for patients with a bodyweight of 15, 30, 55, or 80 kg. **d** Simulated plasma profiles of tacrolimus for patients with plasma hematocrit (Ht) levels of 0.2, 0.25, and 0.3 L/L. **e** Simulated plasma profiles of tacrolimus for patients with an estimated glomerular filtration rate (eGFR) of 10, 50, or 90 mL/min. *conc* concentration

shown in Fig. 3. In total, these covariates explained 41% of the variability in CL/F .

3.5 Dosing Guidelines

The model was used to design a dosing algorithm for the starting dose of tacrolimus after pediatric kidney transplantation. The last measured eGFR and hematocrit before transplantation did not significantly influence the CL/F and because these parameters change tremendously post-transplantation, were not incorporated in the algorithm for

the starting dose. The following equation described the estimation of tacrolimus CL/F (L/h) immediately after transplantation:

$$\frac{CL}{F} = 54.9 * \left(\frac{\text{weight}}{70}\right)^{0.75} * (1.8, \text{ if } CYP3A5^*1/*3 \text{ or } CYP3A5^*1/*1) * (0.74, \text{ if living donor}).$$

The required dose can be calculated using the estimated tacrolimus CL/F and the desired target C_0 . To

calculate this, AUC_{0-12h} were determined for all the available pharmacokinetic time profiles. A C_0 of 10 ng/mL corresponded with an AUC_{0-12h} of approximately 177 ng h/mL, 12.5 ng/mL with 209 ng h/mL, 15 ng/mL with 241 ng h/mL, 17.5 ng/mL with 274 ng h/mL and 20 ng/mL with 306 ng h/mL. Based on the formula: $dose = AUC * CL/F$, it leads to the following formula for a target C_0 of 12.5 ng/mL based on a twice-daily dose:

$$Dose(mg) = 209 \text{ ng h/mL} * 54.9 * \left(\frac{\text{weight}}{70}\right)^{0.75} * (1.8, \text{ if } CYP3A5^*1/*3 \text{ or } CYP3A5^*1/*1) * (0.74, \text{ if living donor})/1000.$$

This formula can be used when dose adjustments are based on AUC; however, most hospitals base dose adjustments on C_0 . Therefore, dosing guidelines were developed using the above formula, which was fine tuned by simulating a 1000 doses in different patients. Table 4 shows the dosing guideline including the simulated median C_0 with the interquartile range at steady state. Dosing guidelines range from 0.27 mg/kg/day for a *CYP3A5* non-expresser weighing 80 kg receiving a kidney from a living donor, to 1.33 mg/kg/day for a *CYP3A5* expresser weighing 10 kg and receiving a kidney from a deceased donor.

4 Discussion

This is the second population pharmacokinetic study of tacrolimus in pediatric renal transplant recipients covering the first 6 weeks after transplantation. Our results showed that a two-compartment model with first-order absorption, lag time, allometric scaling, IIV on CL, V_1 , V_2 , and k_a , and IOV on CL and V_2 was optimal for data modeling. Besides bodyweight, we also demonstrated that *CYP3A5* genotype, hematocrit, eGFR, and donor type all significantly influence tacrolimus CL. Together these covariates explained 41% of the variability in tacrolimus CL. Calculation of tacrolimus dosing guidelines for the initial dose after transplantation showed a factor four difference in daily dose, ranging from 0.27 to 1.33 mg/kg. This is vastly different from the 0.3 mg/kg/day currently used in clinical practice [40]. At first steady state, only 26.1% of patients were within the tacrolimus target C_0 range, 67.4% had subtherapeutic tacrolimus concentrations, and 6.5% had suprathereapeutic concentrations. After a median of 9.8 days (range 1.7–23.8 days), patients were within the tacrolimus target C_0 range.

Genetic polymorphisms in *CYP3A5* partly explain the IIV in tacrolimus pharmacokinetics. In this study, 25.9% of the included children of whom the genotype was known were *CYP3A5* expressers. In agreement with previously published data, we report a significantly lower CL/F in

Table 4 Dosing guidelines for the starting dose of tacrolimus with a target pre-dose concentration (C_0) of 10–15 ng/mL

Weight (kg)	Donor	Pharmacogenetics			
		<i>CYP3A5</i> expresser		<i>CYP3A5</i> non-expresser	
		Dose (mg/kg/day)	C_0 (ng/mL)	Dose (mg/kg/day)	C_0 (ng/mL)
10	Living	0.89	12.5 (8)	0.44	12.4 (7)
	Deceased	1.33	12.3 (8)	0.61	12.2 (7)
20	Living	0.76	12.6 (8)	0.37	12.5 (8)
	Deceased	1.06	12.5 (8)	0.53	12.6 (7)
30	Living	0.63	12.5 (8)	0.33	12.5 (7)
	Deceased	1.00	12.4 (9)	0.46	12.4 (7)
40	Living	0.61	12.6 (8)	0.31	12.4 (7)
	Deceased	0.91	12.5 (9)	0.44	12.6 (7)
50	Living	0.60	12.5 (7)	0.30	12.4 (7)
	Deceased	0.82	12.5 (9)	0.42	12.5 (7)
60	Living	0.55	12.5 (8)	0.30	12.5 (8)
	Deceased	0.80	12.5 (8)	0.40	12.5 (7)
70	Living	0.53	12.5 (8)	0.30	12.5 (8)
	Deceased	0.78	12.5 (8)	0.38	12.5 (7)
80	Living	0.52	12.6 (8)	0.27	12.4 (7)
	Deceased	0.78	12.5 (8)	0.38	12.5 (8)

Dosing guidelines for the starting dose of tacrolimus with tacrolimus median C_0 at first steady state with interquartile range as a result of 1000 simulations per patient
CYP cytochrome P450

children having the *CYP3A5**3/*3 genotype compared with children carrying at least one *CYP3A5**1 allele. Given the wide availability of TDM, genetic testing is most useful prior to initiation of tacrolimus to more rapidly reach the target concentration [41]. In adult kidney transplant recipients, two randomized controlled trials concluded that optimization of the initial tacrolimus dose using *CYP3A5* genetic testing does not improve clinical outcomes when TDM is performed [26, 42]. As the pharmacokinetics of tacrolimus differs between children and adults, these findings cannot be extrapolated. Moreover, our model is more sophisticated than dosing based on the *CYP3A5* genotype only. Therefore, the question remains if genotyping in pediatric transplant recipients prior to the start of tacrolimus therapy adds to adequate dosing of tacrolimus and improvement of clinical outcomes.

Children with a lower bodyweight had a higher weight-normalized tacrolimus dose requirement than children with a higher bodyweight. This is in line with previous findings [10, 11, 20, 30]. Allometric scaling significantly improved the model, substantiating findings from previous published pediatric models [10, 20]. It has been previously reported that younger children have significantly higher weight-normalized dose requirements than older recipients, suggesting the dose should be based on age rather than bodyweight [12, 13]. Incorporation of age in our model did not improve it further. This is in line with previously developed pharmacokinetic models in this population [10, 20, 30].

Contrary to what we expected, kidneys from a deceased donor had a higher tacrolimus CL than kidneys from a living donor. This finding was confirmed in the external validation of the model. A literature search was performed and showed no previous reports substantiating this observation. Dialysis prior to kidney transplantation or preemptive kidney transplantation was not significantly associated with tacrolimus CL, and neither was the number of human leukocyte antigen mismatches. As tacrolimus undergoes hepatic metabolism, higher tacrolimus CL in kidneys from a deceased donor seems highly unlikely. All patients received the same immunosuppressive protocol, regardless of the donor status. Only if there was a slow graft function, was the start of tacrolimus treatment postponed and patients continued treatment with glucocorticoids. This occurred in four patients, but none of them experienced delayed graft function in sensu stricto (i.e., the need for dialysis in the first week after transplantation). However, no correlation between slow graft function and tacrolimus CL was found in these patients. Furthermore, glucocorticoid use was tested as a covariate and did not significantly influence tacrolimus CL. Higher tacrolimus CL in kidneys from a deceased donor is probably caused by other unknown parameters that could not be tested as

covariates and therefore cannot be corrected for. One of these parameters could be the interaction between donor and age. Recipients of a deceased donor kidney tended to be younger than recipients of a living donor kidney transplant, although this was not statistically significant. Another explanation could be other non-investigated parameters such as serum albumin, anemia, and metabolic acidosis. Recipients of deceased donors are usually more catabolic and might therefore have a higher free fraction of tacrolimus. However, this is not supported by our data showing the difference in CL between living and deceased donors does not decrease during the first 6 weeks post-transplantation.

Approximately 70–80% of tacrolimus is distributed in erythrocytes [43]. This indicates that low hematocrit concentrations will reduce the whole-blood concentrations of tacrolimus. In our cohort, children with a hematocrit level <0.30 had an increased CL/F and thus required a higher tacrolimus dose. This is in line with previous findings in pediatric kidney recipients [10, 20]. More than 98% of tacrolimus in the plasma is bound to plasma proteins, and despite measuring the tacrolimus concentration in whole blood, it is actually the unbound concentration that is pharmacologically active [44]. Hematocrit levels do not seem to influence the unbound fraction of tacrolimus in plasma [45]. Low albumin concentrations will increase the tacrolimus unbound fraction. Unfortunately, there were not enough albumin measurements available to determine if there was a relationship between albumin and tacrolimus CL/F. A recently published study reported the validation of an assay specifically developed for measurement of unbound tacrolimus concentrations [46].

In our cohort, children with higher eGFR had higher tacrolimus CL/F. As an increase in eGFR from 30 to 90 mL/min resulted in only 19% higher CL/F, eGFR-based dosage adjustment of tacrolimus seems unnecessary. Tacrolimus undergoes almost no renal elimination; therefore, the explanation for the observed association between tacrolimus CL and eGFR remains unclear. To our knowledge, this is the first pediatric model to include eGFR. Serum creatinine was tested in one model, but was not significantly correlated with tacrolimus CL [29]. In adults, some studies have reported no significant correlation between serum creatinine and tacrolimus CL [47–49], whereas others did find an effect [50, 51]. Recent data showed that the *CYP3A5**1 genotype is associated with a greater extent of renal tacrolimus metabolism and a lower apparent urinary tacrolimus CL as compared with subjects expressing *CYP3A5**3/*3. This is highly indicative of intra-renal *CYP3A5*-dependent tacrolimus metabolism and could possibly explain the influence of eGFR on tacrolimus CL/F [52].

There were no patients expressing *CYP3A4**22, and therefore we could not confirm the reported relationship

between *CYP3A4**22 and tacrolimus CL [22]. An association between the glucocorticoid dose and tacrolimus CL has been reported [53, 54]. We could not substantiate this finding, probably because all children were treated with a glucocorticoid-minimization regimen and prednisolone was only prescribed the first 5 days in a relatively low dose.

The main strength of this study was the extensive evaluation of the final model; bootstrap analyses, VPCs, and an NPDE were performed, and the model was also externally validated. Another strength of the study is the large amount of blood samples per patient, including abbreviated tacrolimus pharmacokinetic curves. An extensive literature search was performed and all covariates known to influence the CL of tacrolimus, were tested. The population pharmacokinetic model was developed in patients treated according to the same immunosuppressive protocol. Other studies included children with different regimens, for example, with azathioprine instead of mycophenolic acid or glucocorticoids in different doses, making it difficult to determine the effect of tacrolimus. The final strength of the study is that dosing guidelines for the starting dose of tacrolimus after renal transplantation were developed. These dosing guidelines will be prospectively tested.

The main limitation of this study is that it is a retrospective analysis and therefore we had to rely on data available in the medical records. Because of this limitation we did not include a patient expressing *CYP3A4**22, nor did we have enough *CYP3A5* expressers in the validation cohort. Another limitation is that during the study period the tacrolimus analysis changed from the immunoassay to an LC-MS/MS. However, this difference was built into the residual error model. Furthermore, the relatively large proportion of Caucasian patients in our center is a limitation. Finally, the developed population pharmacokinetic model is only suitable for children aged between 2 and 18 years receiving immediate-release formulations of tacrolimus, as the once-daily preparation was not tested.

5 Conclusions

The population pharmacokinetics of tacrolimus during the first 6 weeks after renal transplantation can be adequately described using the model presented in this article. Higher bodyweight, lower eGFR, and higher hematocrit levels resulted in lower tacrolimus CL. *CYP3A5* expressers and recipients who received a kidney from a deceased donor had higher tacrolimus CL. The tacrolimus weight-normalized starting dose should be higher in patients with lower bodyweight, in those who are *CYP3A5* expressers, and in patients receiving a kidney from a deceased donor. By

combining these parameters, an individualized tacrolimus dosing regimen has been developed that adequately predicts the target C_0 and hopefully will improve patient outcome.

Compliance with Ethical Standards

Funding No sources of funding were used in the preparation of this article.

Conflict of interest Teun van Gelder has received a study Grant from Chiesi Pharmaceuticals, lecture fees from Chiesi Pharmaceuticals, Astellas Pharma, Roche Pharma, and Novartis Pharma, and consulting fees from Astellas Pharma, Novartis Pharma, and Teva Pharma. Dennis A. Hesselink has received grant support and lecture and consulting fees from Astellas Pharma and Chiesi Pharmaceuticals, as well as a lecture fee from Hikma Pharma. Brenda C. M. de Winter has received travel support from Astellas Pharma. Saskia N. de Wildt has received funding from The Netherlands Organisation for Health Research and Development (Grant No. 90700304). Louise M. Andrews, Roger J. M. Brüggemann, Elisabeth A. M. Cornelissen, Karlien Cransberg, Birgit C. P. Koch, and Ron H. N. van Schaik have no conflicts of interest directly relevant to the content of this article.

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