

ORIGINAL ARTICLE

Inclusion of CYP3A5 genotyping in a nonparametric population model improves dosing of tacrolimus early after transplantation

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Summary

Following organ engraftment, initial dosing of tacrolimus is based on recipient weight and adjusted by measured C_0 concentrations. The bioavailability and elimination of tacrolimus are affected by the patients *CYP3A5* genotype. Prospective data of the clinical advantage of knowing patient's *CYP3A5* genotype prior to transplantation are lacking. A nonparametric population model was developed for tacrolimus in renal transplant recipients. Data from 99 patients were used for model development and validation. A three-compartment model with first-order absorption and lag time from the dosing compartment described the data well. Clearances and volumes of distribution were allometrically scaled to body size. The final model included fat-free mass, body mass index, hematocrit, time after transplantation, and *CYP3A5* genotype as covariates. The bias and imprecision were 0.35 and 1.38, respectively, in the external data set. Patients with functional *CYP3A5* had 26% higher clearance and 37% lower bioavailability. Knowledge of *CYP3A5* genotype provided an initial advantage, but only until 3-4 tacrolimus concentrations were known. After this, a model without *CYP3A5* genotype predicted just as well. The present models seem applicable for clinical individual dose predictions but need a prospective evaluation.

Introduction

Tacrolimus (Tac) is the cornerstone of most immunosuppressive solid organ transplant protocols. Tac has low extraction ratio and low bioavailability. It is metabolized by cytochrome P450 3A (*CYP3A*) enzymes, and *CYP3A5* contributes significantly to the overall *CYP3A* metabolism in patients expressing this enzyme [1,2]. Tac is highly distributed to erythrocytes and bound to plasma proteins with approximately only 1% free fraction [3]. Due to a narrow therapeutic index and large pharmacokinetic variability, therapeutic drug monitoring (TDM) of Tac is mandatory [4]. Concentrations are generally determined in whole-

blood samples because Tac shows a concentration-, temperature-, and hematocrit dependent distribution between plasma and erythrocytes [3].

Two main challenges associated with Tac dosing in renal transplant patients are (i) choosing the correct starting dose and (ii) making adequate dose adjustments to compensate for changing pharmacokinetics with time after transplantation. The starting dose is generally individualized based on body weight and immunological risk. Several publications have presented data, indicating that dosing could be improved by also including patient-specific factors such as *CYP3A5* genotype, age, and sex [5-9]. Subsequent dosing after transplantation is currently managed using Tac trough

concentrations and tacit knowledge. In the early post-transplant phase, Tac is measured 3–4 times per week, less frequently with time after transplantation.

In renal transplant recipients, several population pharmacokinetic models have been developed for Tac [8–18]. None use a nonparametric approach, which is reported to accurately detect outliers better than the commonly used parametric approaches [19]. Furthermore, to our knowledge, no result from using Tac population models in the clinic is available yet.

The primary aim of the present analysis was to develop a nonparametric population pharmacokinetic model for future use for tacrolimus dosing in a clinical prospective setting in renal transplant recipients. There was a special emphasis on the value of *CYP3A5* genotype for the model performance.

Material and methods

Patients

A single-center study was performed. Data from 69 adult renal transplant recipients were used for making and setting up the population model. Intensive sampled data over 44 dose intervals were obtained from 29 patients investigated in three previous clinical trials recruiting patients from 2007 to 2012 and have previously been described in detail [20–23]. In addition, data from 44 patients following standard of care follow-up at our transplant center between 2011 and 2012 contributed to trough concentrations up to 10 weeks post-transplant (four patients contributed to data in both groups). Overall, a total of 1546 Tac measurements were available, one-third intensively sampled. Each patient contributed to an average of 22 samples, ranging from 5 to 50.

Data from 30 adult renal transplant patients >18 years of age were used for validation of the model. A total of 576 Tac trough concentrations were available. There was an average of 19 samples per patient, with a range of 9 to 24.

In addition to Tac dose times and amount, whole-blood concentrations, and sample times, the following data from each patient were evaluated for inclusion in the population model: *CYP3A5* genotype, hematocrit, sex, age, total body weight (WT), body mass index (BMI), predicted fat-free mass (FFM) [24], serum albumin, C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total plasma bilirubin, alkaline phosphatase, and concomitant use of nifedipine, which has a potential interaction with Tac [25]. For the trough concentration data, the time of dosing was set to 8:00 am and 8:00 pm (exact times were not available). Tac concentrations measured during ongoing episodes of diarrhea were excluded from the present data sets [26].

The study has been approved by the Norwegian Regional Committee for Medical and Health Research Ethics South-East.

Immunosuppressive regimen

The standard immunosuppressive regimen consisted of oral Tac (Prograf[®] capsules, Astellas Pharma US Inc., Northbrook, IL, USA) combined with mycophenolate mofetil (1.5 g/day, CellCept[®] F. Hoffman – La Roche, Basel, Switzerland), steroids, and induction with two doses of basiliximab (Simulect[®], Novartis, Switzerland). The steroid protocol used was 250 mg intravenous methylprednisolone at the day of transplantation followed by oral prednisolone: 20 mg/day (day 1 to day 14), 15 mg/day (day 15–28), 10 mg/day (day 29–60), and further tapered to 5 mg by day 180 after transplantation. High-risk patients (defined as panel reactive antibodies >20% and/or presence of donor-specific antibodies) were also given intravenous human immunoglobulins and rituximab in addition to higher doses of steroids and Tac.

The initial Tac dose for the patients contributing with only Tac trough concentrations was 0.04 mg/kg total body weight twice daily, rounded to the closest 0.5 mg dose, followed by dose adjustments according to trough concentration, aiming for 3–7 µg/l (8–12 µg/l in high-risk patients) by changes in the doses up to the discretion of the treating physician. The details of the immunosuppressive protocols have been presented earlier [20,21,23].

Tacrolimus analysis

Tac whole-blood concentrations were measured with immunoassays in all cases except in samples from one clinical trial [23], where the concentrations were determined by HPLC-MS/MS. The HPLC-MS/MS concentrations ($C_{LC-MS/MS}$) were converted to immunoassay equivalent concentrations (C_{immuno}) by the formula $C_{immuno} = (C_{LC-MS/MS} - 0.19) / 0.80$, established by the analytical laboratory performing the analyses.

Assay error was estimated in the population model based on analytical validation data, and specific error polynomials were developed for the immunoassays and the HPLC-MS/MS assay, respectively.

CYP3A5 genotyping

DNA was extracted from ethylenediaminetetraacetic acid (EDTA) anticoagulated whole blood using the MagNA Pure instrument (Roche Applied Science, Penzberg, Germany). *CYP3A5*-genotyping (rs776746; NG_007938.1: g.12083G>A, A=*CYP3A5**1 and G=*CYP3A5**3) was performed by real-time PCR and melt curve analysis with

hybridization probes on the LightCycler[®] 480 instrument (Roche Applied Science, Penzberg, Germany) or by a PCR–restriction fragment length polymorphism assay as previously described [27].

Population pharmacokinetics modeling and validation

The pharmacokinetic modeling was performed in Pmetrics (version 0.40, Laboratory for Applied Pharmacokinetics, Los Angeles, CA, USA) [19] using the algebraic model solver. While all models have equations with parameters, such as volume of distribution, there are two broad approaches to estimate the values of those parameters in a population: parametric and nonparametric. Parametric approaches assume that the study sample is drawn from an underlying defined distribution of parameter values, such as normal or log-normal. Nonparametric approaches do not assume any underlying distributions. We chose to use a nonparametric approach because of certain advantages over parametric methods [19]: (i) to better detect, if present, *outlier* patients, (ii) to detect any unexpected subpopulations such as fast- or slow-metabolizers, (iii) and to build a model that could be used for multiple-model adaptive control as implemented in the BestDose clinical dose optimization software package produced by LAPK (www.lapk.org).

According to a previous analysis of the data set [22], the structural model was set to three compartments with first-order Tac absorption from the dosing compartment into the central compartment after a delay or lag time, and distribution to and from a peripheral tissue compartment. No intravenous data were available so the model was parameterized with apparent central clearance (CL/F), intercompartment clearance (Q/F), and central and peripheral volumes of distribution (V/F, V_p/F).

A relative bioavailability (FA) term was introduced into the different models for each of the following covariates: CYP3A5 genotype, sex, and use/no use of nifedipine. Steady-state situations were modeled by applying the first known concentration in any new dosing period as the initial condition for the concentration in the central compartment.

Both the additive lamda and multiplicative gamma error models in Pmetrics were tested during the model development, using the assay error polynomials as presented above. As many multiples of 80 021 grid points as possible were applied, limited by the hardware used (MacBook Pro, 2.66 GHz Intel Core 2 Duo processor, 8 GB 1067 MHz DDR3 memory and running OS X, version 10.8.2, Apple Inc, CA, USA), and with uniform initial grid distribution.

All pharmacokinetic disposition parameters were allometrically scaled to body size using coefficients of three-fourth for clearances and 1 for volumes, testing the following body size measures; WT, BMI, and predicted FFM [24,28]. Continuous covariates were centralized to the median value

of the present population. Hematocrit was used as a covariate on clearances and volumes to account for differences in the free fraction of Tac [3]. Covariates were included stepwise, followed by a reduction in the resulting model by taking one and one covariate out of the model.

Model selection was based on comparison of the Akaike information criterion (AIC) [29], the fit of both the population and individual predictive versus observed plots and biological plausibility. The R^2 values of the predictive versus observed plots were statistically compared with an F -test and slopes by a linear regression interaction term between the two nested models.

To evaluate the potential benefit of knowing the CYP3A5 genotype of patients for Tac dosing, CYP3A5 genotype was removed as a covariate from the final model to make a “reduced model.” The full model with CYP3A5 genotype and the reduced model were evaluated for their respective predictive accuracy. From the Bayesian prior of the full and reduced models, Pmetrics calculated the Bayesian posterior for each subject in the external validation set ($n = 30$), and Tac trough concentrations were predicted for these patients, given individual Tac dosing and patient covariates. The following statistics were computed: predictive error (PE, predicted minus observed concentrations), bias (mean weighted PE), imprecision (bias-adjusted mean weighted squared PE), and the R^2 and slope of the individual predicted versus observed plots.

To further evaluate the potential advantage of knowing the CYP3A5 genotype of patients when starting them on Tac dosing after transplantation in a clinical setting, the median PEs of the full and reduced models for each patient’s “next” Tac trough concentration were calculated in the external validation population ($n = 30$) after including zero to eight measured Tac trough concentrations from each patient. Specifically, when no Tac concentrations were included, the full and reduced population prior median parameter values were used to predict the first measured Tac trough concentration in each patient, and the median PE was recorded. Then, the first measured Tac trough concentration was used to calculate a Bayesian posterior to predict the second measured Tac trough concentration, and the median PE was again recorded. This process was repeated until the first eight measured Tac trough concentrations were used to predict the final 9th trough concentration. In this way, a realistic clinical scenario was established, where more data become available with time after transplantation and can be used to predict the next dose.

Tacrolimus dose evaluation

To evaluate potential Tac dosing regimens, a Monte Carlo simulation of the typical patient with and without

functional CYP3A5 (*1/*3) was performed using the full model. For this simulation, the typical patient had covariates set to the median of the investigated population, that is, 41 years of age, weighing 78 kg (BMI of 26 kg/m² and a FFM of 59 kg), and having a hematocrit of 36%. Two such patients, with and without functional CYP3A5, served as simulation templates for 1 000 profiles drawn from the full model population joint density. Dosing regimens from 0.5 to 5.0 mg Tac (increased by 0.5 mg) BID were simulated for each of the two typical patients. A total of eight doses of Tac were administered, and the trough concentration 96 h after the first dose was calculated (steady-state conditions).

Results

Patients

Patient demographics of the two populations used in the present analysis are shown in Table 1. All parameters except age (*P* = 0.0014) were similar between the two data sets. The average dose-adjusted steady-state trough concentrations were 41% lower (*P* < 0.00001) in the 17 patients with CYP3A5*1/*3 genotype as compared to the 82 patients not expressing functional CYP3A5 (*3/*3).

Population model development

Table 2 shows a summary of the parameters and covariates included in the full model with CYP3A5 genotype. Table 3 shows the parameter values for the full model and the reduced model without CYP3A5 genotype. Both models were initiated with 12 × 80 021 grid points. The full model converged after 5 285 cycles to 56 support points, and the reduced model converged after 6 142 cycles to 61 support

Table 1. Demographics at the time of first sample in the present analysis.

	Modeling population		Validation population	
	N/median	IQR	N/median	IQR
M: F	50: 19		21: 9	
Age (years)	41	18	57*	23
Body weight (kg)	78	23	76	20
Body mass index (kg/m ²)	26	6	25	5
Fat-free mass (kg)	59	17	58	18
CYP3A5 genotype: *1/*3: *3/*3	10: 59		7: 23	
Hematocrit (%)	34	9	35	7
Time after Tx (days)	1	921	1	0
Using nifedipine	17		11	

**P* = 0.0014.

M, male; F, female; Tx, transplantation; CYP3A5, cytochrome P450-3A5; IQR, interquartile range.

Table 2. Parameters affected by covariates in the final model based on any decrease in AIC and subjective improvement in predicted-observed plots.

Parameter	Covariates				
	CYP3A5	HCT	FFM	BMI	TXT
Apparent clearance (CL/F)	X	X	X		
Apparent intercompartment clearance (Q/F)		X	X		
Apparent central volume of distribution (V/F)		X		X	
Apparent peripheral volume of distribution (Vp/F)		X	X		
Relative bioavailability (FA)	X				
Lag time first week (Tlag1)			X		Day 1–7
Lag time week 2–4 (Tlag2)			X		Day 8–28
Lag time after first month (Tlag3)			X		Day 29–

CYP3A5, cytochrome P450-3A5; HCT, hematocrit; FFM, fat-free mass; BMI, body mass index; TXT, time after transplantation.

points. The final-cycle AIC value for the full model was 2919.4, and for the reduced model, it was slightly lower at 2916.2, with the lower value indicating the more likely model. However, the parameter value estimates in both models were similar, as shown in Table 3. In the full model, CL/F was 25.8% (*P* = 0.08) higher in patients with functional CYP3A5, and they also showed a relative Tac bioavailability of 63% compared with the nonexpressers. The gamma model failed to converge so the lambda model was chosen. The final-cycle lambda was 1.26, indicating moderate process noise, that is, uncertainty of sample times and dose times, which is typical of TDM data. In our case, we had to assume dose times of 8.00 am and 8.00 pm in the absence of specifically recorded dose times for the TDM data set.

Covariate analysis

Allometric scaling to FFM was superior to WT and BMI for CL/F, Q/F, and Vp/F. However, BMI was most appropriate for the scaling of V/F, with a change in AIC of 9.1. This effect was considered big enough to justify the increased model complexity compared with the more simple solution of using the same body size measure for all parameters. Additionally, scaling CL/F, Q/F, V/F, and Vp/F to hematocrit improved the AIC by 16.1. This is biologically plausible given the intracellular distribution of Tac into erythrocytes [3].

CL/F was affected by CYP3A5 genotype, age (linear decrease > 50 years), concomitant use of nifedipine, and sex when these covariates were added individually to the base model scaled to body size and hematocrit. However, in multivariate covariate stepwise addition and subtraction,

Table 3. Parameter values [median and interquartile range (IQR)] for the final full model and the final model after eliminating *CYP3A5* genotype as a covariate. The bioavailability (F) of the *CYP3A5* expressers was tested relative to the *CYP3A5* nonexpressers for which F was set to 1.0 in the *Full model*, while F was included as a parameter affecting all patients in the *No CYP3A5 model*.

Parameter	Full model		No <i>CYP3A5</i> model	
	Median	IQR	Median	IQR
Apparent clearance for <i>CYP3A5</i> expressers (CL/F _{CYP}) [L/h]	26.7	13.2	–	–
Apparent clearance for <i>CYP3A5</i> nonexpressers (CL/F _{noCYP}) [L/h]	21.2	11.0	–	–
Apparent clearance (CL/F) [L/h]	–	–	17.3	13.6
Apparent intercompartment clearance (Q/F) [L/h]	19.5	32.3	13.9	28.5
Apparent central volume of distribution (V/F) [L]	177	295	190	266
Apparent peripheral volume of distribution (Vp/F) [L]	3707	7736	2982	4155
Relative bioavailability (FA)	0.63*	0.12	0.79†	0.26
Lag time first week (Tlag1) [h]	1.00	1.55	1.08	1.59
Lag time week 2-4 (Tlag2) [h]	0.15	0.46	0.16	0.46
Lag time after first month (Tlag3) [h]	0.59	0.57	0.44	0.48
Absorption rate constant (Ka) [h]	1.04	1.27	1.14	1.24

*FA for *CYP3A5* expressers versus nonexpressers (FA = 1).

†FA for the whole population (not relative to any subpopulation).

CYP3A5, cytochrome P450-3A5, IQR, interquartile range.

nifedipine, age, and sex were not retained as significant covariates. Intercompartment clearance was improved by introducing sex as a covariate, but it was not retained in the final model after the stepwise deletion. No covariates other than hematocrit and body size showed any influence on V/F or Vp/F.

In the final model, *CYP3A5* genotype was retained as a covariate on Tac bioavailability and time after transplantation as a covariate on lag time. The most appropriate way to introduce the time effect on lag time was by applying different lag times at the following intervals after transplantation: week 1, week 2–4, and after week 4. Scaling the lag

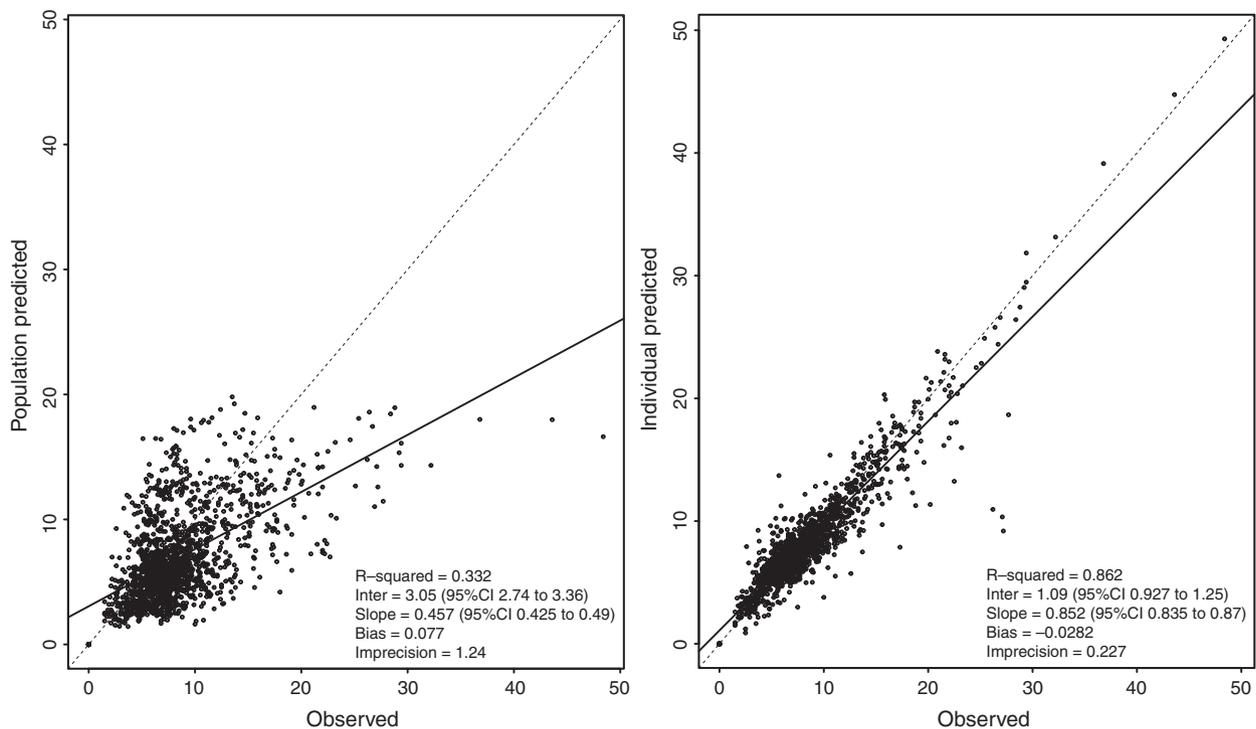


Figure 1 Population (left) and individual (right) predicted versus observed plots of the full model with *CYP3A5* genotype.

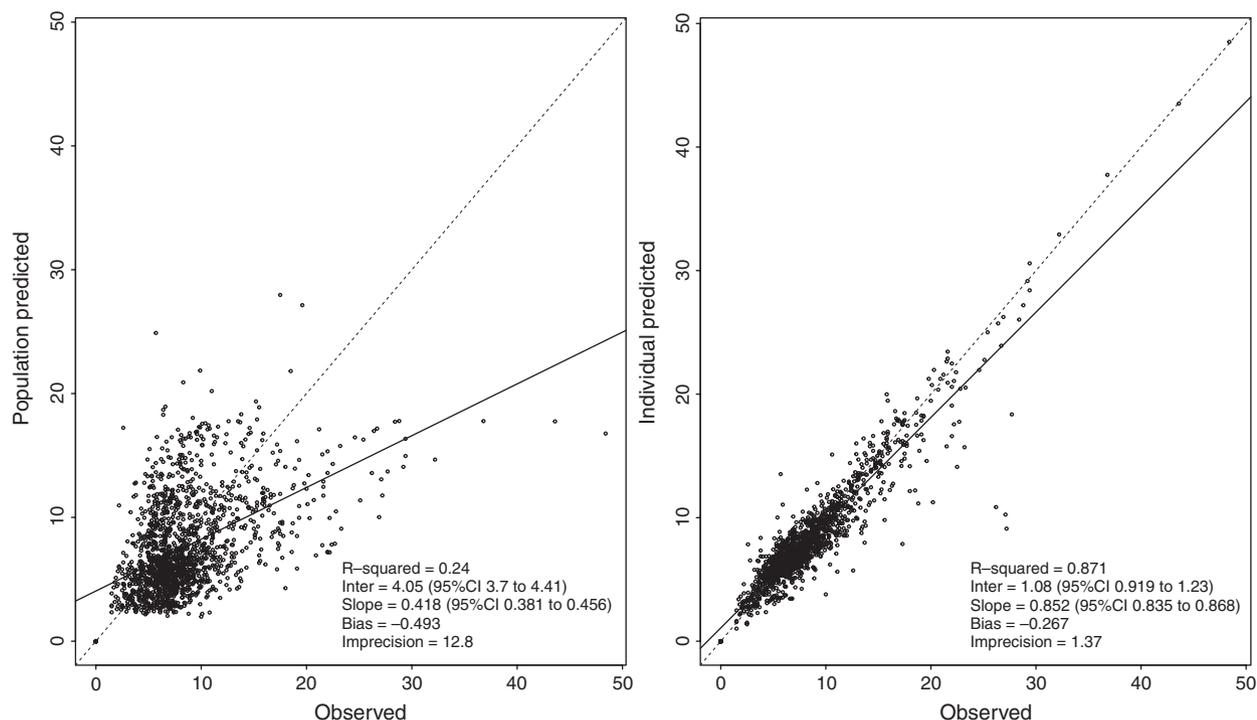


Figure 2 Population (left) and individual (right) predicted versus observed plots of the full model without *CYP3A5* genotype.

time to FFM also improved the AIC by 1.4, and this was retained in the final model.

Prediction versus observation

The population and individual predicted versus observed plots for the two models are shown in Fig. 1 and Fig. 2. The full model showed significantly better R^2 value ($P < 0.00001$), but no significant difference in slope ($P = 0.46$) for the population predictions, and lower bias and imprecision, compared with the reduced model excluding *CYP3A5* genotype. The individual predicted versus observed plot was similar for both models, but the bias and imprecision were still lower for the full model.

Population model validation

The external validation indicates that both models appropriately describe the pharmacokinetics of Tac in renal transplant recipients in the early post-transplant phase. Comparing the data from the 30 new “external” patients with the 44 “internal” patients that provided serial trough concentrations in the population used for developing the model, there were only marginal differences in predictive bias and imprecision. For the full model, bias and imprecision were 0.35 and 1.38 in the external data set and 0.43 and 1.58 in the internal data set, respectively. For the reduced model without *CYP3A5* genotype as a covariate,

external and internal bias and imprecision were 0.41 and 1.65 versus 0.33 and 1.30, respectively.

Effect of CYP3A5

The first Tac trough concentration was obtained on the first day after transplantation in all patients, and the subsequent eight concentrations were collected during the following 9 to 17 days. The full model with *CYP3A5* genotype as a

Table 4. The models (with and without *CYP3A5* genotype as covariate) median (IQR) predictive error for the next concentration (PE, predicted versus observed concentration, of the 1st to the 9th concentrations) when provided all from none to the eight first Tac concentrations after transplantation.

Conc No	With <i>CYP3A5</i>		Without <i>CYP3A5</i>	
	Median PE	IQR	Median PE	IQR
1	-1.47	4.67	-1.89	4.44
2	-0.47	4.46	-0.70	3.37
3	0.11	4.69	-0.21	3.93
4	0.12	3.64	-0.58	2.75
5	0.24	2.93	0.19	2.09
6	0.41	2.54	0.58	2.34
7	-0.49	3.42	0.14	2.54
8	0.28	2.55	-0.13	2.12
9	-0.14	2.36	0.01	1.57

CYP3A5, cytochrome P450 3A5.

Table 5. Percentages of simulated steady-state trough concentration (96 h after first dose) below, within and above the therapeutic window of 3 to 7 µg/l of different BID dosing regimen for Tac in the typical patient with and without functional CYP3A5.

BID dose (mg)	With CYP3A5			Without CYP3A5		
	Below 3 µg/l (%)	Between 3-7 µg/l (%)	Above 7 µg/l (%)	Below 3 µg/l (%)	Between 3-7 µg/l (%)	Above 7 µg/l (%)
0.5	52	37	12	40	42	18
1.0	47	40	13	30	49	21
1.5	42	44	14	22	53	25
2.0	36	48	16	17	54	29
2.5	31	51	18	12	53	35
3.0	27	53	19	9	51	40
3.5	24	55	21	5	48	47
4.0	21	56	23	4	44	52
4.5	17	57	26	3	41	56
5.0	15	57	29	3	37	61

CYP3A5, cytochrome P450 3A5; BID, twice daily (bis in die).

covariate predicted the next concentration with lower bias than the reduced model when no blood concentration information was available (Table 4), that is, only the population prior was used for predictions. This superiority was on average sustained until four subsequent blood concentrations were available (until day 6 post-transplant), and Bayesian posteriors with increasing individual data were used for predictions. With more than three to four samples available, the ability of the two models to predict the next Tac trough concentration was comparable.

Tacrolimus dosing simulations

Results of simulations of dose regimens from 0.5 to 5.0 mg BID are shown in Table 5. The effect of functional CYP3A5 is clear, and a large proportion of the simulated patients are outside of the predefined therapeutic window (trough concentrations between 3 and 7 µg/l) using the standard starting dose of Tac (3 mg BID). The 25th percentile for this dose was 2.6 and 4.6 µg/l and the 75th percentile was 5.6 and 9.0 µg/l for simulated patients with and without functional CYP3A5, respectively.

Discussion

The main finding of the present study was that in renal transplant recipients, this nonparametric model adequately predicts the next Tac trough concentration. To the best of our knowledge, the presented population pharmacokinetics model for Tac, developed in Pmetrics [19], is the first published nonparametric model for this immunosuppressant. Initially, knowledge of each patient's CYP3A5 genotype improves the predictions, but after obtaining three to four Tac trough concentrations, the model does at least as well without the CYP3A5 genotype information.

CYP3A5 genotype greatly affects Tac pharmacokinetics, as our data and those of others clearly demonstrate

[8,9,12,13,17,30–36]. To achieve the same trough concentrations of Tac, expressers of CYP3A5 need approximately twice the dose of nonexpressers, and if not giving higher doses to CYP3A5 expressers at the time of transplantation, they reach the therapeutic window several days later than nonexpressers [35]. The clinical advantage of including knowledge of knowing the individual genotype needs, however, more investigation [36]. Previous reports are conflicting whether the predominant effect of CYP3A5 is on the bioavailability or clearance of Tac. This may be explained by methodological differences and how the applied software treats the available data. In the present analysis, both relative bioavailability and apparent clearance were different between CYP3A5 expressers and nonexpressers. The Tac bioavailability in patients expressing functional CYP3A5 was estimated to 63% of that in patients without this enzyme, and the apparent clearance was about 26% higher in expressers. In this regard, it is interesting to note that in a parametric model based on the same patient data, the CYP3A5 effect was significant on the bioavailability only [22].

Knowledge of CYP3A5 genotype is helpful for more precise selection of the initial Tac doses, but CYP3A5 genotyping alone is not enough to precisely select the optimal initial dose [37]. Furthermore, from the present simulations, it seems that the standard starting dose of “the typical patient” (3 mg BID) is too high in a large proportion of the patients. Different strategies may be applied to better obtain improved individualized dosing. As an example, in addition to CYP3A5 genotyping, pretransplant CYP3A4 phenotyping has been investigated [34]. From the present work, the use of a nonparametric population model for improved Tac dosing looks like an accurate and easily implementable alternative. This was shown with current standard TDM data, including only trough concentrations. The full advantage of using such a population model in

predicting the most appropriate individual dose will, however, first be achieved when blood concentrations are obtained at different time points within a dose interval. In other words, a TDM strategy based only on trough concentrations severely restricts the information about individual Tac pharmacokinetics, because theoretically a range of different time–concentration profiles may fit the same measured trough concentration. More optimal timing of Tac measurements will provide richer information about individual Tac pharmacokinetics. This is information a pharmacokinetic population model, and appropriate software easily can take full advantage of, but which is difficult to evaluate without these tools. Therefore, rather than just obtaining trough concentrations three to four times during the first week after transplantation, as in the current validation data set, more information about each individual will be attained if these samples instead are appropriately timed within the first couple of dose intervals. This would most likely improve the predictive performance of the model. Further investigations are, however, needed to evaluate this and also to elucidate on the knowledge of *CYP3A5* genotype within such a setting [38,39].

Another advantage of a population model for individual dose estimations is that it will make it possible to recommend individual doses based on an identified AUC target rather than merely a trough target concentration. However, a relevant target AUC will first have to be established, but when established, it gives more flexibility for the treating physician both when it comes to Tac sampling times and individualization of target level. Further, despite this relevant contribution to limiting systemic exposure of Tac in patients expressing functional *CYP3A5*, three to four trough concentrations from each individual were sufficiently informative to the nonparametric model to make explicit information about individual *CYP3A5* genotype redundant. This might give the nonparametric model an additional advantage as it might also adjust for genotypes or other factors affecting the pharmacokinetics of a drug that we currently are unaware of. Recently, data have shown that transporters such as MRP2 and ABCC2 also affect Tac pharmacokinetics [40].

Potent concomitant immunosuppressive therapy, such as antibody induction, reduces the need of high levels of Tac in the early phase after transplantation [41]. Whether induction therapy eliminates the risk associated with sub-optimal Tac coverage during the first few days after transplantation is not known, but in such a setting, a pharmacokinetics population model alone might be sufficient for guiding Tac dosing [42–44]. Based on anecdotal data, however, some patients may be in need of adequate Tac coverage already from the first dose. Hence, the safest option until more data are available will be to combine the use of a population model and *CYP3A5* genotyping for

individual initial dose selection and subsequent dose adaptation in renal transplantation.

Even though this is one of the larger combined data sets of detailed 12-h data and trough TDM data so far used for development of a pharmacokinetic population model for Tac, a limitation is the relative low number of patients who express functional *CYP3A5* (10 of 69 and 7 of 30, respectively), and none of these were *CYP3A5**1/*1 homozygotes. A large proportion of the data are also trough concentrations without exact dosing times known, and 12-h pharmacokinetic profiles were not determined in the early phase after transplantation.

In conclusion, the present nonparametric population model for Tac described both the internal and external data set well. Including *CYP3A5* genotype improved the dose predictions until three to four Tac trough concentrations were available, after which the reduced model did at least as well. The performance of the model needs to be further investigated in combination with more optimal sample collection design and tested prospectively for its ability to estimate optimal individual dosing of Tac in renal transplant recipients.

Authorship

ÅÅ: performed the clinical trials, designed and performed the population modeling, and wrote the article. KM: the principal investigator in the clinical trials and reviewed the modeling and article. MvG, ES, RJ, and MNN: designed and performed the population modeling and reviewed the article. ES: collected some of the patient data. SaB and StB: performed pharmacological and genotyping analyses and reviewed the article. AH: performed the clinical trials and reviewed the article.

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