Short title: Tacrolimus PopPK

Population pharmacokinetics of tacrolimus in adult kidney transplant patients: impact of CYP3A5 genotype on starting dose

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Abstract

Objectives: The aims of this study were to develop a population pharmacokinetic model of tacrolimus in adult kidney transplant recipients; to use this model to compare cytochrome P450 3A5 (CYP3A5) genotype based initial dosing of tacrolimus to standard per-kilogram based dosing; and to predict the best starting dose of tacrolimus based on patient genotype to achieve a trough concentration between 6 and 10 µg/L by day 5 post-transplantation.

Methods: Population analysis was performed using the software program NONMEM®. Tacrolimus dosing regimens were compared by predicting tacrolimus trough concentrations in a simulated dataset by running NONMEM® with population parameters fixed at the final model estimates. Data from 173 patients with 1,554 tacrolimus concentration-time measurements were modelled.

Results: Tacrolimus disposition was well described by a two-compartment model with first-order elimination and first order-absorption following a lag-time. Patient *CYP3A5* genotype (rs776746), weight, haematocrit and post-operative day were identified as significant covariates effecting tacrolimus apparent clearance (CL/F), with higher CL/F in *CYP3A5 *1* allele carriers, heavier patients, patients with low haematocrit and in the immediate post-transplantation period. Typical population estimates for tacrolimus CL/F in *CYP3A5 *1* allele carriers and non-carriers were 40.8 L/h and 25.5 L/h, respectively.

Conclusion: In patients carrying the *CYP3A5*1* allele, a per-kilogram dose of 0.075 mg/kg twice daily appeared to be much too low with approximately 65% of simulated subjects predicted to achieve a trough below 6 µg/L at day 5 post-transplantation. To reduce the risk of under immunosuppression in the immediate post-transplantation period, carriers of a *CYP3A5*1* allele are likely to benefit from a tacrolimus starting dose of either 10 mg or 0.115 mg/kg twice daily.

INTRODUCTION

Tacrolimus is a potent immunosuppressant agent used to prevent and treat rejection in adult kidney transplantation. Usage of tacrolimus is complicated by its narrow therapeutic index and wide between and within-subject pharmacokinetic variability. Adequate exposure is imperative for the prevention of rejection, while over-exposure risks serious toxicities that reduce tolerability and impact long-term allograft and patient survival. The dosage of tacrolimus needed to achieve therapeutic concentrations in adult kidney transplant recipients is highly variable between patients and difficult to predict, especially in the immediate post-transplantation period.

Regular therapeutic drug monitoring (TDM) of tacrolimus with accurate dosage modification is mandatory. Largely for reasons of practicality and convenience, pre-dose trough concentration (C_0) values are typically used to guide tacrolimus dosing. However, evidence regarding the correlation of C_0 with tacrolimus exposure over a dosing interval as measured by the area-under the concentration-time curve (AUC_{0-12}) is conflicting ($r^2 = 0.04$-0.91). It has been suggested that AUC monitoring might be preferable, however a lack of comparative data in the literature prevents firm conclusions from being reached (1). Of note, TDM provides no information on the optimal starting dose of tacrolimus to use in the immediate post-transplantation period in a given individual or on how to adjust drug dosage under different clinical circumstances.

Information needed for dosage individualisation can be obtained through population pharmacokinetic modelling (2). During such analysis, typical values for pharmacokinetic parameters are estimated together with between- and within- subject variability associated with these estimates. The influence of multiple factors (covariates) on pharmacokinetic parameters can also be examined. An appropriate population pharmacokinetic model can be used to assist in predicting the optimal starting dose for a given patient and can be used as *a prior* information in a Bayesian dosage prediction program to assist with repeat dosage prediction and adjustment, and the

most appropriate dosage regime to reach or maintain a desired drug concentration can be predicted faster and with less dosage adjustments (2, 3). To be useful however, a population pharmacokinetic model must characterise the patient group in which it is intended to be used, as defined by factors such as graft type, time post-transplantation and analytical technique used for drug measurement.

Several factors have previously been identified as affecting the pharmacokinetics of tacrolimus. Tacrolimus apparent clearance (CL/F) is known to be dependent on a patient's *CYP3A5* 6986A>G genotype (rs776746). Heterozygous or homozygous carriers of the *CYP3A5*1* allele (*1/*1 and *1/*3) express high levels of functional CYP3A5 protein (CYP3A5 expressers) and are generally faster metabolisers of tacrolimus compared to homozygous carriers of the CYP3A5*3 allele who produce very low or undetectable levels of functional CYP3A5 protein (CYP3A5 non-expressers) (4). Furthermore, numerous studies have reported a decrease in tacrolimus dosage requirements to maintain similar trough concentrations with increasing time post-transplantation. A reduction in corticosteroid exposure (an inducer of tacrolimus metabolism) and increased haematocrit and plasma protein levels with time (leading to decreased tacrolimus free fraction and decreased apparent clearance based on total drug measurement) have been postulated as reasons for this (5-7).

The primary goal of this study was to develop a population pharmacokinetic model of tacrolimus in a large cohort of adult kidney transplant recipients that could eventually be used to assist future drug dosing through Bayesian forecasting. Secondary goals were to employ this model in simulation experiments to compare *CYP3A5* genotype based initial dosing to standard per-kilogram based, and to predict the best tacrolimus starting dose based on patient genotype to achieve a tacrolimus trough concentration between 6 and 10 µg/L by day 5 post-transplantation.

MATERIALS AND METHODS

Patients and data collection

De-identified demographic and pharmacokinetic data were merged from two consecutive clinical studies in adult kidney transplant recipients performed at the Princess Alexandra Hospital in Brisbane, Australia (8, 9) to create a large data set for population pharmacokinetic modelling. In the first study, a full concentration-time profile characterising tacrolimus and prednisolone therapy was collected from 20 patients with blood samples taken pre-dose and 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 9 and 12 hours (h) post dose. Half of the patients were sampled in their first post-transplant week, the other half were > 90 days post-transplantation at the time of sampling (range: 90 days to 6½ years). In the second study, a limited concentration-time profile characterising tacrolimus and prednisolone therapy was collected from 153 patients with blood samples taken pre-dose and 1, 2 and 4 h post-dose on at least one occasion between day 4 and 12 months post-transplantation. Both studies were performed in compliance with the declaration of Helsinki and were approved by Princess Alexandra Hospital and University of Queensland Ethics Committees. All participants provided written informed consent.

Immunosuppressant therapy

Patients received immunosuppressant therapy as per unit protocol. Induction therapy included 20 mg intravenous basiliximab (Simulect®, Novartis, East Hanover, New Jersey) pre- and 4 days post-operatively and 500 mg intravenous methylprednisolone (Solu-Medrol®, Pfizer, New York, USA) pre- and 12 h post-operatively. Oral tacrolimus (Prograf®, Janssen-Cilag, MacQuarie Park, Australia) was initiated pre-operatively at a dose of 0.075 mg/kg and continued twice daily to achieve individualised target trough concentrations according to recipient immunological and toxicity risk status (generally 6 to 10 µg/L over the first three months). Oral prednisolone (Panafcortelone®, Aspen Pharmacare, St Leonards, Australia) was initiated on the first post-operative day at a dose of 0.3 mg/kg ideal body weight once daily (up to a maximum of 30 mg

daily) and maintained at this dose until one month post-transplantation. It was subsequently tapered by 1 mg per day every week down to a maintenance dose of 5 to 10 mg daily. Oral mycophenolate mofetil (Cellcept®, Roche, Dee Why, Australia) was initiated pre-operatively at a dose of 1000 mg twice daily with dose adjustment for toxicity or allograft rejection.

Tacrolimus and prednisolone analysis

Tacrolimus concentrations were determined in whole blood using high performance liquid chromatography (HPLC) with tandem mass spectrometric detection (10, 11). This assay was linear over the range 0.5 to 50 µg/L. Assay accuracy ranged from 101.3 to 103.4% and imprecision was <5%. Total and free prednisolone concentrations were determined in plasma and plasma ultrafiltrate, respectively, using ultra HPLC with tandem mass spectrometric detection (12). Plasma ultrafiltrate was prepared by temperature-controlled ultrafiltration. This assay was linear over the range 1.0 to 2000 nmol/L. Intra-assay coefficient of variation (CV) was <5% and inter-assay CV was <10%.

Free and total prednisolone area under the concentration-time curve (AUC) was estimated from drug concentration-time measurements taken at 1, 2 and 4 h post-dose using limited sampling strategies (LSSs) previously developed or validated in this transplant population (8). For the purpose of this study, the highest free and total prednisolone concentration value at these time points was taken as the maximum concentration (C_{max}) over a dosing interval.

Genotyping

CYP3A5 6986A>G (rs776746) allelic discrimination genotyping was performed on genomic DNA extracted from whole blood samples using a QIAamp deoxyribonucleic acid mini kit (Qiagen, Hilden, Germany). Real-time polymerase chain reaction (PCR) was performed with a 7900 Real

Time PCR System (Applied Biosystems, Melbourne, Australia). PCR conditions were: 10 minutes at 95°C, then 50 cycles of 15 seconds at 92°C and 1 minute 30 seconds at 69°C. Allelic discrimination was undertaken with a pre-designed SNP genotyping assay by Applied Biosystems, Melbourne, Australia. A Pearson's χ^2 test was performed to assess the deviation of *CYP3A5* 6986A>G genotype frequencies from Hardy-Weinberg equilibrium.

Population pharmacokinetic analysis

Software

Population pharmacokinetic analysis was performed using NONMEM® version 7.12 (Icon Development Solutions, Ellicott City, MD, USA) with an Intel FORTRAN compiler aided by Pearl Speaks NONMEM® (PsN) version 3.5.3 (13). NONMEM® output was visually explored by graphics produced by R (v2.15.0) with Xpose® version 4.3.5 (14) and by STATA® version 10.1 (StataCorp®, College Station, Texas, USA). Pirana® v2.5 (15) was used for run record management.

Structural and stochastic model

One-, two-, and three-compartment structural disposition models with first-order elimination and bioavailability fixed to 1 were tested during model building. First-order absorption models with and without lag-time and a more advanced Erlang absorption model (16) were also tested. Inter-individual variability (IIV) was assumed to be a normally distributed random variable with a mean of zero. IIV was tested on all parameters in the model and included in the next model building step if significant. Correlations between individual parameter estimates were also examined. Inter-occasion variability (IOV) was explored on pharmacokinetic parameters after the structural and covariate model had been established. Proportional, additive and combined error models were tested to describe residual unexplained variability (RUV). Typical population pharmacokinetic parameter

estimates, IIV, IOV and RUV were estimated using the first-order conditional estimation method with interaction (FOCE-I).

Competing models were discriminated during the model building process by a decrease in the objective function value (OFV) of more than 6.63 with the addition of one model parameter for nested models.

Covariate model

Clinical factors investigated for an influence on the pharmacokinetics of tacrolimus were: patient age, donor age, ethnicity, gender, total body weight, height, organ donor status (live/deceased donor), serum albumin, serum creatinine, serum urea, total bilirubin, aspartate aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, creatinine clearance (calculated using the Cockroft-Gault equation), haematocrit, use of CYP3A4 inhibitors and proton pump inhibitors and free and total prednisolone exposure (AUC and C_{max}). It was decided *a priori* to include *CYP3A5* genotype in the base model as a covariate on tacrolimus CL/F. First, all covariates were evaluated visually with scatter plots and through examination of covariate-covariate correlation (Spearman rank coefficient) and frequency distributions. All but one covariate of two or more related covariates with a Spearman rank correlation coefficient ≥ 0.5 were discarded (keeping the covariate with least missing data). In the case of missing covariate values, the median population value was used for that individual. Next, all remaining covariates were screened for PK parameter association by calculating the Spearman rank coefficient for all covariate-parameter combinations. Only combinations with a correlation coefficient > 0.2 were tested in NONMEM® for statistical significance using a stepwise covariate model building (SCM) strategy. Covariates were considered to be statistically significant if their inclusion in a nested model resulted in an OFV drop of 6.63 or more (χ^2, df =1, p<0.01) and their exclusion from the full model resulted in an OFV

rise of 7.88 (χ^2, df =1, p<0.005) or more. At the same time, changes in parameter IIV were examined, with the expectation of a 5% or more decrease in unexplained IIV with covariate addition. A greater than 15% change in parameter values at the extremes of the covariate values in the data set was also required.

The covariate-parameter relationships for continuous variables (e.g. haematocrit, body weight) were initially modelled as a linear relationship, but for each step the covariates competing for inclusion were checked for improved fit using piece-wise linear, exponential and power function models. The effect of a covariate on a parameter was estimated relative to the median covariate value in the dataset.

Model evaluation and internal validation

Models were evaluated using goodness of fit (GOF) plots and their predictive performance was assessed using prediction-corrected visual predictive checks (pcVPCs)(Figure 1) based on 1,000 simulations (17). The median and nonparametric 90% prediction interval (5th to 95th percentile) from the simulated plasma concentrations together with the 95% confidence intervals around these percentiles were compared visually with the same percentile lines and confidence interval obtained from the original raw data. Uncertainty in the pharmacokinetic parameter estimates was quantified by performing a nonparametric bootstrap of the final model with 1,000 replicates using PSN®.

Examining starting dose recommendations

To compare the current tacrolimus dose-per-kilogram strategy used at the Princess Alexandra Hospital to a same-dose-fits-all approach and *CYP3A5* genotype and weight based schemes over the first 5 days of therapy, a series of simulations (n=100) using the final full model

were performed. Frequency distributions of patients achieving tacrolimus trough concentrations below 6 µg/L, between 6 and 10 µg/L and above 10 µg/L for the different dosing regimens were simulated by running the original dataset in NONMEM® with the final model estimates. For the per-kilogram dosing regimen, tacrolimus dose was calculated according to patient weight (0.075 mg/kg) and rounded to nearest 0.5 mg. For the same-dose-fits-all approach, tacrolimus dosage was sequentially fixed in 0.5 mg increments from 3 to 12 mg twice daily for all patients. For a genotype-based dosing regimen, patients were divided into two groups depending on whether they were CYP3A5 expressers or not and then a per-kilogram and same-dose-fits all approach was examined across the groups. The best tacrolimus starting dose based on patient genotype was considered to be one associated with the maximum number of patients having a steady-state tacrolimus trough concentration between 6 and 10 µg/L. Simulated tacrolimus concentrations were considered to be steady-state trough values if the sampling time was 12 hours (±1.5 hours) post-dose on post-operative day 5 (±1 day).

RESULTS

Patients and data collection

Data from 173 adult kidney transplant recipients with 1,554 tacrolimus concentration-time measurements were collated for population analysis. Demographic, clinical and pharmacogenetic characteristics of patients involved are summarized in Table 1. The vast majority of data collected in this study come from the early post-transplantation period with 83% of measurements taken between 0 and 90 days post-transplantation. The different dosing and sampling regimens available in the dataset meant that the entire concentration-time profile of tacrolimus was well characterised. Initial examination of the data showed a poor relationship between tacrolimus dosage and trough concentrations (Spearman correlation coefficient 0.14). Frequency of the *CYP3A5* 6986A>G

genotype in the population was in Hardy-Weinberg equilibrium. 85% of patients were identified as CYP3A5 non-expressers (*3/*3 genotype), 13% carried a single functional allele (*1/*3) and 2% carried two functional alleles (*1/*1).

Population pharmacokinetic analysis

Structural and stochastic model

A 2-compartment disposition model, with first-order absorption after a lag time and first order elimination was selected as the best structural model to describe tacrolimus pharmacokinetics. The best base model allowed estimation of IIV associated with CL/F and the central (V1/F) and the peripheral (V2/F) compartment volume of distribution. RUV was best described using a proportional error model. Adding IOV on CL/F and V1/F on the full covariate model caused a further significant reduction in the model OFV and was therefore included in the final model (see Table 2).

Covariate model

Following *a priori* inclusion of patient *CYP3A5* genotype in the base model, haematocrit and body weight on CL/F and free prednisolone C_{max} on V1/F were identified in the SCM as being significant (Table 3).

The influence of *CYP3A5* genotype on tacrolimus CL/F was modelled using a categorical relationship. Three patients were homozygous for the functional *1 allele and these were pooled with the 23 heterozygous patients during modelling. Genotype could not be established in one individual, who was arbitrarily assigned to the most common genotype *CYP3A5*3/*3*. For completeness, a model was tested without CYP3A5 as covariate in it; this resulted in an OFV increase of 31 points and unexplained IIV in CL/F increasing from 48.4% to 53.9%. In the final

model, carriers of at least one functional *CYP3A5* *1 allele typically had 60% higher tacrolimus CL/F than non-carriers (Figure 2).

The influence of haematocrit on tacrolimus CL/F was best described using a linear relationship. Addition of haematocrit on CL/F resulted in an OFV drop of 36 points and a reduction in unexplained IIV associated with CL/F of 17.6%. In the final model, every 0.01 change in haematocrit fraction from the median haematocrit fraction of 0.33 resulted in a 1% change in CL/F from typical CL/F, with higher tacrolimus CL/F in patients with lower haematocrit.

The influence of body weight on tacrolimus CL/F was best described using a power function model, which in line with allometric scaling theory, had a fixed exponent of 0.75 (18, 19). Heavier patients had higher tacrolimus CL/F. Addition of body weight influence on CL/F resulted in an OFV drop of 32 points and a reduction in unexplained IIV associated with CL/F of 6% points.

The influence of free prednisolone C_{max} on tacrolimus V1/F was best described using a linear relationship. Addition of a free prednisolone C_{max} influence on V1/F resulted in an OFV drop of 33 points and a reduction in unexplained IIV associated with V1/F of 7.9%. In the final model every 1 nmol/L change in prednisolone C_{max} from the median value resulted in a 0.28% change in V1/F from typical V1/F, with higher V1/F in patients with lower prednisolone C_{max}.

Post-operative day (POD) was not included in the SCM but was tested afterwards with the intension of examining whether there were further time-varying influences that could not be described by the covariates already modelled. Addition of a post-operative day influence on CL/F resulted in a further OFV drop of 53 points and a reduction in unexplained IIV associated with CL/F of 4.6% while addition of post-operative day as a covariate on V1/F had no significant influence. The influence of post-operative day on CL/F was best described using a linear relationship with capping of values greater than 180 days to 180 days. In the final model, every 1

day change in post-operative day from the median value of 23 days resulted in a change in CL/F from typical CL/F of 0.21%, with higher CL/F in patients in the immediate post-operative period.

Total reduction in unexplained IIV from base to final covariate model was from 49% to 30% for CL/F and from 114% to 47% for V1/F. All covariates remained significant during the backward elimination step. Base and final model parameter estimates are given in Table 2. A summary of significant covariate models is shown in Table 3. Eta-shrinkage was high in the final model: 25.9% for CL/F and 59.7% for V1/F. RUV associated with the final model was 18.3%.

Model evaluation and internal validation

No trends that suggested misspecification in the final model were identified in the GOF plots and a pcVPC (Figure 1). Results of the non-parametric bootstrap to estimate uncertainty associated with the final model are given in Table 2. The non-parametric 95% confidence interval was relatively narrow for all parameters, suggestive of a robust model. For covariate effects, none of the confidence intervals included zero, indicative of statistically significant relationships.

Examining starting dose recommendations

One hundred and fifty-six tacrolimus concentrations in the dataset were identified as 'early, steady-state' trough values (C_0) as defined by being taken between 10.5 and 13.5 hours post-dose on day 5 (± 1 day) post-transplantation. Nine individuals had more than one trough value. Considering only one 'early, steady-state' trough value per patient concentrations ranged from 2.5 to 33.6 µg/L with 58 concentrations (42 %) in the targeted range of 6-10 µg/L. Twenty concentrations (14 %) were below and 61 (44 %) were above this target range. Of 21 carriers of the functional CYP3A5*1 allele, 6 (29 %) had trough concentrations below 6 µg/L and only 3 (14 %) had trough concentrations above 10 µg/L (P-value 0.002 for the non-parametric test for departure from the

fitted trend).

Simulations showed that a fixed dose regimen of 6 mg twice daily given to all patients over the first 5 days post-transplantation without any TDM adjustments would result in 37%, 32% and 32% of patients having a tacrolimus trough concentration below, in and above the 6-10 µg/L target range, respectively. Results for a per-kilogram dose regimen of 0.075 mg/kg twice daily given to all patients were almost identical with 35%, 33% and 31% of patients achieving a trough below, in and above the target. In patients carrying the functional *CYP3A5*1* allele, both a fixed tacrolimus dose of 6 mg twice daily (Figure 3b) and a per-kilogram dose of 0.075 mg/kg twice daily (Figure 3d) was clearly much too low, with more than 66% of simulated subjects predicted to achieve a trough concentration below 6 µg/L. Increasing the tacrolimus dose in *CYP3A5*1* carriers to 10 mg twice daily or 0.115 mg/kg reduced the risk of sub-therapeutic trough concentrations to less than 40%. It should be noted, however, that under this scenario, the risk of supra-therapeutic trough concentrations increased from approximately 10% to 30%.

DISCUSSION

This prospective pharmacokinetic modeling study of tacrolimus in 173 adult kidney transplant recipients represents the largest study of its type in this patient category to date. As expected, possession of a *CYP3A5*1* allele was associated with increased tacrolimus CL/F and increased risk of sub-therapeutic tacrolimus trough concentrations in the immediate post-transplantation period. The performance of tacrolimus weight-based and fixed-dose starting regimens was essentially equivalent, suggesting that much of the pharmacokinetic variability associated with tacrolimus therapy is not weight dependent. Based on simulations from the population analysis, a *CYP3A5* genotype-based dosing regimen is proposed, in which carriers of the functional allele *CYP3A5*1* are initiated on either 10 mg or 0.115 mg/kg of tacrolimus twice daily

and non-carriers are initiated on either 6 mg or 0.075 mg/kg twice daily. This approach should in theory reduce the occurrence of sub-therapeutic tacrolimus C_0 values in *CYP3A5*1* carriers, but will at the same time increase the risk of supra-therapeutic trough concentrations. This increases susceptibility to tacrolimus-related toxicities, including acute nephrotoxicity, neurotoxicity and hyperglycaemia. However, each of these complications is usually reversible with reduction of tacrolimus dosing, as would occur with the onset of TDM. Thus, their development may be preferable to the development of acute rejection, which has the potential to impact long-term graft outcomes (20). A multicenter study recently examined genotype based dosing in a clinical trial where patients were randomized to either a fixed tacrolimus dose of 0.2 mg/kg/day or a *CYP3A5* genotype determined dosage of 0.30 mg/kg/day for *1 carriers and 0.15mg/kg/day for *1 non-carriers. In the genotype dose differentiated arm of the study 43.2% of the patients achieved a tacrolimus C_0 value within the target range compared to 29.1% in the fixed dose treatment arm (P=0.03) (21). However, it remains to be shown if the improvements also affect the clinical outcome. The population model developed in this study can be used in simulation analysis to predict the optimal starting dose of tacrolimus to achieve any desired C_0 value at any time point post-transplant (eg. a tacrolimus C_0 value of 10 µg/L on day 2, if preferred). The model can also be employed in a Bayesian forecasting program to assist with repeat dosage prediction and adjustment based on observed drug concentration-time measurements.

Typical pharmacokinetic parameter values and covariate findings obtained in this study are in good agreement with past investigations. Previous studies in adult kidney transplant recipients have involved from 12 to 73 subjects (5, 7, 16, 22-27). One and two-compartment distribution models with first-order elimination have typically been used to describe tacrolimus disposition. First-order absorption models, with or without a lag time, and Erlang-type absorption models with a fixed number of transit compartments have been used to describe drug uptake. Patient *CYP3A5*

genotype, haematocrit, post-operative day, prednisolone dose, aspartate aminotransferase and tacrolimus formulation used (Prograf versus Advagraf) have formally been identified as having a significant influence on tacrolimus CL/F. Typical estimates of CL/F across previous studies range from approximately 25 to 30 L/h in CYP3A5 non-expressers and 40 to 45 L/h in CYP3A5 expressers and typical estimates of V1/F range from approximately 150 to 500 L compared to values of 25.5 L/h, 40.8 L/h and 113 L respectively in this study.

In the presented study, patient CYP3A5 genotype, weight, haematocrit and post-operative day were identified as significant covariates effecting tacrolimus CL/F. All models tested throughout the development process consistently estimated *CYP3A5*1* carriers to have much higher CL/F than non-carriers with little fluctuation across different models. Patients who carried a CYP3A5*1 allele typically had 60% higher tacrolimus CL/F than non-carriers. As the *1 allele is associated with increased CYP3A5 function, one would expect even higher tacrolimus dosing requirements in homozygous compared to heterozygous carriers. However, as only 3 patients involved in this study had the CYP3A5*1/*1 genotype, this could not be adequately examined. The clear effect of *CYP3A5* genotype on tacrolimus CL/F regardless of patient body weight underscores the potential importance of an individualised initial dosing strategy in this population.

The pregnane X receptor, which is encoded by the *NR1I2* gene, is responsible for up-stream regulation of *CYP3A5*. In a recent study by our group, adult kidney transplant recipients who possessed a *NR1I2* 8055T allele (rs2276707) had higher tacrolimus-dose adjusted exposure compared to non-carriers based on a non-compartmental analysis involving some of the patients included in this current study (28). When patient *NR1I2* 8055C>T genotype was introduced as a categorical covariate on CL/F in our final model, the estimated tacrolimus CL/F was approximately 9% lower in patients with the CT or TT genotype compared to patients with the CC genotype corresponding with the results of the non-compartmental analysis. However, the OFV only dropped

2.6 points indicating that the effect is not statistically significant. Additionally, a recent study has identified a further single nucleotide polymorphism in the *CYP3A4* gene which also likely impacts on tacrolimus pharmacokinetics (29); clearly there is a need for larger pharmacogenetic/genomic studies of tacrolimus in the future with newer information incorporated into population modeling as it becomes available.

Decreasing tacrolimus CL/F with increasing haematocrit is well described (30). As tacrolimus binds strongly to red blood cells and drug concentrations are measured in whole blood, low haematocrit would be expected to result in an increase in total body clearance due to an increase in free drug fraction; unbound drug clearance however is unlikely to change. Haematocrit fraction is generally lower in kidney transplant recipients immediately post-surgery and then increases significantly as kidney function is restored.

Increasing tacrolimus CL/F with increasing body weight has been reported previously in organ transplant recipients (31, 32) and would be theoretically expected. For future modeling studies of tacrolimus, it would be worthwhile including patient weight as a covariate on CL/F as a power function *a priori*. This would especially be helpful in studies with low power where statistical significance of established covariates cannot always be achieved. Influence of patient weight on V1/F was also examined as it would also be expected to have a potential effect. Its introduction resulted in an OFV drop of 3 points only and it was thus not retained.

Decreasing tacrolimus dose with increasing time post-transplantation is well described (5-7, 33-35). A decrease in tacrolimus clearance with time is generally believed to be the reason for this, although increasing bioavailability must also be considered. A reduction in corticosteroid dosage and increased haematocrit and albumin concentrations with time have been postulated as causes for this. The continued significance of post-operative day even after inclusion of haematocrit suggests that other physiological-based covariates not examined or not well characterized in this study are

likely to be important (eg. fluid status, alpha-1-acid glycoprotein and lipoproteins levels, gastrointestinal tract motility, kidney and liver function). Influence of post-operative day on V1/F was also examined. Its introduction resulted in an OFV drop of 7.7 points, but as IIV in V1/F was reduced by less than 2%, it was not retained.

Higher prednisolone dose has previously been associated with increased tacrolimus clearance (7, 24, 36). It has been suggested that corticosteroids may increase tacrolimus metabolism and thereby decrease exposure through induction of CYP3A enzymes. This was able to be tested in the present study in more depth by testing prednisolone exposure (C_{max} and AUC of free and total prednisolone) as a covariate on tacrolimus CL/F and V1/F. Interestingly C_{max} of free prednisolone was found to have a significant influence on the apparent central volume of distribution (V1/F) of tacrolimus, with higher V1/F in patients with lower free prednisolone C_{max} values. This is a novel finding. Prednisolone has well known anti-inflammatory properties and this possibly causes changes in membrane permeability and influences fluid retention. However, this explanation does not account for why tacrolimus V1/F was relatively constant over time, while C_{max} of free prednisolone decreased with time in accordance with the steroid tapering. Also, taking into account the lipophilic nature of tacrolimus, it would be expected to be largely insensible to subtle shifts in membrane water permeability. Another possibility is that higher tacrolimus V1/F is an indicator for some unknown factor that leads to lower free prednisolone exposure. The possibility that the finding is spurious must also be considered. The clinical utility of prednisolone C_{max} is likely to be very limited so long as the measuring of prednisolone concentrations is not done in routine clinical practice.

Data collection in this study was performed prospectively. At least four concentration-time measurements were taken in each patient over a dosing interval, generally across multiple occasions. Tacrolimus concentrations were measured via high performance liquid chromatography.

This technique is highly sensitive and specific for the parent drug compared to immunoassays, which are sometimes associated with non-specific binding of the antibody to metabolites and endogenous compounds (10). Study results, however, should not be extrapolated beyond the patient group in which they were derived, as defined by factors such as graft type, time post-transplantation and analytical technique used for drug measurement.

It should be noted that the final population model developed in this study explained 82% (RUV 18%) of the observed pharmacokinetic variability associated with tacrolimus therapy. Multiple small effects from a large number of other variables may be undetected (eg. other genetic effects not tested, environmental factors such as patient diet, other concomitant drugs not examined). In the present case, the dosing strategy suggested is likely to be superior to empiric 'one dose fits all' approaches but needs to be further tested before wide scale introduction. The inter occasion and inter individual variability associated with tacrolimus CL/F was both approximately 30% indicating that the additional benefit from target concentration intervention might be limited at this stage (37).

In conclusion, this study presents a population pharmacokinetic model of tacrolimus from a large body of data collected from adult kidney transplant recipients. The model is a firm base for employing Bayesian forecasting in the future. Simulations have shown that a fixed tacrolimus dose or a dose per kilogram regimen performs equally well, but leaves ample room for improvement. The importance of *CYP3A5*1 carrier status on tacrolimus exposure is confirmed. However, the need for TDM remains, with simulations revealing that a starting regimen based on CYP3A5 genotype alone, without TDM, cannot deliver desired target exposure to more than 30% of the population in the early post-transplant period. There is clearly still information missing on other factors that predicted tacrolimus pharmacokinetic variability in this population. It is important to note that the suggestions arising from simulation are based on population-based predictions and

apply to the typical individual. When dealing with a particular patient, the clinician always needs to assess individual factors and risks when deciding dosing strategies.

Table 1. Demographic, clinical and pharmacogenetic characteristics of study patients

Characteristic	Value[a]
Number of patients (n)	173
Age (y)	50 [33-64]
Gender male / female (n)	115 / 58
Body weight (kg)	79 [59-101]
CYP3A5 6986A>G genotype[b] (n)	
AA (*1/*1)	3
GA (*3/*1)	23
GG (*3/*3)	146
Haematocrit (fraction)	0.33 [0.25-0.40]
Total bilirubin (µmol/L)	11 [8-17]
Albumin (g/L)	34 [27-41]
Alkaline phosphatase (U/L)	62 [41-114]
Aspartate aminotransferase (U/L)	17 [11-34]
Gamma-glutamyltransferase (U/L)	25 [14-66]
Creatinine clearance[c] (mL/min)	53 [15-86]
Tacrolimus dose (mg)	5 [3-8]
Tacrolimus trough[d] concentrations (µg/L)	9.33 [5.56-15.97]
Total prednisolone AUC_{0-6h}[e] (nmol*h/L)	5269 [3638-7112]
Free prednisolone AUC_{0-12h}[e] (nmol*h/L)	853 [412-1231]
Total prednisolone C_{max} (nmol/L)	1119 [753-1567]
Free prednisolone C_{max} (nmol/L)	162 [85-260]
Post-operative day (POD) (days)	23[f] [2-104][f]

a – Median or number [10th-90th percentile]

b – Genotypes are in Hardy-Weinberg equilibrium. For one patient the genotype could not be established. That patient was arbitrarily assigned to the most frequent genotype

c – Calculated using the Cockroft-Gault equation

d – Based on tacrolimus concentrations in whole blood from a sample taken between 10.5 and 13.5 hours post-dose on day 5 (±1 day) post-transplantation

e – Based on a limited sampling strategy (13)

f – Identical for both POD and POD capped at 180 days

AUC = area under the concentration-time curve, C_{max} = maximum concentration, CYP3A5 = Cytochrome P450 3A5

Table 2. Parameter estimates for the base and final models and bootstrap results for the final model

Parameter	Base model (OFV= 6643)		Final model (OFV=6054)	
				Bootstrap (n=1,000)
	Estimate	**RSE%**	**Estimate**	**Median (95% non-parametric confidence interval)**
CL/F (L/h) ($\theta_{CL/F}$)	23.9	6.4	25.5	26.2 (24.0 to 28.5)
IIV$_{CL/F}$ (%)	48.6	8.5	29.5	28.9 (20.6 to 36.6)
IOV$_{CL/F}$ (%)			29.9	29.1 (24.4 to 33.9)
θ_{CYP3A5}	1.95	-	1.60	1.64 (1.41 to 1.92)
θ_{HAEM}			-1.01	-1.13 (-2.06 to -0.32)
θ_{POD}			-0.21%	-0.21% (-0.31 to -0.08%)
V1/F (L) ($\theta_{V1/F}$)	145	18.1	113.0	106.7 (70.6 to 158.2)
IIV$_{V1/F}$ (%)	113.6	10.6	46.8	51.0 (19.4 to 96.1)
IOV$_{V1/F}$ (%)			126.5	115.6 (91.7 to 148.4)
θ_{Pred}			-0.28%	-0.30% (-0.30 to -0.28%)
Q/F (L/h)	101	8.7	67.9	67.0 (48.8 to 86.3)
V2/F (L)	1500	12.3	1060	986 (648 to 1268)
IIV$_{V2/F}$ (%)	91.4	8.7	89.4	102.9 (79.3 to 137.6)
ka (h^{-1})	0.51	12.5	0.35	0.35 (0.25 to 0.46)
IIV$_{ka}$ (%)	54.9	19.3	47.6	44.9 (31.9 to 55.7)
Lag-time (h)	0.30	17.9	0.44	0.44 (0.37 to 0.47)
Correlation (%)				
V1/F, ka			67.7	
V1/F, V2/F			-4.9	
ka, V2/F			-1.3	
Proportional RUV (%)	29.5	3.9	18.3	19.0 (17.2 to 21.2)

Final model:

$$CL/F_i = \theta_{CL/F} \times \theta_{CYP3A5}^{X} \times (1 + \theta_{HAEM}(HAEM - 0.33)) \times \left(\frac{WT}{70}\right)^{0.75} \times (1 + \theta_{POD}(POD - 22.7))$$

$$V1/F_i = \theta_{V1/F} \times (1 + \theta_{PRED}(Pred_{Cmax,free} - 155.5))$$

Where x = 1 for CYP3A5 expressers (*1/*1 and *1/*3 genotype) and x = 0 for CYP3A5 non-expressers (*3/*3 genotype); CL/F = tacrolimus apparent clearance; CYP3A5 = cytochrome P450 3A5; HAEM = haematocrit (fraction); OFV = objective function value; IIV = inter-individual variability; IOV = inter-occasion variability; ka = absorption rate constant; POD = Post-operative day (capped at 180 days); $Pred_{Cmax,free}$ = C_{max} value of free prednisolone (nmol/L); Q/F = apparent inter-compartmental clearance; RSE = relative standard error; RUV = residual unexplained variability; V1/F = apparent central volume of distribution; V2/F = apparent peripheral volume of distribution; WT = total bodyweight (kg).

Table 3. Summary of significant covariate models

Model	Description	OFV	IIV CL/F	IIV V1/F
0	Model with no covariates	6674	54%	110%
1	Base model (with *CYP3A5* genotype on CL/F)	6643	49%	114%
2	Model 1 + Haematocrit on CL/F	6607	44%	114%
3	Model 2 + Body weight on CL/F	6575	38%	117%
4	Model 3 + Free prednisolone C_{max} on V1/F	6542	39%	112%
5	Model 4 + POD on CL/F	6489	38%	106%
6	Model 5 + IOV on CL/F and V1/F	6054	30%	47%

Models 1 to 4 are based on subsequent stepwise covariate modelling (SCM) steps. POD and IOV were added after the SCM (models 5 and 6). CL/F = apparent oral clearance; C_{max} = maximum concentration; CYP3A5 = cytochrome P450 3A5; IIV = inter-individual variability; IOV = inter-occasion variability; OFV = objective function value; POD = post-operative day; V1/F = apparent volume of distribution of the central compartment

Figure 1. Prediction corrected visual predictive check based on the final model in patients who are (a) CYP3A5 non-expressers and (b) CYP3A5 expressers
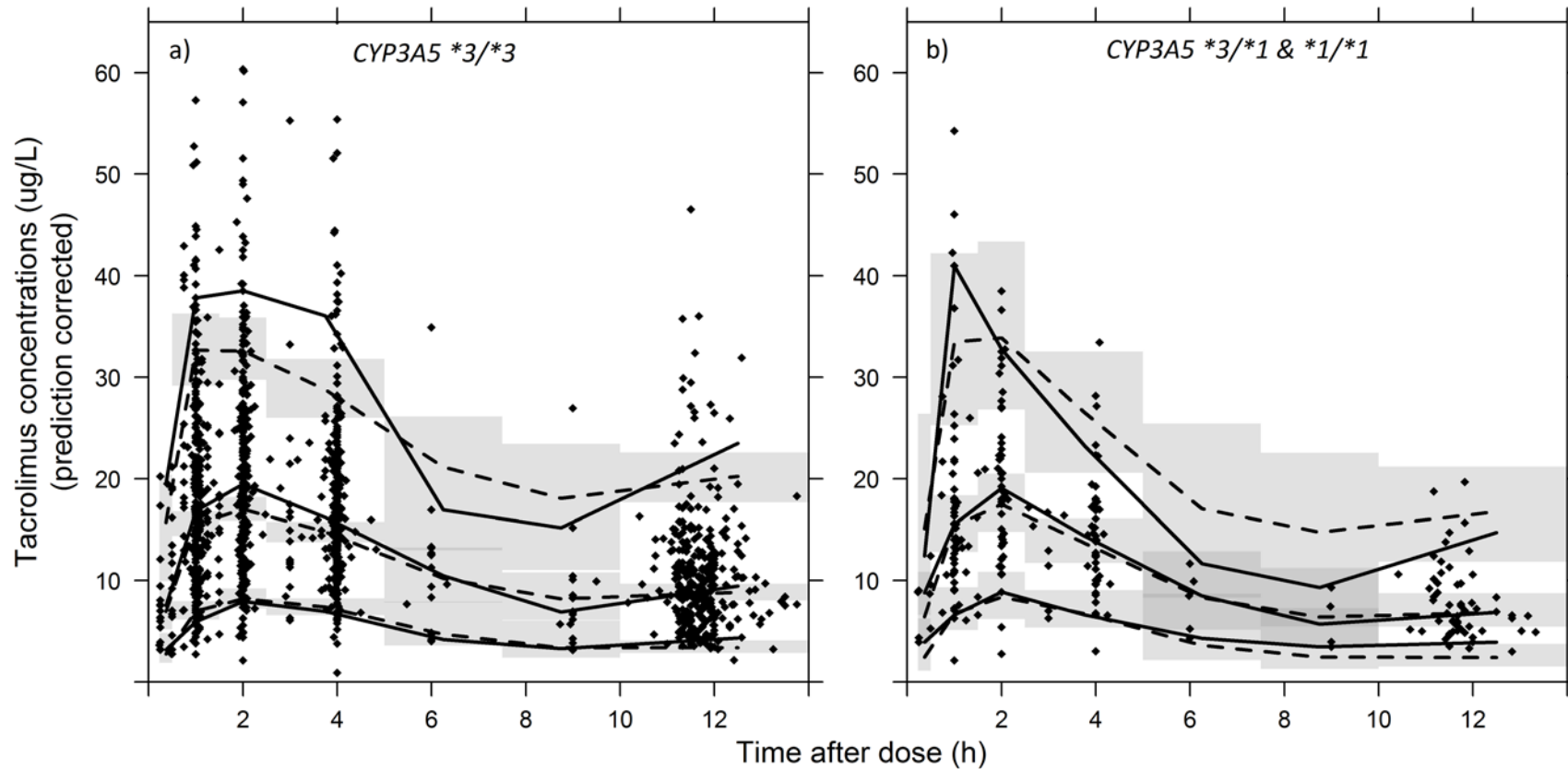
The upper, middle and lower continuous lines represent the 95th, 50th and 5th percentiles of the observations. The upper, middle and lower dashed lines represent the 95th, 50th and 5th percentiles of the simulated data from the final model. The grey shaded areas are the 95% confidence intervals for the percentiles of the simulated data for each bin.

Figure 2. Box and whisker plot of individual's tacrolimus CL/F (L/h) estimate stratified by *CYP3A5* genotype
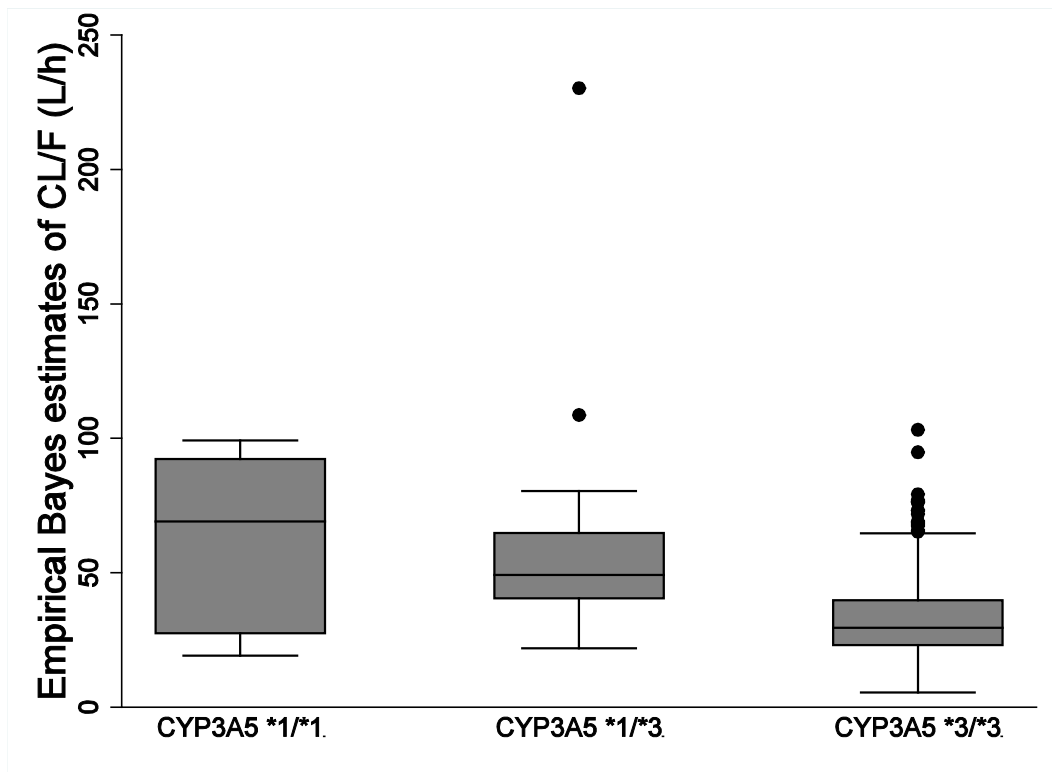
Boxes represent upper and lower quartile and median values, whiskers mark the extremes that are not outliers, black dots represent outlier values that are greater than 1.5 times the interquartile range

CL/F = apparent clearance; CYP3A5 = cytochrome 450 3A5;

Figure 3. Fraction of patients below, in and above the recommended tacrolimus trough concentration target range of 6-10 µg/L, based on 100 simulated datasets.
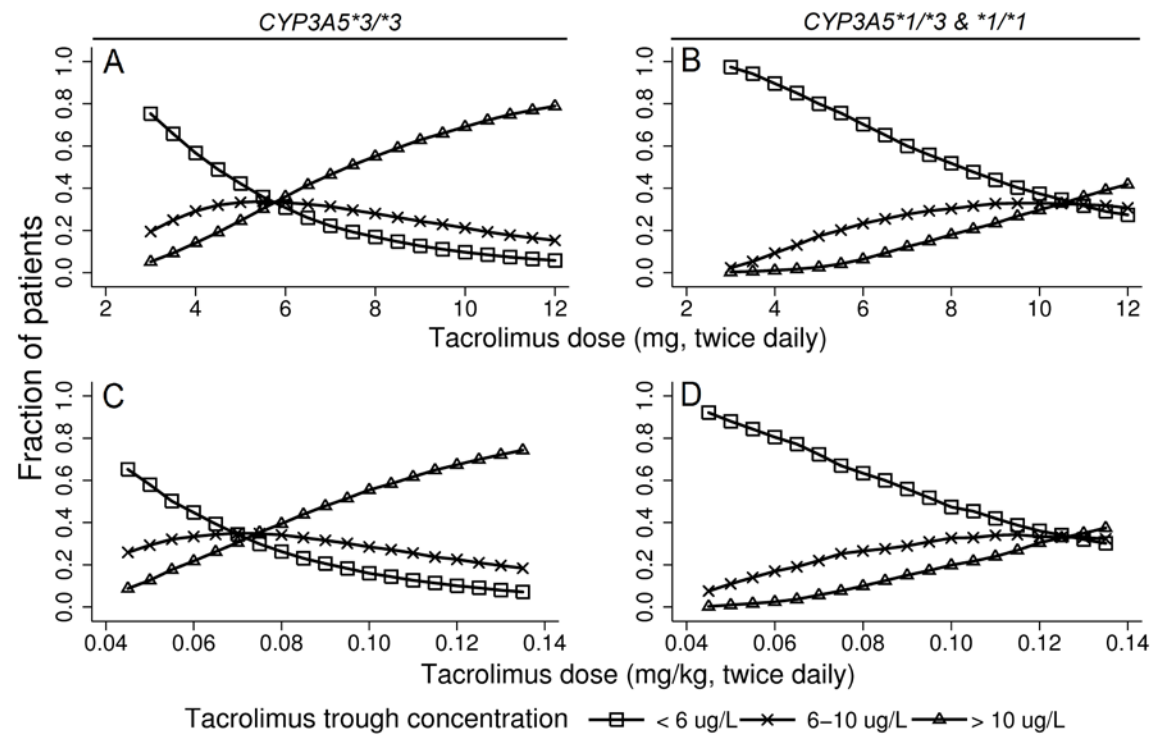
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