POPULATION PHARMACOKINETICS OF TACROLIMUS IN KIDNEY TRANSPLANT RECIPIENTS

A model for individual dosing

by

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ABSTRACT

Background: Population pharmacokinetics is the study of pharmacokinetic variability in the population. One goal of such studies is to improve individual drug treatment by identifying relationships between pharmacokinetic parameters and patient characteristics. Tacrolimus is an immunosuppressive drug used in kidney transplantation. Tacrolimus has a narrow therapeutic window and large pharmacokinetic variability both between and within patients. In addition, dose-normalized whole blood concentrations tend to increase during the first months after kidney transplantation. Therefore, individual dosing of tacrolimus is a major clinical challenge. The objective of this study was to develop a population pharmacokinetic model for tacrolimus to aid in prediction of initial dose and individual dose requirements during the first ten weeks after kidney transplantation.

Methods: Twenty-nine kidney transplant recipients contributed full pharmacokinetic profiles of tacrolimus at 44 dosing occasions, and 44 patients contributed trough concentrations from the first ten weeks after kidney transplantation. A total of 1546 blood samples were analyzed. Demographic, clinical and pharmacogenetic patient characteristics were evaluated as covariates. Population pharmacokinetic modeling was performed in NONMEM 7.2®.

Results: A two compartment model with first order absorption and lag time adequately described the data. Relative bioavailability was 24 % lower in females than in males and 49 % lower in CYP3A5 expressers than in CYP3A5 nonexpressers. Fat free mass was the most predictive body size metric. Whole blood concentrations of tacrolimus increased linearly with increasing hematocrit. An underlying increase in hematocrit with time after kidney transplantation largely explained the apparent time-varying pharmacokinetics of tacrolimus. In addition, relative bioavailability was 122 % higher than its lowest value immediately after transplantation, decreased to its lowest value during the first four days and subsequently increased by 31 % with an asymptote at day 60.

Conclusions: Initial dose of tacrolimus should be predicted from sex, *CYP3A5* genotype, fat free mass and hematocrit. Hematocrit is an important factor to predict changes in tacrolimus whole blood concentrations over time. The model may potentially aid in individual dosing of tacrolimus. Its predictive performance must be evaluated before application in clinical practice.

Key words: Tacrolimus, hematocrit, population pharmacokinetics, target concentration intervention, kidney transplantation

SAMMENDRAG

Bakgrunn: Populasjonsfarmakokinetikk er studien av farmakokinetisk variasjon i befolkningen. Et mål med slike studier er å forbedre individualisert legemiddelbehandling ved å kartlegge sammenhenger mellom pasientegenskaper og farmakokinetiske parametere. Takrolimus er et immundempende legemiddel som benyttes etter nyretransplantasjon. Takrolimus har et smalt terapeutisk vindu og stor farmakokinetisk variabilitet både mellom og innen pasienter. I tillegg stiger dosenormaliserte fullblodkonsentrasjoner de første månedene etter nyretransplantasjon. Individuell dosering av takrolimus er derfor en stor klinisk utfordring. Hensikten med denne studien var å utvikle en farmakokinetisk populasjonsmodell for takrolimus som kan benyttes til å bedre forutsi riktig oppstartsdose og individuelle doseringsbehov de første ti ukene etter nyretransplantasjon.

Metode: Tjueni nyretransplanterte pasienter bidro med totalt 44 fulle konsentrasjons-tidskurver, mens 44 pasienter bidro med bunnkonsentrasjoner (C_0) de første ti ukene etter nyretransplantasjon. Totalt 1546 fullblodkonsentrasjoner av takrolimus ble analysert. Demografiske, kliniske og farmakogenetiske pasientegenskaper ble undersøkt som potensielle kovariater. Farmakokinetisk populasjonsmodellering ble gjennomført i NONMEM 7.2®.

Resultater: En to-roms modell med forsinket første ordens absorpsjon beskrev de observerte konsentrasjonene. Relativ biotilgjengelighet var 24 % lavere hos kvinner enn hos menn og 49 % lavere hos pasienter som uttrykte funksjonelt CYP3A5-enzym enn hos pasienter uten funksjonelt CYP3A5-enzym. Farmakokinetikken var sterkere relatert til fettfri vekt enn til total kroppsvekt. Fullblodkonsentrasjoner av takrolimus steg lineært med stigende hematokrit. En underliggende stigning i hematokrit med tid etter nyretransplantasjon forklarte en stor andel av de tilsynelatende tidsrelaterte farmakokinetiske endringene for takrolimus. I tillegg var relativ biotilgjengelighet 122 % høyere enn den laveste observerte verdien umiddelbart etter transplantasjonen, sank til den laveste verdien i løpet av de neste fire dagene og økte deretter med 31 % mot en asymptote ved dag 60 etter transplantasjonen.

Konklusjon: Oppstartdose bør beregnes fra kjønn, *CYP3A5* genotype, fettfri vekt og hematokrit. Hematokrit er en viktig faktor for å forutsi fullblodkonsentrasjoner av takrolimus over tid. Modellen kan potensielt bidra til å forbedre individuell dosering av takrolimus. Den må imidlertid ytterligere valideres før den kan tas i bruk i klinisk praksis.

Nøkkelord: Takrolimus, hematokrit, populasjonsfarmakokinetikk, legemiddelmonitorering, nyretransplantasjon

FORORD

Dette prosjektet ble utført som avsluttende masteroppgave ved Institutt for Samfunnsmedisinske Fag, Universitetet i Bergen, januar-oktober 2012. Oppgaven utgjør siste del av tre års videreutdanning for reseptarer til graden master i farmasi. Veiledning ble gitt av professor Anders Åsberg ved Farmasøytisk Institutt, Universitetet i Oslo og dr. Reidun Kjome ved Institutt for Samfunnsmedisinske Fag, Universitetet i Bergen. Jeg fikk også gode innspill fra professor Nick Holford ved University of Auckland i forbindelse med modellutviklingen og utforming av artikkelen. Jeg ønsker å takke alle mine veiledere for fantastisk hjelp og støtte gjennom hele prosessen.

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Jeg ønsker også å takke

alle involverte ved Rikshospitalet som alltid tok meg godt i mot og bidro til datainnsamling av *CYP3A5* genotype.

storebrødrene mine, spesielt Kjetil som hjalp meg med bilderedigering.

alle modelleringsvenner i Uppsala og rundt omkring i verden som fikk meg til å forstå at man tross alt ikke bør modellere helt alene. Dere er vidunderlige og en stor inspirasjon.

Og Stein, som sørger for å minne meg på alle gledene som finnes, også utenfor modelleringsverdenen.

Elisabet, oktober 2012

PREFACE

The report from this project was written in the format of a research article with the intention of publishing in the scientific journal *Therapeutic Drug Monitoring.* The thesis is therefore constructed by four parts.

- Chapter 1 includes an introduction to population pharmacokinetic modeling. Figures, Tables and Equations in chapter 1 are named with the prefix 1.

- Chapter 2 includes characteristics of the drug of interest, tacrolimus. Figures are named with the prefix 2.

- The research article is placed between chapter 2 and 3 and has a separate list of references. Figures, Tables and Equations are not associated with a prefix and are shown subsequent to the article text. The research article may be read independently.

- Chapter 3 consists of an extended discussion on methodological considerations in light of the study results. Figures are named with the prefix 3. The literature list provided at the end of the document includes references from chapter 1, 2 and 3.

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LIST OF ABBREVIATIONS

ALAT	Alanine aminotransferase
ALB	Albumin
ALP	Alkaline phosphatase
ASAT	Aspartate aminotransferase
BILI	Bilirubin
BMI	Body mass index
BOV	Between occasion variability
BQL	Below quantification limit
BS	Bootstrap
BSA	Body surface area
BSV	Between subject variability
C_0	Trough concentration
CL	Clearance
CL/F	Apparent clearance
CL_{GRP}	Group value of clearance
CL_i	Individual value of clearance
CMIA	Chemoluminescent microparticle immunoassay
CRP	C-reactive protein
CV	Coefficient of variation
CYP3A4	Cytochrome P450 3A4
CYP3A5	Cytochrome P450 3A5
CWRES	Conditional weighted residual
DSA	Donor-specific antibody
E_H	Hepatic extraction ratio
F	Bioavailability
F_G	Intestinal availability
F_H	Hepatic availability
F_i	Individual bioavailability
FFM	Fat free mass
Hct	Hematocrit
k_a	Absorption rate constant
LC-MS/MS	Liquid chromatography tandem mass spectrometry
MEIA	Microparticle enzyme immunoassay
NONMEM	Nonlinear Mixed Effects Modeling
OFV	Objective function value
P-gp	P-glycoprotein
pcVPC	Prediction corrected visual predictive check
PD	Pharmacodynamics
PK	Pharmacokinetics
PRA	Panel reactive antibody
Q/F	Apparent intercompartmental clearance
SNP	Single nucleotide polymorphism
Tx	Transplantation
Txt	Time after transplantation
TBW	Total body weight
TCI	Target concentration intervention
TDM	Therapeutic drug monitoring
Tx	Transplantation
Txt	Time after transplantation
V1/F	Apparent central volume of distribution
V2/F	Apparent peripheral volume of distribution
VPC	Visual predictive check
WT	Weight

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1 POPULATION PHARMACOKINETIC MODELING

1.1 INDIVIDUALIZED DRUG THERAPY

It is generally recognized that patients respond differently to medical treatment and that ideally, treatment should be personalized [1]. One approach towards personalized treatment is to adjust dosage regimens of drugs based on the individual response [2]. When the therapeutic response of a drug is not easily measured and there is a known relationship between drug concentration in blood and drug effects, the measured drug concentrations may serve as surrogates for the individual response [3]. The drug concentration associated with the most efficient therapy while minimizing risk of toxicity is selected as the target concentration. If the acceptable target concentration range (therapeutic window) is narrow and pharmacokinetic variability is large, dosage regimens must be adapted for each individual to achieve and maintain the selected target concentration [3].

To characterize the time course of drug concentration and to select appropriate dosage regimens, information about the primary pharmacokinetic parameters (bioavailability, absorption rate, clearance and volume of distribution) are required. Determination of these parameters in a single individual is dependent on multiple sampling within the dose interval [4]. There are however financial and ethical obstacles to frequent blood sampling in the target population who may be ill, old, very young or perhaps have received an organ transplant [5, 6]. Average pharmacokinetic parameters are therefore traditionally derived from pharmacokinetic studies on a relatively small number of healthy volunteers [7]. However, dosage recommendations based on these results are not necessarily optimal for the target population who will be receiving the drug [4]. The primary pharmacokinetic parameters vary between patients because they depend on physiological properties. Examples of such physiological properties include organ function, enzymatic activity, blood flow to eliminating organs and binding properties in blood and tissues. Some of these are not easily measurable. They are however reflected by measurable factors, such as body weight or body composition, age, genotype and laboratory values [3]. By characterizing the quantitative relationship between these measurable patient factors and pharmacokinetic parameters, sub-populations with different dose requirements may be identified prior to treatment initialization. Furthermore, these relationships can be translated into more optimal initial dose recommendations for specific groups of patients [8]. In contrast to the traditional methods for

pharmacokinetic analysis, population pharmacokinetic analysis offers a powerful approach to investigate these relationships based on sparse data available from the patient group of interest [4].

Population pharmacokinetics is defined as "*the study of the sources and correlates of variability in the drug concentrations among individuals who are the target population"* [9]. A population pharmacokinetic model consists of a mathematical model that relates dose, drug concentration and patient characteristics. In addition, it consists of a statistical model that quantifies the unexplained pharmacokinetic variability and residual variability, assuming that these arise from specific distributions [7]. Information about this variability can be used to estimate more reliable pharmacokinetic parameters for each individual based on sparse drug concentrations available from the clinical setting and thereby further facilitate dose individualization. Population pharmacokinetic studies constitute an important part of the science of quantitative pharmacology (pharmacometrics) [10]. Although population pharmacokinetic studies have greatest application in drug development, they are also used to extend knowledge about pharmacokinetic mechanisms of drugs already on the market [4]. They provide a framework for improved drug therapy both on the group level and on the individual level. Hence, such analyses are important towards the overall goal of personalized medical treatment.

1.2 NONLINEAR MIXED EFFECTS MODELING

Several methods exist for performing population pharmacokinetic analyses. The "Naïve Pooled Data Method" treats data as if all concentrations were derived from the same individual and therefore cannot be used to estimate pharmacokinetic variability between patients. The "Standard Two-Stage Method" requires richly sampled data from each individual to estimate individual parameters in the initial stage and parameter variability in the second stage [6]. In contrast, the Nonlinear Mixed Effects Modeling approach allows estimation of all parameters simultaneously and is typically the method of choice due to several advantages. First, it distinguishes between true biological variability in the pharmacokinetic parameters and random residual variability. Second, it does not require intensive sampling within the dosage interval. Third, it handles situations when individuals contribute unbalanced amounts of data and allows integration of data arising from several sub-studies, including data from the routine clinical setting [5]. Thereby, it naturally reflects the patient group of interest [4].

Nonlinear mixed effects modeling was introduced to the pharmacokinetic field in the end of the 1970s to analyze sparse, heterogeneous data arising from therapeutic drug monitoring of digoxin [5]. *Nonlinear* refers to that the mathematical function describing the system is nonlinear in its parameters [7, 11]. Notably, these nonlinearities are not related to whether the pharmacokinetic model is linear or nonlinear. *Mixed effects* refer to *effects* contributing to the overall variability. These effects are of two different types: *Fixed* effects are predictable effects on a variable (e. g. typical clearance of 20 L/h in the population), whereas *random* effects describe unpredictable variability (e. g. 50 % variability between subjects around the typical value 20 L/h in the population). In population pharmacokinetics, fixed and random effects parameters are referred to as thetas (θ's) and etas (η_i's), respectively [12]. A population pharmacokinetic model normally consists of three components:

i) a fixed effects structural model to describe the typical pharmacokinetic parameters of the drug in the population
ii) a random effects statistical model to describe variability in the parameters
iii) a fixed effects covariate model to describe predictable effects of patient characteristics on the pharmacokinetic parameters

The model components may be viewed as separate models, but they also interact with each other to constitute the full population model. This is illustrated in Figure 1-1 together with questions that are typically asked during development of each component.

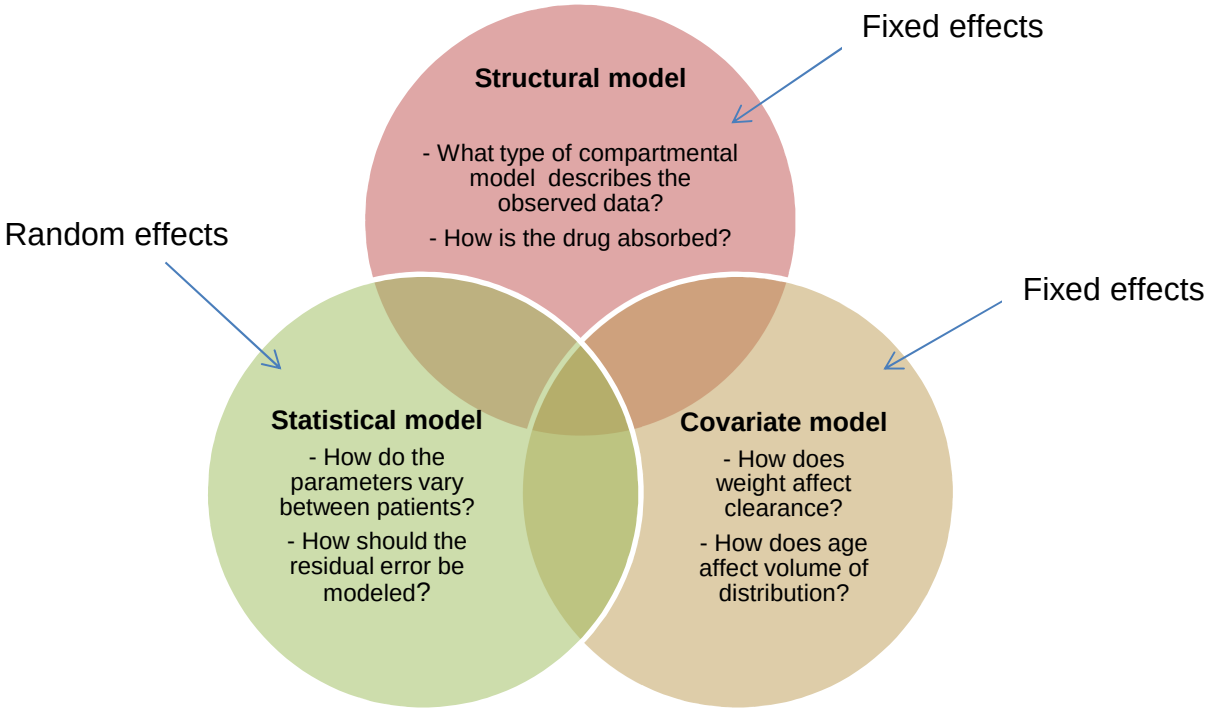

Figure 1-1. Components of a mixed effects population pharmacokinetic model and examples of questions that may be asked during development of each particular model component. The structural model and covariate model are built by fixed effects, and the statistical model is built by random effects. They can be considered as separate model components, but also interact with each other to constitute the full population model.

The structural model

The analysis typically begins with describing the data using a pharmacokinetic compartmental model. The most common parameterization is by primary pharmacokinetic parameters (clearances and volumes of distribution) rather than by rate constants or coefficients and exponents [13]. The adequate number of compartments (e. g. one-, two- or three-compartment model) and the absorption profile for orally administered drugs (e. g. zero order or first order absorption with or without a lag time) are determined [7].

The statistical model

The statistical model consists of sub-models describing between subject variability (BSV), between occasion variability (BOV) and residual error.

Between subject variability (BSV)

All individuals have unique pharmacokinetic parameters due to random biologic variability. Individual clearance is described by the deviance from the typical value (Eq. 1-1):

$$CL_i = \theta_{CL} + \eta_i \,, \qquad \text{Eq. 1-1}$$

where CL_i is the individual value of clearance, θ_{CL} is the parameter estimate of the typical value of clearance in the population and η_i is the individual deviation from the typical value [13]. Although η_i is unknown, its variance (ω^2) is estimated by assuming that individual deviations from the typical value are normally distributed around a mean of zero in the population [12]. However, biological parameters are generally not normally distributed, because negative values are not possible in biology. In addition, the distribution tends to be right skewed. The natural logarithm (ln) of biological parameters is however often approximately normally distributed [14]. Therefore, BSV in population pharmacokinetic models is described exponentially (Eq. 1-2):

$$CL_i = \theta_{CL} \times e^{\eta i}. \qquad \text{Eq. 1-2}$$

For interpretation of the variability, the variance of η_i's is transformed to the apparent coefficient of variation: $\sqrt{\omega^2} \times 100\ \%$, to generate an expression of the variability using the same scale as the parameter (e. g. L/h for clearance) [7].

Between occasion variability (BOV)

Pharmacokinetic parameters are not constant over time within a patient. When patients are sampled at more than one occasion (i. e. more than one hospital visit), BOV must be taken into account [15]. BOV is estimated by using the following expanded model for individual clearance (Eq. 1-3):

$$CL_{ik} = \theta_{CL} \times e^{\eta i + \eta k} \,, \qquad \text{Eq. 1-3}$$

where $e^{\eta k}$ is the deviance between the individual parameter CL_i and the occasion-specific individual parameter CL_{ik} at the k^{th} occasion. In this equation, η_k's are assumed to be normally distributed with mean zero and variance π^2.

Residual variability

The residual variability (ε) is the discrepancy between the predicted concentration and the measured concentration after taking BSV and BOV into account. The size of the residual error is dependent on [15]:

- model misspecifications
- analytical assay errors
- inaccuracy in dosing time
- inaccuracy in sampling time
- within subject variability

The most commonly used models for the residual error are additive (Eq. 1-4) and proportional (Eq. 1-5) [12]:

$$Y_{ij} = Conc_{ij} + \varepsilon, \quad \text{Eq. 1-4}$$

$$Y_{ij} = Conc_{ij} + (\varepsilon \times Conc_{ij}), \quad \text{Eq. 1-5}$$

where Y_{ij} and $Conc_{ij}$ are is the model predicted concentration with and without residual error, respectively, for the i^{th} individual at the j^{th} measurement. The error term ε is assumed to be a normally distributed random term with mean zero and variance σ^2. Whereas additive error components are estimated in the same unity as the concentration (e. g. µg/L) and assume a constant error over all concentrations, proportional error components are estimated in percentages and allows the size of the residual error to increase in proportion to increasing concentration [13]. Oftentimes, these models are combined (Eq. 1-6):

$$Y_{ij} = Conc_{ij} + (\varepsilon_1 \times Conc_{ij} + \varepsilon_2), \quad \text{Eq. 1-6}$$

The combined error model is commonly used because it describes a constant error model at low concentrations and a predominant proportional error model at high concentrations [10].

If the analyzed data arise from more than one study, the residual error may be different across these studies. This is expected if the sub-studies applied different analytical assays to determine the concentrations or if they were performed under different circumstances, for

example with and without compliance control [7]. Such differences are accounted for by estimating a study-dependent fixed effect (θ_{studyi}) (Eq. 1-7):

$$Y_{ij} = Conc_{ij} + \theta_{study_i} \times (\varepsilon_1 \times Conc_{ij} + \varepsilon_2), \quad \text{Eq. 1-7}$$

Finally, when heterogeneous data are used or if non-compliance is expected in some individuals, individual contribution to the residual error ($e^{\eta err_i}$) can be estimated to prevent patients with larger residual errors to have profound impact on the overall residual error (Eq. 1-8) [16]:

$$Y_{ij} = Conc_{ij} + \theta_{study_i} \times (\varepsilon_1 \times Conc_{ij} + \varepsilon_2) \times e^{\eta err_i}, \quad \text{Eq. 1-8}$$

where ηerr_i's describe BSV in the residual error and are assumed to be normally distributed with mean zero and variance ω_{err}^2. Figure 1-2 illustrates a typical concentration-time profile and its associated variabilites.

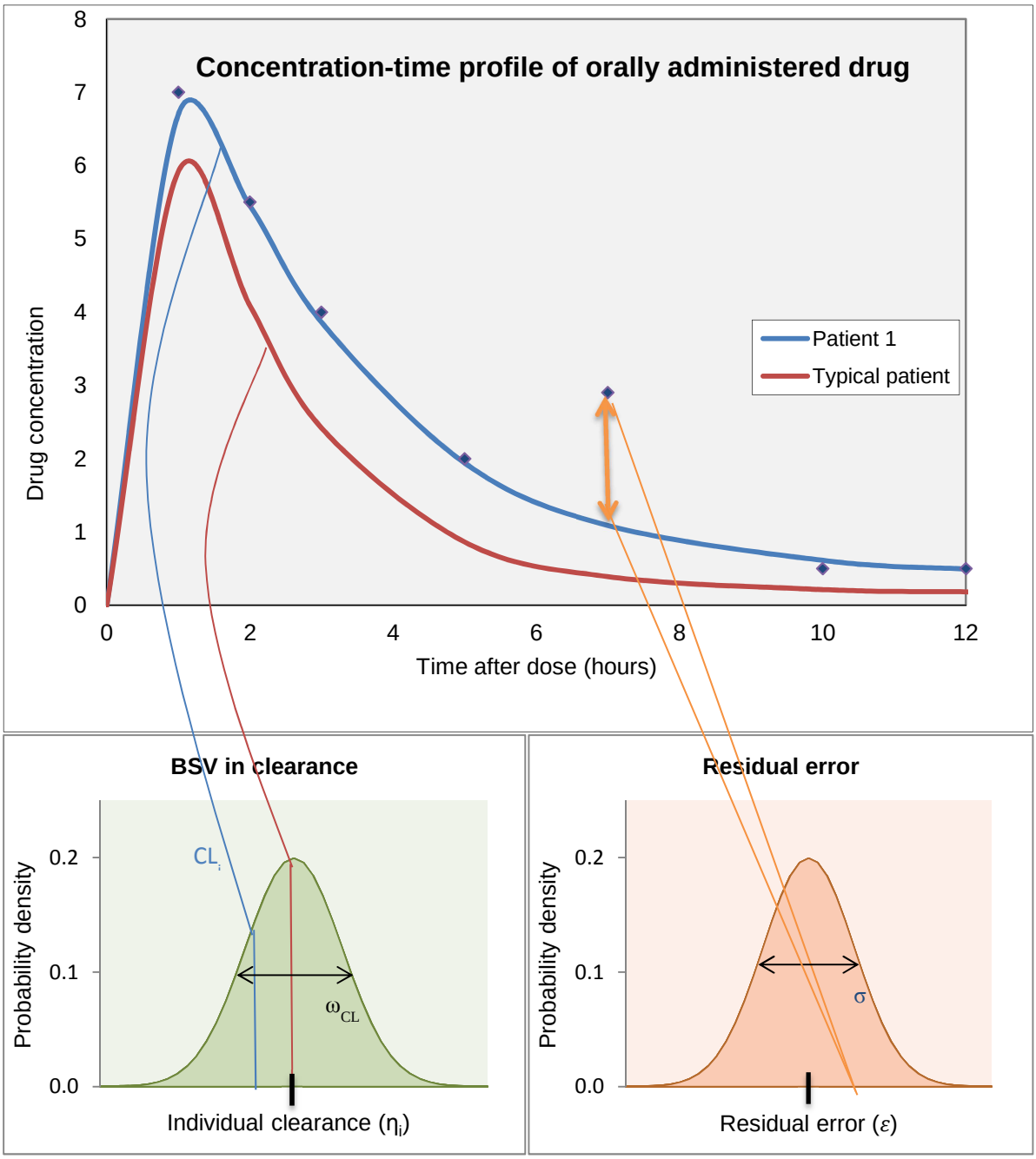

Figure 1-2. Example of concentration-time profile and its associated variabilities. *Top panel*: Drug concentration-time profile using the population estimates of pharmacokinetic parameters (red line) and an example of the observed drug concentrations in an individual (blue diamonds). The blue line is fit to the observed concentrations and represents the individual concentration-time profile. In this example, the patient differs from the typical patient because it has a different value of clearance. This difference is modeled exponentially as in Eq. 1-2: $CL_i = \theta_{CL} * e^{ni}$. *Bottom left panel* shows the estimated distribution of individual clearances, where η_i's are assumed to be randomly distributed with a mean value of 0. The standard deviation ω_{CL} reflects the estimated variability in clearance ($\eta=N(0,\omega^2)$). A concentration measurement differs from the individual predicted concentration by the residual error $\varepsilon_{ij,}$ due to, for example, an error in the analytical assay. *Bottom right panel* shows the estimated residual error distribution with mean zero and standard deviation σ ($\varepsilon=N(0,\sigma^2)$). BSV, between subject variability. The values used in the plot are only for demonstration and do not correspond to actual values.

The covariate model

Covariates represent measurable patient characteristics. Examples include

- demographic variables, such as total body weight, fat free mass, age and sex
- genetic variables, such as polymorphisms in genes coding for metabolizing enzymes
- laboratory values, such as liver function test values, serum creatinine and hematocrit, which typically reflect elimination organ function or drug binding components in blood
- disease status, such as gastrointestinal disease affecting absorption
- environmental factors, such as smoking habits or concomitantly administered drugs that interact pharmacokinetically with the drug of interest

Other factors that are plausible contributors to pharmacokinetic variability may also be investigated as covariates [7]. A covariate is introduced to the model in the form of a mathematical function. For example, a fraction of the total variability in drug clearance in the population may be explained by body size. Thus, a measure reflecting body size, such as total body weight, is modeled by using a biologically plausible mathematical function of the relationship between body weight and clearance [13]. The fit of the population model may improve, and the total population variability in clearance can be divided into the variability explained by total body weight and the remaining unexplained, random variability [12].

Covariate search strategies

Traditionally, covariate search has been based on graphical evaluation. Scatter plots of individual pharmacokinetic parameter estimates as a function of the covariate in question are generated for this purpose [17, 18], exemplified in Figure 1-3 by plotting clearance as a function of body weight.

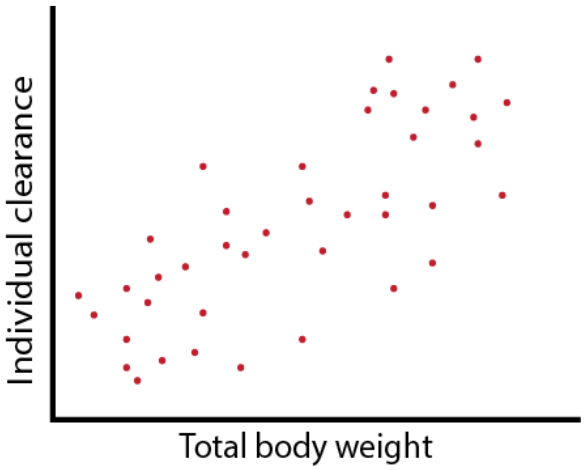

Figure 1-3. Example of individual estimates of clearance as a function of body weight. Each individual is represented by one red dot. The analyst observes a trend of increasing clearance with weight, and implements the relationship in the model by a corresponding mathematical equation to evaluate whether variability in clearance is explained by body weight.

If a covariate has a predictable effect on a parameter, it should appear as a trend in the scatterplot. There are, however, two main disadvantages to this method. First, it is dependent on that the individual parameter estimates are accurate [19]. The quality of the individual parameter estimates depends on the study design. Second, in longitudinal studies, covariates may change over time within individuals. When the first recorded covariate is used to generate the scatter plot, the plot is not representative for the entire period of the study. Due to these disadvantages, implementation of covariates directly in the modeling software (*direct covariate testing)* is often preferred as an alternative method [20]. This method does not suffer from shrinkage in the individual parameter estimates and allows for time-varying covariates because the modeling software updates the covariate effect for every new record in the dataset [12]. It is however more time consuming, because a greater number of models are typically tested [7].

Coding of covariates

Continuous covariates are most often introduced to the model in the form of additive or power functions [13], exemplified by weight as covariate on clearance (Figure 1-4). In the additive model, θ_{CL} represents base clearance and θ_{WT} represents the change in clearance introduced by a change in weight. A nonlinear relationship allows this change to be more pronounced at certain values of the covariate. For example, clearance may change more dramatically per kg at lower weight compared with at higher weight, because gained fat does not contribute

directly to increased clearance [21]. The covariate parameter is typically centered to a normal value (e.g. 70 kg) for easier interpretation of the estimate of $\theta_{CL,}$ which then will apply to a patient of 70 kg instead of a patient with a weight of zero. A variety of functional forms may be evaluated, such as exponential or sigmoidal models (not shown) [22]. If several functions for the same covariate improve the model, the model with the greatest improvement in goodness of fit is selected [7] (see later section on model evaluation).

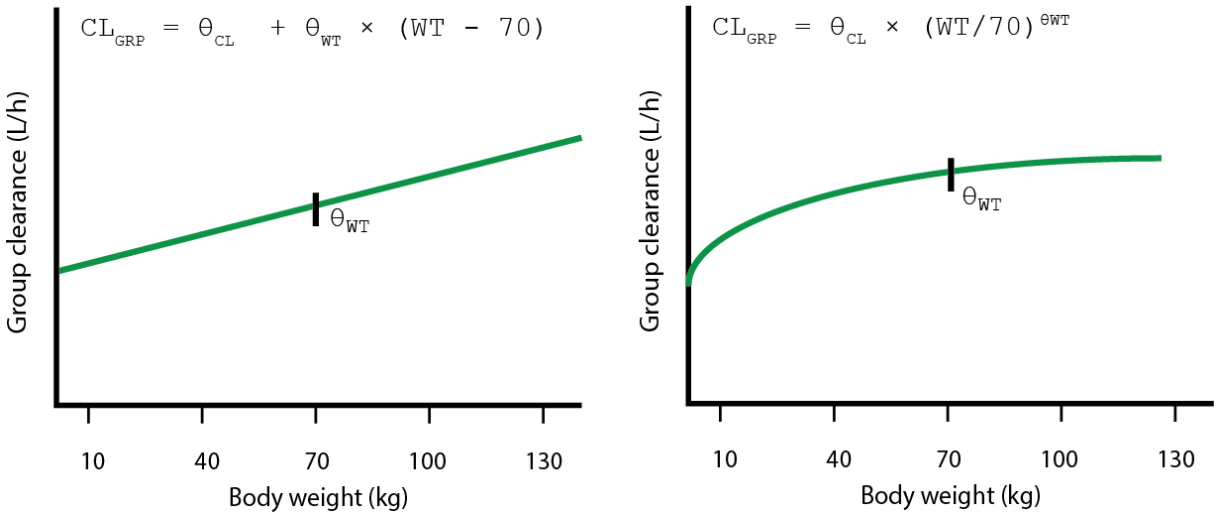

Figure 1-4. Mathematical functions for modeling of covariates. *Left panel*: Additive model for the effect of weight (WT) on clearance. Clearance increases linearly with weight. θ_{CL} represents group value of clearance (CL_{GRP}) when weight is 70 kg, and θ_{WT} is the estimated change in CL_{GRP} per unit change in weight. *Right panel*: Power model for the effect of weight (WT) on clearance. Clearance changes more per kg (θ_{WT} is between 0 and 1 for this shape). θ_{CL} represents typical value of clearance (CL_{GRP}) when weight is 70, and θ_{WT} is the estimated change in log (CL_{GRP}) per unit change in log weight.

For dichotomous covariates, the fractional change in a parameter in one group compared to the reference group can be estimated. In the modeling software NONMEM (see later section), this is achieved by if-else statements, exemplified in Box 1-1 by coding the effect of sex on clearance [12]:

BOX 1-1. Coding of dichotomous covariates in the modeling software NONMEM

Interpretation	Code
If the patient is male (sex equals 0), then the group value of clearance (CLGRP) is θ_{CL}.	`IF (SEX.EQ.0) THEN` `GRPCL = θCL`
Else,	`ELSE`
if the patient is female (sex does not equal 0), then the group value of clearance is the group value in males with a fractional change of θ_{female}.	`GRPCL = θCL × θfemale` `ENDIF`

Because covariates are fixed effects, they only partly explain the overall variability. Although body weight may be recognized as a covariate, the same value of clearance will not apply to all individuals with similar weight. Nevertheless, covariates provide the best guess for the initial dose. To account for the remaining unexplained variability, individual pharmacokinetic parameters must be estimated, which requires at least one measured drug concentration [23]. Drug concentrations arising in the clinical setting are often sparsely sampled and therefore insufficient to calculate individual parameters when viewed independently. However, they can be used in conjunction with the typical pharmacokinetic parameters and their population distributions to estimate individual parameters with more reliability [24]. This method allows dose individualization beyond the information obtained by covariates.

1.3 BAYESIAN ESTIMATION OF INDIVIDUAL PHARMACOKINETIC PARAMETERS

The method used to estimate individual pharmacokinetic parameters is called *Bayesian* estimation because it is based on *prior* information provided by a population model [24]. The algorithm for estimation of Bayesian individual parameters balances information from the population model (prior knowledge) with information obtained from the individual concentration measurements to obtain the most likely individual parameters (Eq. 1-9 and Figure 1-5).

$$OBJ_{BAYES} = \sum_{i=1}^{p} \frac{(P_{i-} \hat{P}_i)^2}{\omega_i^2} + \sum_{j=1}^{n} \frac{\left(C_{j-} \hat{C}_j\right)^2}{\sigma_j^2} \quad , \qquad \text{Eq. 1-9}$$

where OBJ_{BAYES} is the a Bayesian objective function, P_i is the estimated individual parameter, $\hat{P}_i$ is the population parameter, ω^2 is the variance of the parameter in the population, C_j is the observed concentration, $\hat{C}_i$ is the predicted concentration from the individual parameters and σ^2 is the variance of the residual error in concentration measurements [24]. These estimates are also called Empirical Bayes Estimates (EBEs), *Maximum a Posteriori* Bayesian estimates or *posthoc* estimates and are calculated by a modeling software such as NONMEM (see later section).

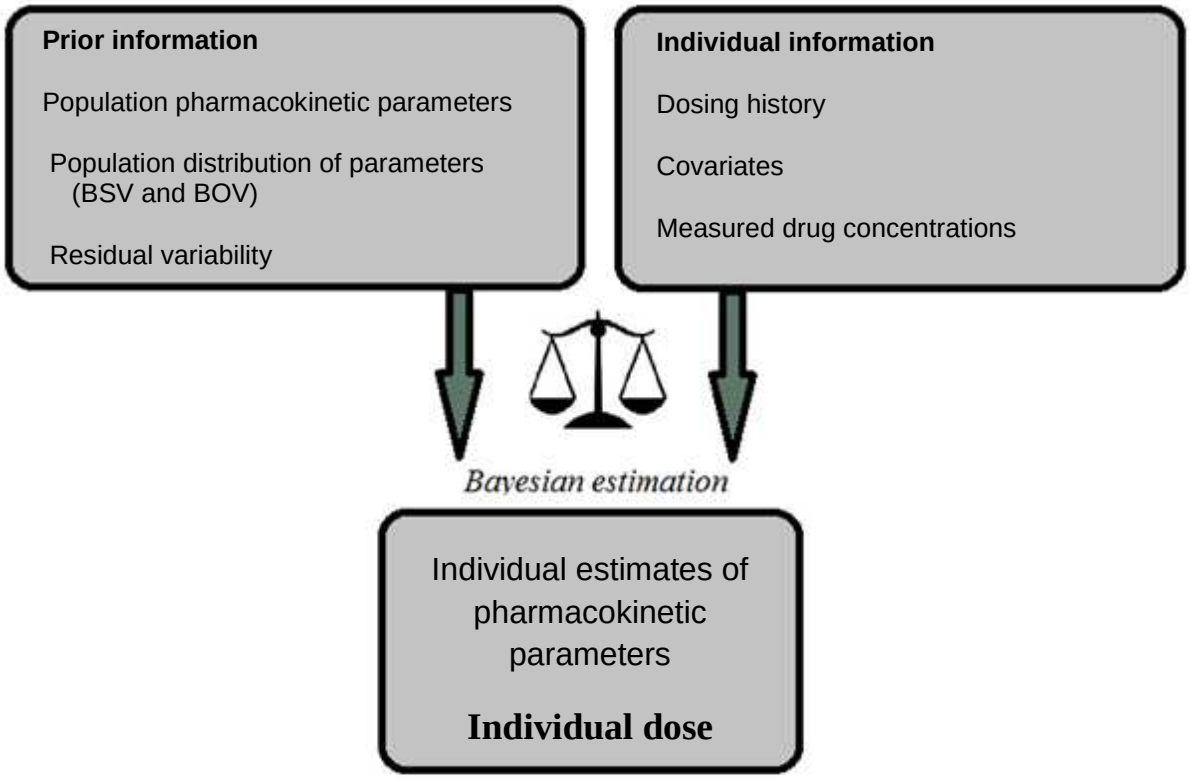

Figure 1-5. Illustration of Bayesian estimation of individual pharmacokinetic parameters. The final estimates are obtaind by balancing prior information and individual information.

If no concentration measurements are available, only prior information from the population model is used, and the individual parameters will be equal to the population parameters because these parameters are the most likely values. The more individual concentration measurements that become available, the less will the individual estimates rely on the population parameters [7]. Although Bayesian individual parameter estimates are generally more reliable than parameters derived solely from the individual observations, they will not be realistic if the sampling design only offers sparse or uninformative drug

concentrations. This phenomenon is illustrated in Figure 1-6 and is referred to as shrinkage [19]. The consequences of individual dosing based on shrunk individual parameters are discussed in the extended discussion section (chapter 3).

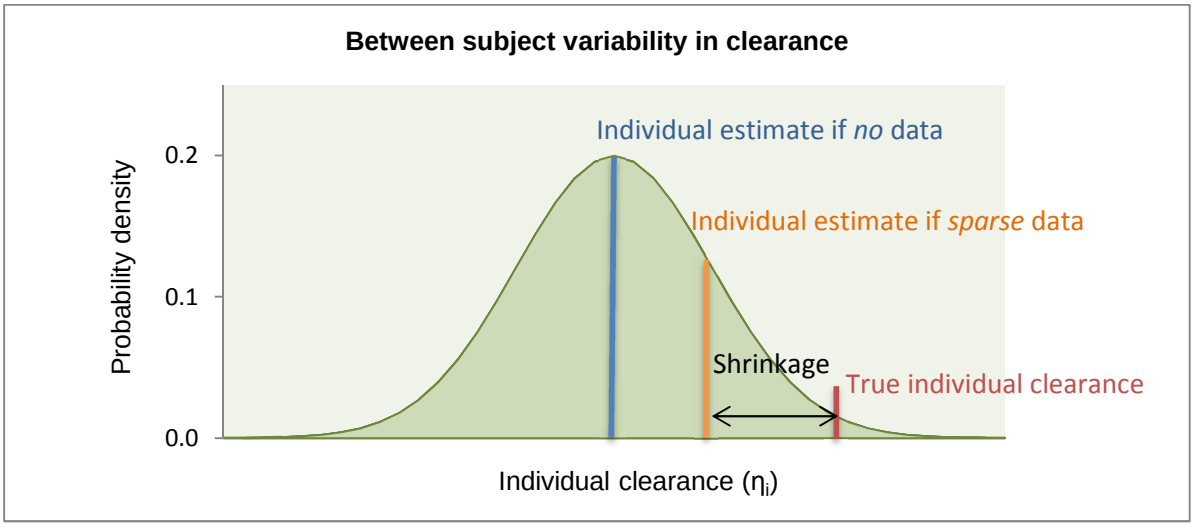

Figure 1-6. Illustration of shrinkage in individual parameters. The individual estimate of clearance (orange) is shrunk towards the population mean (blue) when individual parameter estimates are based on sparse or uninformative data. The shrinkage is the difference between the true value of clearance (red) and the individual estimate. *Figure was created by inspiration from presentation at PAGE-meeting 2007: Savic & Karlsson, Shrinkage in Empirical Bayes Estimates for Diagnostics and Estimation.*

1.4 A MODELING SOFTWARE: NONMEM

Introduction to NONMEM

NONMEM is a software package that performs parametric nonlinear mixed effects modeling [12]. It is the most commonly used software in population pharmacokinetic analyses [6, 7]. To perform an analysis in NONMEM, a dataset and a user-defined control stream are required. In the dataset, each individual is listed with administered doses, dosing times, observed drug concentrations and available covariates (an example is provided in appendix H). In the control stream, the user defines the structural, statistical and covariate model, selects initial parameter estimates, estimation method and other user-defined options (described in more detail in appendix G). The results are output in several text files. Among these results are the pharmacokinetic parameter estimates and variances that were requested in the control stream [13].

The objective function and the likelihood ratio test

Typical parameter values and several levels of random variability are estimated simultaneously by maximum likelihood estimation [5]. NONMEM employs the user-defined initial parameter estimates as starting points and iteratively suggests combinations of alternative parameter values. In each step, the likelihood of observing the drug concentrations in the dataset given the parameters under evaluation is calculated. The procedure is complete when the parameters are found that maximizes the likelihood of observing the data [12]. This may however occur at both local and preferably the global maximum of the function [7].

The modeler typically defines a variety of different models and wishes to compare them. For example, a base model is compared with an alternative model that includes an effect of weight on clearance. This comparison is based on calculating the ratio between the likelihoods obtained by fitting each model separately. This is called the Likelihood Ratio Test [12] (Eq. 1-10):

$$Likelihood\ ratio = \frac{L_{alt}}{L_{base}} \qquad \text{Eq. 1-10}$$

where L_{alt} is the likelihood of observing the data using the alternative model (e. g. the model with an effect of weight on clearance) and L_{base} is the likelihood using the base model. However, NONMEM does not output the likelihood directly. It rather calculates an objective function value (OFV) proportional to *"– 2 x log likelihood"*. The OFV therefore represents the likelihood indirectly. Importantly, the absolute OFV has no meaningful interpretation. The OFV is only meaningful when models are compared. Eq. 1-11 is an extension of Eq. 1-10 by including *"– 2 × log"*. It shows how the expression becomes equal to the difference in OFV:

$$\begin{aligned} -2\log\frac{L_{alt}}{L_{base}} = & -2\log L_{alt} - (-2\log L_{base}) = \\ & -2\log L_{alt} + 2\log L_{base} = OFV_{alt} - OFV_{base} = \Delta\text{OFV} \end{aligned} \qquad \text{Eq. 1-11}$$

The transformation is practical: The difference in OFV (ΔOFV) is approximately χ^2-distributed, and the contribution of an added parameter can be evaluated at a selected significance level according to the χ^2-distribution (Figure 1-7) [10].

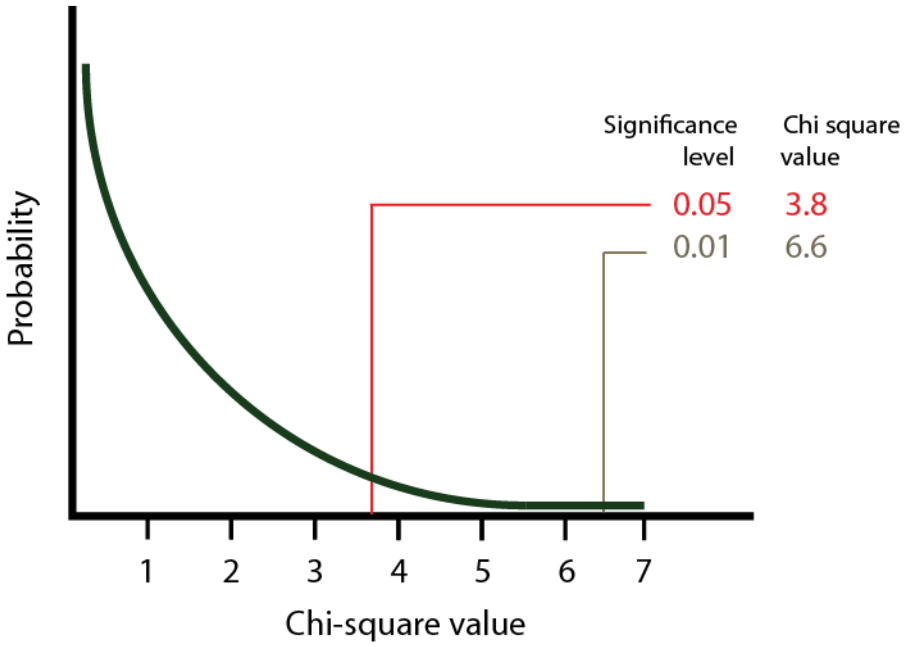

Figure 1-7. χ^2-distribution with one degree of freedom and associated selected significance levels.

A reduction in OFV of > 3.8 and > 6.6 are associated with significance levels of $p<0.05$ and $p<0.01$, respectively, with one degree of freedom (one added parameter) [8]. The likelihood ratio test is restricted to comparison between *nested* models. Two models are nested if the alternative model is identical to the base model when the added parameter (θ_{WT}) is fixed to 0, as exemplified in Box 1-2 for the effect of weight on the group value of clearance (CL_{GRP}) (shown without random effects and centered to a normal weight):

Box 1-2. Example of nested models

Base model	$CL_{GRP} = \theta_{CL}$
Alternative model	$CL_{GRP} = \theta_{CL} + \theta_{WT} \times (\text{Weight} - 70)$

In this example, if the OFV is decreased by at least 3.8 points for the alternative model, it is concluded that the effect of weight on clearance is significant ($p<0.05$). Importantly, the OFV is comparable only for models describing identical datasets using the same estimation method [12]. Covariates are normally included stepwise by initially selecting the model with the greatest decrease in OFV and subsequently reevaluating models by adding new covariates. This is done repeatedly until no covariates improves the model statistically significant based on the likelihood ratio test [20]. Subsequent to selecting a full covariate model, the significance of each covariate is evaluated by removing them sequentially (backward elimination) [20].

1.5 MODEL EVALUATION

The above-described likelihood ratio test gives the statistical significance of a model component and provides a general model-building criterion. However, decisions in population model development are also based on subjective evaluation of biological plausibility of parameters, precision of the parameter estimates and graphical evaluation of the model's goodness of fit [10].

Biological plausibility

Relationships are biologically plausible if they are explained by known biological principles. A typical biologically plausible relationship is increasing clearance and volume of distribution with increasing body weight [21]. Such relationships are sometimes recommended to include in models even when not statistical significant because they are expected to apply to the general population. If a covariate relationship is identified that is inconsistent with biological principles it may be rejected even if it is statistically significant [7].

Precision of the parameter estimates

All parameter estimates will have a degree of uncertainty [8]. Symmetric confidence intervals are estimated by NONMEM, but these are only approximate. To generate asymmetric standard errors and confidence intervals in population pharmacokinetic modeling, the nonparametric bootstrap procedure is normally employed [25]. During a bootstrap procedure, the dataset is resampled with replacement to create new datasets with the same size but with randomly selected combinations of individuals [26]. This procedure is repeated 500-1000 times, and the parameter estimates resulting from each dataset are ranked in increasing order. The 2.5th to 97.5th percentile for each parameter estimate is interpreted as the 95 % confidence interval [25] (example of bootstrap results are shown in appendix B). If the 95 % confidence interval of a covariate parameter includes the null value (e. g. if the confidence interval of the covariate coefficient of sex on clearance is 0.8-1.3) the covariate is normally not included because the effect is uncertain [7].

Graphical evaluation of goodness of fit

Graphical evaluation is essential at all model building stages [7]. Traditionally, the most commonly considered graphics are plots of population predicted or individually predicted concentrations versus observed concentration (Figure 1-8 and appendix C). Ideally, all concentrations are randomly scattered around the line of unity with no sign of bias [7]. A large spread around the line of unity in the plot of population predicted concentrations is expected due to unexplained BSV [27]. In contrast, the plot of individually predicted concentrations versus observed concentrations is usually considerably more precise because it is predicting the concentrations from the individual Bayesian parameter estimates.

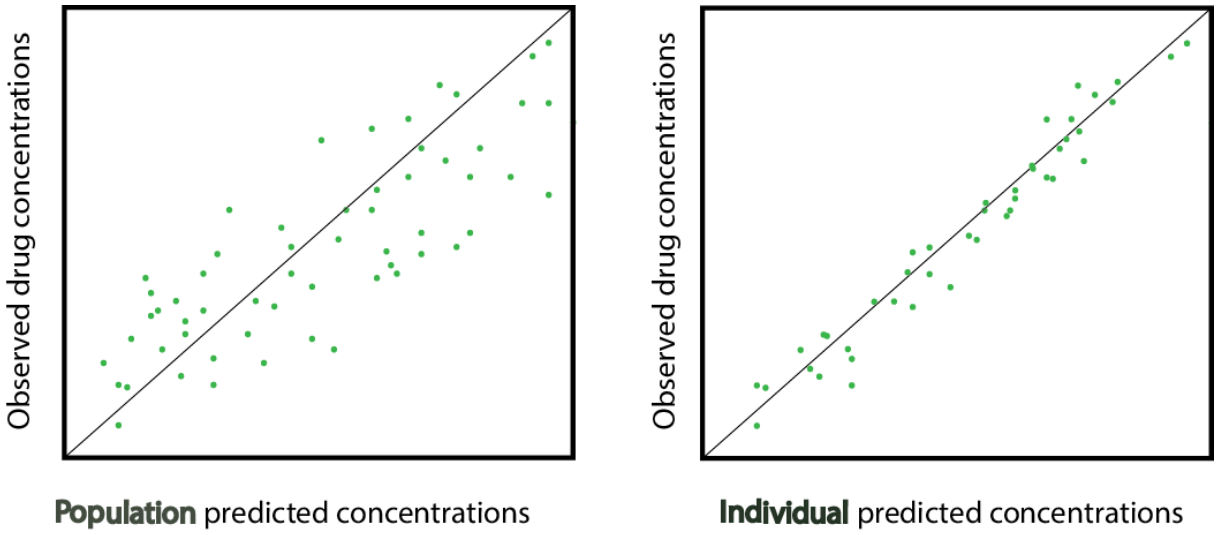

Figure 1-8. Example of traditional goodness of fit plots. Observed concentrations are plot versus predicted concentrations. *Left panel:* Population parameter estimates are used to predict the concentrations. *Right panel*: Individual parameter estimates are used to predict the concentrations.

The usefulness of these plots is limited by the fact that they do not include the aspect of time and give little information about how to improve the model if bias occurs [27]. Other traditional diagnostic plots include residual error plots, plots of the distribution of the estimated individual parameters and individual plots of goodness of fit. Examples of these plots are provided in chapter 3 (Figures 3-1, 3-2, 3-3, 3-5). However, the recently introduced visual predictive checks (VPCs) are now widely accepted as the most informative plots [28]. To create VPCs, at least 100 datasets with the same structure as the original dataset are

simulated based on the selected model. The analyst visually checks whether 90 % of the original observations fall within the 90 % prediction interval of the simulated concentrations. Observation intervals and prediction intervals that overlap indicates that the model describes the data well [28]. VPCs may be stratified by categorical covariates or created with continuous covariates on the x-axis to investigate whether the model predicts well over the range of the covariate (Figures 3 and 5 in the Research Article). In contrast to some of the traditional plots, VPCs provides information about how to improve the model if misspecifications occur.

When the data arise from a clinical setting of therapeutic drug monitoring, it is important to take into account that doses are adapted according to patient characteristics. This means that there will be an inherent relationship between clearance and dose. This relationship is not maintained during simulation. Therefore, *prediction corrected* visual predictive checks (pcVPCs) must be generated in such situations [28].

1.6 ACCEPTING A FINAL MODEL

Before accepting a final model, the statistical assumptions should be tested (see discussion on methodological considerations) [29]. Furthermore, *the principle of parsimony* also applies to population modeling, which means that the final model should be the simplest model that is adequate for the predefined purpose [7]. A model may be simplified by removing unnecessary random error terms or by combining fixed effects if they have overlapping confidence intervals [10]. Finally, the clinical relevance of the covariates and whether the covariates are easily measured in clinical practice should be considered [25].

By combining all the above-described criterions during model development, the modeling process becomes an overall subjective process. The correct final model does not exist because all models are approximations and simplifications of reality [7]. The question is not whether the model is right or wrong, but whether it is useful for the purpose it was developed [8]. When the model is developed for predictive purposes, it is important to evaluate whether the model is also able to predict drug concentrations in patients that were not included in the model building dataset [8, 9]. Therefore, validation on an external dataset must be performed before a model can be accepted for clinical application [9].

2 TACROLIMUS

2.1 CALCINEURIN INHIBITORS IN KIDNEY TRANSPLANTATION

Kidney transplantation is the preferred treatment for patients experiencing end-stage kidney disease [30]. When a patient receives a kidney transplant, lifelong treatment with immunosuppressive drugs is essential to prevent allograft rejection [31]. Calcineurin inhibitors (cyclosporine and tacrolimus) have been cornerstones in this treatment for several decades. Still, there is currently no clinically available biomarker reflecting the immunosuppressive effect of these drugs [32]. Therefore, whole blood concentrations are used as surrogates for the drug effect, assuming that whole blood concentrations reflect the pharmacodynamic response [3].

Calcineurin inhibitors are characterized by narrow therapeutic windows and large unexplained pharmacokinetic variability between and within patients. Therefore, it is essential to frequently measure the drug concentrations to ensure that they are within the acceptable target concentration range that adequately suppresses the immune response while toxicity is minimized. However, due to unexplained variability, maintaining drug concentrations within the acceptable therapeutic range is difficult even with frequent blood sampling, especially in the early post-transplant phase [33]. Tacrolimus has recently become the calcineurin inhibitor of choice at several kidney transplantation centers, including the National Norwegian Transplantation Center (Oslo University Hospital, Rikshospitalet) from January 2012 [34] because of a lower acute rejection rate combined with a more beneficial adverse effect profile [35]. Still, treatment with tacrolimus frequently leads to serious adverse effects, including nephrotoxicity, neurotoxicity, post-transplant diabetes mellitus, hypertension, infections and cancer [36].

2.2 PHARMACOKINETIC CHARACTERISTICS

General pharmacokinetics

Tacrolimus is a lipophilic macrolide compound, normally rapidly absorbed with absorption lag times of 0-2 hours [36]. Oral bioavailability is generally poor (mean value is typically 15-25 %, but differs across studies [36]), and highly variable (range 4 - 89 %) [37]. In blood,

tacrolimus binds strongly to erythrocytes and plasma proteins. The unbound fraction is less than 1 % [38]. Tacrolimus is a substrate of P-glycoprotein (P-gp) and the cytochrome P-450 isoenzymes CYP3A4 and CYP3A5. These enzymes are expressed in the liver and intestinal cells. Clearance is approximately 2 L/h, which means that the hepatic extraction ratio (E_H) is very low (3 %) [39]. Less than 1 % of unchanged tacrolimus is renally excreted [37].

The low bioavailability of tacrolimus is a result of poor solubility in the gastrointestinal fluids and presystemic metabolism [40]. Intestinal CYP3A metabolism is the main contributor to the first pass loss of tacrolimus, and the estimated intestinal availability (F_G) is 14 % (Figure 2-1) [41]. The additional first pass loss caused by hepatic metabolism is modest because of the low hepatic extraction ratio [3].

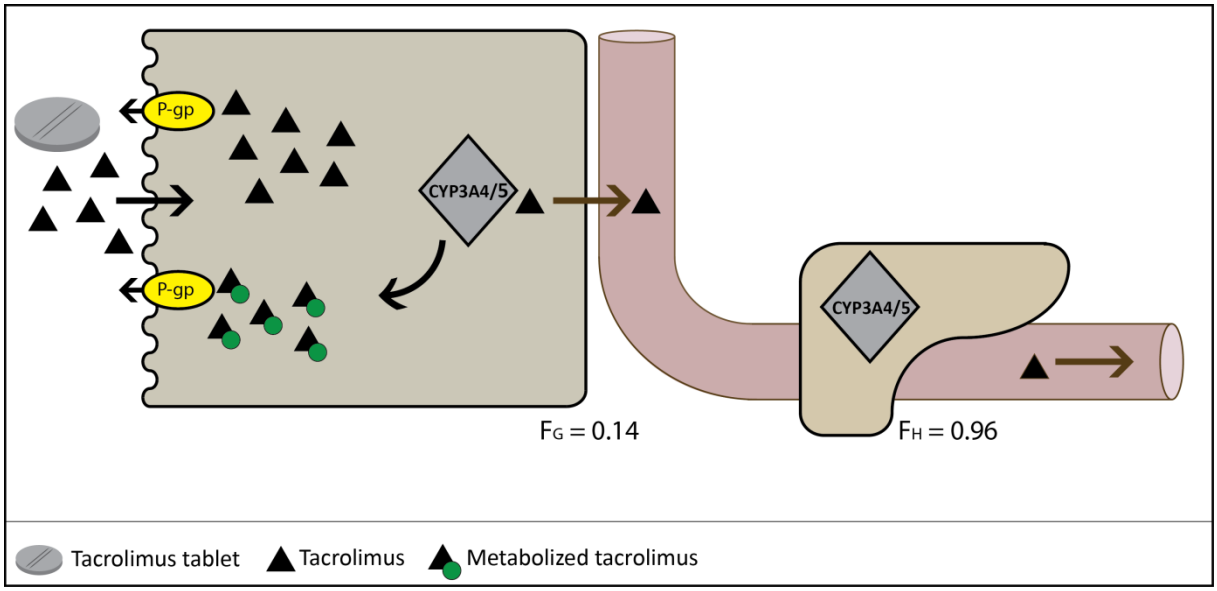

Figure 2-1. First pass loss of tacrolimus. Metabolism mainly takes place in intestinal cells due to activities of P-glycoprotein and CYP3A4. In patients expressing CYP3A5, this isoenzyme also contributes to first pass loss (not shown). The fraction passing the enterocytes (intestinal availability, F_G) is 14 %. A modest additional loss is caused by first pass hepatic metabolism (hepatic availability, F_H). Numbers are taken from Galetin et al. [41].

Distribution in blood

Tacrolimus readily passes the membrane of erythrocytes and binds to intracellular proteins with similar structure as the target protein in T-lymphocytes (FK-binding proteins) [42]. Temperature dependent blood cell partitioning is the main reason why whole blood concentrations are used rather than plasma concentrations [43]. The blood:plasma concentration ratio is also dependent on hematocrit, plasma protein concentration, tacrolimus concentration and binding affinity to intracellular proteins in erythrocytes [37]. Of these,

hematocrit is the most important factor [44]. The erythrocyte bound concentration is inactive and protected against elimination [45]. An assumption during therapeutic drug monitoring is that the measured total concentration reflects the pharmacologically active unbound concentration. However, if any of the variables influencing the blood:plasma concentration ratio change, this assumption may not hold [3].

Figure 2-2 illustrates the theoretical blood distribution of tacrolimus at two situations where the hematocrit levels are 20 % and 40 %. Both levels are plausible in kidney transplant recipients [46]. Dosing rate and unbound drug clearance are assumed to be the same in both situations. Therefore, the unbound concentration is theoretically similar [47]. The whole blood concentration, however, changes in proportion to hematocrit.

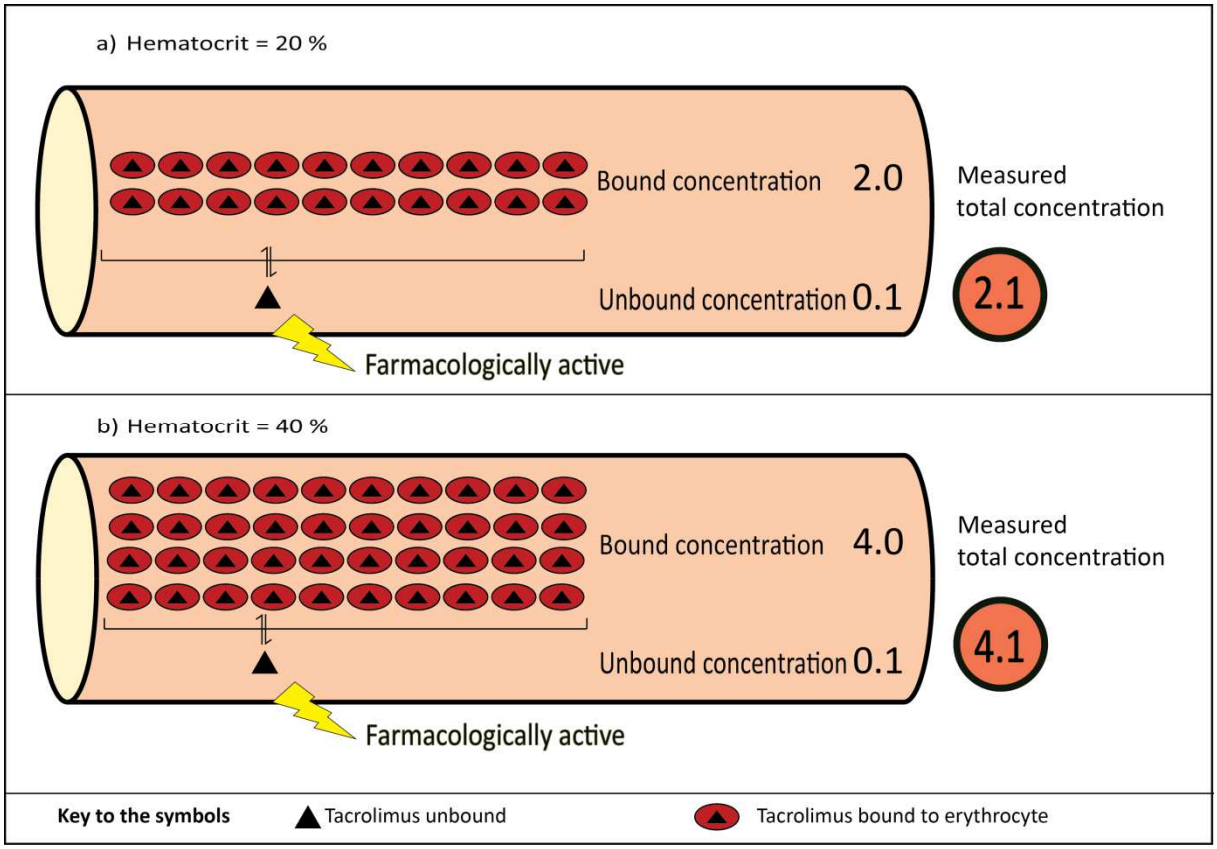

Figure 2-2. The theoretical influence of hematocrit on tacrolimus whole blood concentrations. Two situations are compared with the same dosing rate and unbound clearance and consequently similar unbound concentrations (represented by a black triangle constituting 0.1 µg/L). *Upper panel:* 20 red circles are representing erythrocytes corresponding to a hematocrit of 20 %. *Lower panel*: 40 red circles represent a erythrocyte concentration corresponding to a hematocrit of 40 %. Although the unbound, pharmacologically active concentration is the same in both situations, the measured whole blood concentration has increased due to an increase in hematocrit. The figure illustrates the importance of hematocrit to interpretation of the measured total whole blood concentration. The figure is simplified by not including plasma proteins.

CYP3A5 polymorphism

Several studies have been performed to investigate alterations in tacrolimus pharmacokinetics caused by single nucleotide polymorphisms (SNPs) in genes coding for the proteins involved in tacrolimus metabolism and distribution [48-51]. Polymorphic expression of CYP3A5 is the most consistently reported pharmacogenetic covariate for tacrolimus in literature [51, 52]. In Caucasians, 90 % are homozygous carriers of the variant allele CYP3A5*3 that is coding for enzyme products without enzymatic activity due to an mRNA splicing defect [53]. Individuals carrying the CYP3A5*1 allele express CYP3A5 with enzymatic activity in several tissues, including intestinal and hepatic cells [53]. In theory, expression of functional CYP3A5-enzymes contributes to both higher hepatic clearance and higher presystemic metabolism of tacrolimus. However, the relative contribution of these events to the observed higher dose requirements in CYP3A5 expressers have not been established [51].

Population pharmacokinetic models in literature

The first published population model in kidney transplanted adults was developed by Staatz et al. in 2002. A one-compartment model described the pharmacokinetics, and aspartate aminotransferase (ASAT) and time after transplantation were identified as covariates on apparent clearance (CL/F) [45]. From 2009, the number of published models for tacrolimus per year for tacrolimus increased in parallel with an increasing focus on pharmacogenetic covariates. *CYP3A5* genotype was consistently recognized as a covariate on tacrolimus CL/F [54-59]. Other frequently identified covariates include hematocrit [54, 57, 59, 60], corticosteroid dose [55, 61] and an effect of time after transplantation both during the initial 1-2 weeks [56, 61] and for the time aspect of weeks and months [45, 56, 59]. In contrast, the majority of population studies did not find any relationship between tacrolimus pharmacokinetics and demographic covariates.

Population models have also been developed for patients undergoing other types of transplantation. In hematopoietic stem cell transplanted children, serum creatinine was found to affect clearance, despite the fact that tacrolimus is not renally cleared [62]. In liver transplant patients, both total body weight and alkaline phosphatase were found significant [63]. Neither of these covariates have been identified in kidney transplant patients.

The structural model is almost exclusively modeled using two compartments [54, 55, 57, 60, 64, 65]. Only authors who developed models based on solely trough concentrations

found one-compartment models more appropriate [45, 61]. The absorption phase is most frequently described by first order absorption with lag time [57, 65] or transit compartments [54, 60].

Table 1-1 lists the population studies of tacrolimus in kidney transplanted adults found in a literature search in PubMed in September 2012. An overview is given on the general aspects of the study designs, the basic pharmacokinetic results, the evaluated covariates that were not found significant (marked in the table with ×) and identified covariates (relationship stated). Of notice, only one of these models is reported to be clinically applied for Bayesian estimation of individual pharmacokinetic parameters [66].

TABLE 1-1. Review of population pharmacokinetic models in kidney transplanted adults available in literature

Population pharmacokinetic study (reference)	Staatz 2002 [45]	Antignac 2007 [61]	Press 2009 [55]	Benkali 2009 [60]	Musuamba 2009[58]	Velickovic 2010 [67]	Woillard 2011 [54]	Passay 2011 [56]	Musuamba 2011 [57]	Han 2012 [59]
STUDY DESIGN										
Modeling software	NONMEM	NONMEM	NONMEM	NONMEM	NONMEM	NONMEM	NONMEM	NONMEM	NONMEM	NONMEM
Number of patients	70	83	31	32	19	63	73	681	65	80
Sample type	Troughs	Troughs	Troughs and full profiles	Full profiles	Full profiles	Troughs	Full profiles	Troughs	Full profiles	Troughs
Time period for sampling following tx	Day 2-1475	Day 1-60	Week 2-52	Week 1 and 2 + month 1, 3 and 6	NA	NA	Month 1, 3 and 6	Month 1-6	Day 15	Day 1-400
Analytical assay	LC-MS/MS	MEIA	MEIA	LC-MS/MS	MEIA	MEIA	TFS-MS/MS	LC-MS/MS	MEIA	MEIA
External validation?	Yes	Yes	No	No	No	No	No	No	No	No
Reported to be in clinical use?	No	Yes	No	No	No	No	No	No	No	To be evaluated
PHARMACOKINETICS										
Adequate number of compartments	1	1	2	2	2	1	2	Not used	2	1
CL/F (L/h) in typical patient	23.6	CL=1.81 (minimum value)	16.1	19.2 (Hct=45 %)	29	1.03	21.2 (Hct=35 %)	38.4	26.8	22.9
COVARIATES										
Demographic covariates										
Total body weight	×	×	×	×	×	×	×	×	×	Vd (linear)
Fat free mass										
Sex	×	×	×	×	×	×	×	×	×	×
Age	×	×	×	×	×	×	×	$CL/F=(Age/50)^{-0.4}$	×	×
Laboratory tests										
Serum creatinine	×	×		×			×		×	×
ASAT	CL linearly decreasing with increasing ASAT	×				×			×	×
ALAT	×	×				×			×	×
ALP	×								×	
Total bilirubin	×								×	
Hematocrit	×		×	CL= 863/Hct			$CL=(Hct/35)^{-1.14}$		Effect on CL/f not interpreted	Higher CL when Hct<33 %
Hemoglobin				×		×	×			×
Plasma protein concentration(albumin)		×	×						×	×
HDL, LDL and total cholesterol*			×							
C-reactive protein										

Concomitant medication										
Corticosteroids (prednisolone)	× (All used < 10 mg)	60 % higher CL with > 25 mg	15 % lower F with > 10 mg			1.6 L/h higher CL with> 25mg	×			×
Nifedipine								6 % lower CL/F if user		
Lansoprazole										
Cinacalcet										
Single nucleotide polymorphisms										
CYP3A5 *1/*1								100 % higher CL/F		
CYP3A5 *1/*3			~ 49 % higher CL/F	× * (only1 expresser in dataset)	150 % higher CL/F		100 % higher CL/F	69 % higher CL/F	57 % higher CL/F	~ 118 % higher CL/F
PXR (2385C>T)			×	×	NC					
ABCB1 (C1236T)			×		45 % higher CL		×		×	
ABCB1 (G2677T/A)			×		×		×		×	×
ABCB1 (C3435T)			×		×				×	
Other covariates										
Time after tx (early phase)	×	×	Sigmoid increase in CL first 7 days with highest increase at day 4.			×		14 % decrease during the first 6-10 days		
Time after tx (late phase)	CL/F linearly decreasing first 3 months, then plateau		×			×	×	29 % decrease during day 11-180 relative to day 3-5		Decrease CL/F first 21 days, plateau after 30 days
Circadian (time of the day)					K_a=2.18 at day time and 0.16 at night time					
Formulation (Advagraf®/Prograf®)			K_a=3.7 for Advagraf® and 1.8 for Prograf®				Different transit absorption model			

Blank field indicates that relationship was not tested. × indicates that the relationship was tested but not found significant. Significant relationships are stated in text.
Tx, transplantation; NA, not available; LC MS/MS, Liquid chromatography tandem mass spectrometry; MEIA, Microparticle enzyme immunoassay; TFS MS/MS, Transmition fluctuation spectrometry tandem mass spectrometry; CL/F, apparent oral clearance; CL, clearance; Vd, volume of distribution; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; ALP, alkaline phosphatase; HDL, High density lipoprotein; LDL, low density lipoprotein; CYP3A5, Cytochrome P450 3A5, PXR, Pregnane X receptor; ABCB1, ATP-binding cassette, subfamiliy B, member 1

RESEARCH ARTICLE

The Importance of Hematocrit for
Tacrolimus Target Concentration Intervention

Selected journal:
Therapeutic Drug Monitoring

The Importance of Hematocrit for Tacrolimus Target Concentration Intervention

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ABSTRACT

Introduction: Prediction of individual dose requirements of tacrolimus in kidney transplantation is a major clinical challenge due to large pharmacokinetic variability between patients and increasing dose-normalized whole blood concentrations during the first post-transplant months. The objectives of this study were to identify predictable differences between kidney transplant recipients to improve initial dosing, to investigate whether the apparent time-varying pharmacokinetics of tacrolimus may be explained by changes in hematocrit and to evaluate the importance of hematocrit for tacrolimus target concentration intervention.
Methods: Twenty-nine patients contributed full pharmacokinetic profiles at 44 occasions, and 44 patients contributed trough concentrations from the first ten weeks after kidney transplantation. A total of 1546 blood samples were analyzed. Demographic, clinical and pharmacogenetic patient characteristics were evaluated as covariates.
Results: Relative bioavailability was 24 % lower in females than in males and 49 % lower in CYP3A5 expressers than in CYP3A5 nonexpressers. Fat free mass was the most predictive body size metric. Whole blood concentrations of tacrolimus increased linearly with increasing hematocrit. An underlying increase in hematocrit with time after kidney transplantation largely explained the apparent time-varying pharmacokinetics of tacrolimus. . In addition, relative bioavailability was 122 % higher immediately after transplantation compared to its lowest value, decreased to its lowest value during the first four days and subsequently increased by 31 % with an asymptote at day 60.
Conclusions: Initial dose of tacrolimus should be predicted from sex, *CYP3A5* genotype, fat free mass and hematocrit. Hematocrit is an important factor to predict changes in tacrolimus whole blood concentrations with time after kidney transplantation. The model may potentially aid in individual dosing of tacrolimus. Its predictive performance must be evaluated before application in clinical practice. The relationship between hematocrit-standardized tacrolimus concentrations and clinical effects should be further investigated.

Key words: Tacrolimus, hematocrit, population pharmacokinetics, target concentration intervention, kidney transplantation

INTRODUCTION

Tacrolimus is a cornerstone immunosuppressive agent in kidney transplantation [1]. Although tacrolimus effectively prevents acute rejection, its nephrotoxic adverse effects contribute to restricted long-term graft survival [2]. Variability in tacrolimus exposure is an additional risk factor for early graft failure [3]. It is therefore important to keep tacrolimus concentrations stable at a target concentration to minimize risk toxicity while maintaining adequate immunosuppression.

Tacrolimus has a narrow therapeutic window and large pharmacokinetic variability both between and within patients [4]. In addition, dose-normalized whole blood concentrations tend to increase during the first months after transplantation [4-7]. Consequently, both accurate initial dosing and managing time-varying dose requirements are major clinical challenges. It is important to understand how patient characteristics influence the pharmacokinetics of tacrolimus in order to individualize and optimize treatment. Tacrolimus is metabolized in the intestines and liver by cytochrome P450 3A (CYP3A) isoenzymes [4]. The most consistently identified covariate for tacrolimus dose requirement is CYP3A5 activity predicted from *CYP3A5* genotype [8-11]. At some centers, CYP3A5 genotyping was recently introduced to guide initial dosing of tacrolimus in the clinical setting [12]. However, large pharmacokinetic variability also exists between patients who do not differ with respect to *CYP3A5* genotype. For example, overweight patients and elderly are prone to higher tacrolimus concentrations after standard initial dosing proportional to total body weight [13, 14]. This variability should be explained and accounted for in order to optimize dosing across all patient groups. Furthermore, pharmacogenetics cannot explain the apparently time-varying pharmacokinetics of tacrolimus. Hence, a dosing strategy for tacrolimus should include a time-varying component to help predict changes in dose requirement over time.

Whole blood concentrations of tacrolimus are typically used to guide dosage adjustments. Tacrolimus is strongly distributed into and bound to erythrocytes. Normally, less than one percentage is unbound in blood [15]. Hematocrit is a factor that provides a theoretical basis for understanding time-varying pharmacokinetics based on whole blood. Hematocrit levels are generally low at the time of kidney transplantation because of the underlying kidney disease, blood loss during surgery and postoperative fluid therapy. During the months after transplantation, hematocrit levels increase due to restoration of endogenous erythropoietin production, although with large variability between individuals [16]. Since tacrolimus is a low extraction ratio drug [4], whole blood concentrations are expected to increase in proportion to hematocrit while the unbound concentration and unbound clearance remains unchanged. Apparent decrease in whole blood clearance of tacrolimus with time after kidney transplantation have been attributed to tapering of corticosteroid dose with time after transplantation, hypothesizing that corticosteroids induce CYP3A enzymes [5, 17]. However, increasing tacrolimus concentrations also occurred in patients receiving fixed corticosteroid

doses [6]. The relative importance of hematocrit, corticosteroid tapering and other factors on the apparent decrease in tacrolimus whole blood clearance with time remains unclear.

It is important to investigate the influence of hematocrit on whole blood based pharmacokinetics of tacrolimus to understand and predict how the whole blood concentrations will change in relation to changes in hematocrit. Furthermore, understanding this relationship may have important clinical implications because a dosage reduction followed by increased whole blood concentration caused by increased hematocrit may lead to unintended changes in the unbound concentration, which is the concentration expected to be more closely related to the therapeutic effect [18].

The first objective of the present study was to develop a population pharmacokinetic model of tacrolimus in kidney transplant recipients and investigate predictable differences for improving initial dosing. The second objective was to investigate whether the apparent time-varying pharmacokinetics of tacrolimus may be explained by changes in hematocrit and to evaluate the importance of hematocrit for tacrolimus target concentration intervention.

MATERIALS AND METHODS

Patients and Data Collection

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

Data were collected from patients who underwent kidney transplantation at Oslo University Hospital, Rikshospitalet. Combined-organ recipients and patients who received drugs that were known to pharmacokinetically interact with tacrolimus were excluded. However, if such drugs were used by more than ten percent of the patients, they were included and the drug was analyzed as a covariate. A total of 69 patients were eligible for the analysis. Forty-four 12-hour concentration-time profiles of tacrolimus in 29 patients were available from three previously published clinical studies [19-21] (Figure 1a, Intensive Group). Tacrolimus trough concentrations (C_0) measured during the first ten weeks following kidney transplantation were collected in 44 patients (Figure 1b, TDM Group), four of which were also represented in the Intensive Group. Dosing was coded by 12 hour intervals and assuming 100 % compliance unless any missed doses were recorded in the patient chart. Patients were informed to be fasting at the time of drug intake.

Data on *CYP3A5* genotype, sex, height and the following time-varying characteristics were collected: Hematocrit, total body weight, age, serum albumin, serum creatinine, C-

reactive protein (CRP), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), total serum bilirubin, alkaline phosphatase, oral prednisolone dose, intravenous methylprednisolone dose, acute rejection episodes, concomitant use of potential interactive drugs and time after transplantation. Covariates that were missing on the day of transplantation were imputed by carrying back the first known value. Covariates missing at any later time point were imputed by carrying forward the last known value. If more than two hematocrit values were missing in sequence, values were derived by linear regression between all known hematocrit and hemoglobin values within the patient. A summary of the study designs is listed in Table 1.

Therapeutic Drug Monitoring

According to the center transplant-protocol, initial tacrolimus (Prograf® capsules, Astellas Pharma US Inc.) dose was 0.04 mg/kg total body weight twice daily, adjusted to the nearest 0.5 mg per dose. Subsequent doses were adapted according to measured whole blood trough concentration, with an acceptable target range of 3-7 µg/L in standard risk patients and 8-12 µg/L in high-risk patients (defined as presence of panel reactive antibody (PRA) > 20 % and/or presence of donor-specific antibodies (DSA)). Trough concentrations were initially measured 3-4 times per week. The frequency decreased in parallel with increasing time since transplantation and depending on clinical status. The acceptable target concentration range was adjusted to 3-5 µg/L six months after transplantation in all patients.

Concomitant Immunosuppressive Medication

The immunosuppressive regimen consisted of mycophenolate mofetil (1.5 g/day), intravenous methylprednisolone (day 1: 250 mg), oral prednisolone (day 2-15: 20 mg, day 16-29: 15 mg, day 30-61: 10 mg, day 62-180: 7.5 mg and from day 181: 5 mg) and induction therapy with basiliximab (day 1 and 5: 20 mg). High-risk patients received mycophenolate mofetil (1.5 – 2 g/day), oral prednisolone (day 2: 80 mg, tapered to 20 mg daily during day 3-8, day 9-29: 20 mg, day 30-61: 15 mg, day 62-180: 10 mg and from day 181: 5 mg), intravenous methylprednisolone (day 1: 500 mg, day 2: 80 mg) and additional induction therapy with human immune globulins and rituximab. Day 1 was defined as the day of transplant.

Analytical Assay

Table 1 shows the analytical assay details in each sub-study and the respective performance data. Immunoassay measurements of tacrolimus tend to be higher than concentrations determined by liquid chromatography due to cross-reactivity with tacrolimus metabolites

[22]. Concentrations determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) were converted to corresponding immunoassay values by the aid of the following equation (Eq. 1), established by the laboratory that performed all analyses:

$$CM = \frac{(LC - 0.19)}{0.80}, \quad \text{Eq. 1}$$

where CM is the chemoluminescent microparticle immunoassay tacrolimus concentration in µg/L and LC is the LC-MS/MS tacrolimus concentration in µg/L.

CYP3A5 Genotyping

DNA was extracted from EDTA anti-coagulated 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 PCR and melt curve analysis with hybridization probes on the LightCycler® 480 instrument (Roche Applied Science, Penzberg, Germany). Primers were designed using LightCycler Probe Design software version 2 (Roche) and hybridization probe sequences were derived from Cheung *et al*. [23].

Population Pharmacokinetic Modeling

Structural Model

Models with one or two compartments, zero or first order absorption and with or without an absorption lag time were investigated. Only oral data were available. Therefore, apparent parameter estimates were obtained (e. g. CL/F, V/F). The population value of bioavailability (F) was defined as 1, and F was estimated relative to this value. Random effects in the pharmacokinetic parameters were modeled in terms of between subject variability (BSV) and between occasion variability (BOV). One hospital visit was defined as one occasion for the Intense Group. For the TDM Group, a new occasion was defined when a dose change took place. BSV and BOV were estimated using exponential models (Eq. 2):.

$$P_{ik} = P_{GRP} \times e^{\eta i + \eta k}, \quad \text{Eq. 2}$$

where P_{ik} is the value of parameter P for the i^{th} individual at the k^{th} occasion, P_{GRP} is the group value of P in the population, $e^{\eta i}$ is the deviation between P_{GRP} and the individual value P_i and $e^{\eta k}$ is the deviance between the individual value P_i and the occasion-specific value P_{ik} [24]. The following model for the residual error was used throughout the analysis (Eq. 3):

$$Y_{ij} = Conc_{ij} + \theta_{study_i} \times (\varepsilon_1 \times Conc_{ij} + \varepsilon_2) \times e^{\eta err_i}, \quad \text{Eq. 3}$$

where Y_{ij} and $Conc_{ij}$ are the model predicted drug concentrations with and without residual error, respectively, for the i^{th} individual at the j^{th} measurement, θ_{studyi} is a study-specific fixed effect, ε_1 is a residual error term proportional to the measured concentrations, ε_2 is an additive error term and $e^{\eta erri}$ is the individual random difference in residual error for the i^{th} individual [25]. The random variables η_i, η_k, ε_1, ε_2 and η_{erri} are assumed to be normally distributed with mean zero and variances ω^2_P, π^2_P , $\sigma_1{}^2$, $\sigma_2{}^2$ and $\omega_{err}{}^2$, respectively.

Covariate Model

All recorded patient characteristics were evaluated as covariates on the parameters where an effect was biologically plausible or if an effect was found in previous studies. To investigate the influence of body size, fat free mass was predicted from total body weight, height and sex [26]. Pharmacokinetic parameters were related to fat free mass using allometric coefficients of 3/4 for clearances and 1 for volumes of distribution, respectively [27]. The additional effect of fat mass on pharmacokinetic parameters was estimated as described by Anderson *et al.* [28] (Eq. 4):

$$\mathrm{NFM} = \mathrm{FFM} + \theta_{\mathrm{FFAT}} \times (\mathrm{TBW} - \mathrm{FFM}), \quad \text{Eq. 4}$$

where NFM is the normal fat mass, FFM is the predicted fat free mass, θ_{FFAT} is an estimable parameter describing the additional effect of fat mass and TBW is total body weight. Binary covariates were modeled by estimating a fractional change in one group compared to the other group. To initially investigate the shape of the effect of continuous covariates on pharmacokinetic parameters, subjects were categorized by covariate value and a mean pharmacokinetic parameter were estimated within each category. The relationship was subsequently modeled using linear, power, exponential or sigmoidal functions.

The following general equation can be used to calculate standardized concentrations accounting for varying hematocrit [29] (Eq. 5):

$$\mathrm{Cb_{std}} = \mathrm{Cu} + \frac{\mathrm{Hct_{norm}}}{\mathrm{Hct}} \times (\mathrm{Cb_{tot}} - \mathrm{Cu}), \quad \text{Eq. 5}$$

where Cb_{std} is the standardized whole blood concentration, Cu is the unbound concentration, Hct_{norm} is a normal hematocrit value (e. g. 45 %), Hct is the measured hematocrit and Cb_{tot} is the measured total whole blood concentration. However, because Cu was unknown and because Cb_{tot} will be considerably greater than Cu, the equation was simplified to Eq. 6:

$$\mathrm{Cb_{std}} = \frac{\mathrm{Hct_{norm}}}{\mathrm{Hct}} \times \mathrm{Cb_{tot}}. \qquad \text{Eq. 6}$$

This simplified equation assumes that hematocrit is a surrogate for erythrocyte mass and the number of protein binding-sites within erythrocytes. To evaluate whether erythrocytes become saturated with tacrolimus within the therapeutic concentration range, nonlinear functions were investigated (not shown). It was also attempted to estimate between subject variability in a parameter relating hematocrit and whole blood concentrations, as described by Wahlby *et al.* [30]. To account for binding to serum albumin, the following equation using the same principles as described above was used (Eq. 7):

$$\mathrm{C_{std}} = \frac{\mathrm{Alb_{norm}}}{\mathrm{Alb}} \times \mathrm{Cb_{std}}, \qquad \text{Eq. 7}$$

where C_{std} is standardized for both albumin and hematocrit, Alb_{norm} is a normal albumin concentration (e. g. 45 g/L) and Alb is the measured serum albumin concentration.

To initially investigate the shape of the time-varying pharmacokinetics, twelve time intervals were defined after day 1 (day of transplant): day 2, 3, 4-5, 6-7, 8-10, 11-14, 15-21, 22-28, 29-42, 43-56, 57-70 and >70 days. For each interval, a CL/F or F interval-specific parameter was estimated. Empirical models were subsequently used to match the discrete distribution of parameter values as a function of time.

Model Evaluation and Statistical Analysis

Pharmacokinetic modeling was performed in NONMEM® (Nonlinear Mixed Effects Modeling, version 7.2, ICON Development Solutions, Elliott City, MD, USA) [31], using the first order conditional estimation method with interaction. Model selection was guided by biological plausibility and prediction-corrected visual predictive checks (pcVPCs) as function of time and relevant covariates, comparing observed data with 90 % prediction intervals constructed from 100 simulated subsets of the original dataset [32]. Statistical significance was evaluated using the likelihood ratio test with a required significance level of 0.05 (difference in objective function value (ΔOFV) = -3.8 for 1 parameter) [31]. Covariates were included stepwise and subsequently deleted from the full model (backward elimination) to evaluate their final statistical contribution. Confidence intervals of the parameters were generated from 500 nonparametric bootstrap replicates [33]. Random effects were retained if the lower 2.5th percentile did not approach zero. Covariate coefficients were retained if the 95 % confidence interval did not include the value equivalent to no effect.

Models and bootstrap procedures were run using Wings for NONMEM® (Holford NHG. Wings for NONMEM v. 720 for NONMEM 7.2. http://wfn.sourceforge.net, 2011). The statistical package R® (v. 2.15.0) was used for statistical and graphical analyses. Means for normally distributed covariates was compared using two-tailed t-tests with assumption of equal variance. If not stated otherwise, descriptive statistics are expressed as mean ± standard deviation.

RESULTS

Patients and Data Collection

Demographic and clinical characteristics, details about tacrolimus treatment and the number of missing values are presented in Table 2. Patients using fluconazole and carbamazepine were excluded because of drug interaction potential with tacrolimus while patients using nifedipine (26 patients), lansoprazole (12 patients) and/or cinacalcet (5 patients, interactive drug according to sub-study 1) were retained. Eighty percent of tacrolimus trough concentrations were outside the defined acceptable concentration range. Hematocrit increased from 31±5 to 38±3 from day 1 to day 70 post-transplant with large variability between patients (Figure 2). Mean hematocrit did not vary by sex (p=0.28) or *CYP3A5* genotype (p=0.51). Mean total body weight was 19 kg higher in males (86 ± 21 kg) compared to females (67 ±15 kg) (p<0.001), but not different across *CYP3A5* genotypes (p=0.93). Mean fat free mass was 19 kg higher in males (64 ± 10 kg) compared to females (46 ±5 kg) (p<0.001), but not different across *CYP3A5* genotypes (p=0.37).

Population Pharmacokinetic Modeling

Structural Model

Tacrolimus pharmacokinetics were best described by a two-compartment model with first order absorption and a lag time. A study specific absorption rate constant (k_a) and lag time improved the description of sub-study 2. BSV and BOV were tested for all pharmacokinetic parameters except lag time, which was assumed to have no random variability. When BOV in F was included, the lower 2.5th percentile of the 95 % confidence intervals of BOV in the four disposition parameters approached zero and were removed (ΔOFV +7.3 for four parameters, p=0.12). For k_a, only BOV was retained as random effect (ΔOFV +3.4 by removing BSV, p=0.07 and ΔOFV +28.7 by removing BOV, p<0.001), indicating that the variability in

absorption rate is mainly dependent on dosing occasion rather than being a subject specific process. Estimating a full correlation matrix between CL/F, V_1/F, Q/F and V_2/F (ΔOFV=-59.0 for 6 added parameters, p<0.001) was chosen rather than retaining BSV in F (ΔOFV=-32.4 for 1 added parameter, p<0.001). Figure 3 shows the pcVPC of predictions of the base model with a systematic prediction bias over the range of hematocrit.

Covariate Model

Standardization of whole blood concentrations to a hematocrit of 45 % improved the model markedly (ΔOFV = -78.4) and removed the systematic prediction bias over the range of hematocrit values (Figure 3c). Accounting for the possibility of saturated erythrocyte proteins, BSV in the relationship between tacrolimus whole blood concentrations and hematocrit or simultaneous standardization to albumin concentrations did not improve the model. The time interval-specific value of F changed with time after transplantation (Figure 4). The changes in the individual value of F (F_i) were describable using two distinct sigmoid functions of time after transplant (Txt), including a random effect describing the BSV in the extent of change at late time (Eq. 8):

$$F_i = \left(\mathrm{FF_{baseline}} + \frac{\mathrm{Fmax_{early}}}{1+\left(\frac{\mathrm{Txt}}{\mathrm{F_{early_{50}}}}\right)^{\mathrm{Hill_{F_{early}}}}} \right) \times \left(\frac{\mathrm{Fmax_{late}} \times e^{\eta_i}}{1+\left(\frac{\mathrm{Txt}}{\mathrm{F_{late_{50}}}}\right)^{-\mathrm{Hill_{F_{late}}}}} \right), \qquad \text{Eq. 8}$$

where $FF_{baseline}$ is a relative term fixed to 1, $Fmax_{early}$ is the maximum increase in F immediately after transplantation, $F_{early50}$ is the day with half maximum early effect on F with the associated shape coefficient $Hill_{Fearly}$ describing the steepness of this change, F_{late50} is the day with half maximum later effect on F with the associated steepness coefficient $Hill_{Flate.}$ $Fmax_{late}$ is the asymptotic maximum value of the late change in F and $e^{\eta i}$ is the difference between the individual asymptote and the population asymptote, where η_i's are assumed to be normally distributed with mean zero and variance ω_{Flate}^2. The six fixed effects parameters replaced the 12 time interval parameters without loss in model goodness of fit (ΔOFV = -64.6 vs -66.5 respectively), and BSV in F_{late} resulted in a further decrease (ΔOFV = -47.1 for 1 parameter, p<0.001). Estimating BSV in the remaining parameters in Eq. 8 was not supported by the data. The remaining time-varying covariates, including prednisolone dose, did not replace the empirical time model for F without loss in model goodness of fit.

During covariate inclusion, *CYP3A5* genotype had a significant effect on both CL/F and F (p<0.001). The additional effect of fat mass (θ_{FFAT}) to the effect of fat free mass on

pharmacokinetic parameters indicated that fat free mass was a more appropriate size metric than total body weight (θ_{FFAT}=0.1, 95 % confidence interval (-0.7-1.9) for clearances, θ_{FFAT}=0.3, 95 % confidence interval -0.7-2.4 for volumes of distribution). It was noted in the pcVPCs that in patients with fat free mass < 55 kg tacrolimus concentrations were overpredicted, whereas in those with fat free mass > 60 kg concentrations were slightly underpredicted. This prediction error over the range of fat free mass was reduced by including sex as a covariate on F (ΔOFV =-6.2, p=0.01). No other covariate, including the evaluated drugs, was found to affect pharmacokinetics of tacrolimus.

Model Reduction and Evaluation

Based on 95 % confidence intervals generated by the bootstrap procedure it was decided to retain a proportional residual error model (ΔOFV +41.1 for removing BSV in the residual error and ΔOFV +1.7 for removing the additive error term) with joint study specific component for sub-study 1 and 4 (ΔOFV +1.0). In addition, only the random effect correlation between CL/F and Q/F was retained (ΔOFV = +17.5, 5 parameters). The effect of *CYP3A5* genotype on CL/F was removed because the 95 % confidence interval of 0.61-1.23 did not support its inclusion in addition to an effect on F (ΔOFV +0.01, p=0.91). Table 3 shows the results from backward elimination of covariates.

The final model parameter estimates are presented in Table 4. Based on bootstrap averages, relative bioavailability was 49 % lower in CYP3A5 expressers and 24 % lower in females. Relative bioavailability was 122 % higher immediately after transplantation relative to its lowest value, followed by a steep decrease ($F_{early\ Hill}$ = 8.7) to a baseline with half of the change at day 2.4 after transplantation. Relative bioavailability then increased more slowly ($F_{late\ Hill}$ = 2.4) to an asymptote 31 % higher than the baseline (BSV 104 %) with half of the change after 30 days (Figure 4). According to the pcVPCs, the model predicted the observed tacrolimus concentrations well within the 12-hour dose interval for the Intensive Group and during the first 70 days after transplantation in the TDM Group (Figure 5 a, b). It also predicted without sign of bias over the range of covariates (Figure 5 c-h), except for some overprediction in females.

DISCUSSION

In the present study, a population pharmacokinetic model was developed for tacrolimus using data from 69 kidney transplanted adults. A wide range of covariates were investigated, and

the results suggest using hematocrit, *CYP3A5* genotype, fat free mass, sex and time post-transplant to predict the initial dose. By standardizing tacrolimus concentrations in proportion to hematocrit the model improved markedly. The apparent time-varying pharmacokinetics of tacrolimus after kidney transplantation were largely explained by a parallel increase in hematocrit, suggesting that hematocrit, which is normally measured during routine post-transplant care, can help to predict changes in tacrolimus whole blood concentrations over time. Kidney transplant patients are more prone to extreme hematocrit values than the normal population both during the first year and at later times after transplantation [16], pointing out the particular importance of considering hematocrit levels for this patient group.

Hematocrit has been identified as a covariate in several previously performed population pharmacokinetic analyses of tacrolimus [10, 11, 34, 35], but these applied hematocrit either to clearance or volume of distribution. In theory, all pharmacokinetic disposition parameters based on total concentrations should be similarly influenced by a change in unbound fraction for a low extraction ratio drug such as tacrolimus, which was the rationale for modeling hematocrit on the whole blood concentrations rather than on any single parameters. The linear relationship appeared as simple and practical. There was no sign of saturation of erythrocytes at the evaluated concentration range (1.5-48.4 µg/L).

Tacrolimus is also bound to albumin, α_1-glycoprotein and lipoproteins [36]. Variability in plasma concentrations of these proteins represents another potential source of variability in tacrolimus whole blood concentrations. Ideally, standardization of whole blood concentrations should account for both binding to proteins in erythrocytes and binding to plasma proteins [15]. However, only albumin concentrations were recorded in the present study. Standardization to albumin did not improve the fit. The potential influence of albumin was possibly obscured by missing and imputed albumin values for 32 % of the samples or by the correlated increase in albumin and hematocrit following kidney transplantation.

The fact that fat free mass appeared as the more suitable body size metric is biologically plausible because fat mass is not expected to contribute directly to metabolic capacity [28]. It provides an explanation to the higher tacrolimus concentrations observed in overweight patients when initial dose is calculated in proportion to total body weight [13, 14]. This result is therefore of particular relevance to prevent risk of toxicity in obese patients.

The result of a 24 % lower relative bioavailability in females compared with men has some support by previous studies [7, 14]. Although the effect was small in terms of ΔOFV, the visual predictive checks over the range fat free mass with and without a sex effect

confirmed its importance. Sex differences in the pharmacokinetics of CYP3A substrates remain controversial in literature, and one study used intravenous data to demonstrate a higher clearance of the CYP3A substrate midazolam without an additional effect on bioavailability [37]. In our study, there was little evidence for an effect on clearance, but an underlying contributing effect cannot be excluded based on the relatively large fraction of sparse sampling. The importance of body size and sex as covariates are in conflict with previous population studies of tacrolimus in kidney transplanted adults [8, 34, 38, 39]. These studies did however not investigate fat free mass as body size metric. In addition, they applied the stepwise covariate inclusion method, which only retains covariates improving the model independently [40]. The contribution of correlated covariates may be masked using this method.

On the day following surgery (day 2), bioavailability was estimated to be about two times higher than on day 4. Others found a pattern of a rapid change in clearance during the first post-transplant week [38], which might also be the underlying explanation to our observations. Higher bioavailability may result from low food intake following surgery [4]. Alternatively, both clearance and bioavailability might be affected by a potential immediate effect on CYP3A transcription by inflammatory cytokines [41] or methylprednisolone administration in conjunction to surgery [42]. The later increase in bioavailability up to around day 60 after transplantation have also been described by others [6, 39]. During this period, prednisolone daily dose was tapered from 20 mg to 10 mg in most patients, but prednisolone failed to serve as a covariate. Prednisolone administration should not thereby be rejected as the underlying cause. Traditional covariate models do not explicitly model the induction/de-induction of CYP3A/P-glycoprotein transcription and prednisolone exposure is not well reflected by dose [43]. The observed time related changes in bioavailability in this study are interesting from a population perspective, but of limited value in an individual clinical setting due to the large BSV (apparent CV of 104 %) in the asymptotic value of relative bioavailability.

The results from this study support the previous recommendations of doubling initial dose of tacrolimus to CYP3A5 expressers [12, 44]. Previous population studies identified *CYP3A5* genotype solely as a covariate on clearance (CL/F) [10, 34, 45]. In contrast, our results indicate 49 % decreased relative bioavailability in CYP3A5 expressers without an additional effect on clearance, suggesting that CYP3A5 activity in the gut wall is the more important factor leading to higher dose requirement in this patient group. However, only five

twelve-hour pharmacokinetic profiles were available in CYP3A5 expressers. These results should therefore be carefully interpreted.

There was a large fraction of tacrolimus trough concentrations (80 %) outside the defined acceptable concentration range of 3-7 µg/L, demonstrating that the currently applied method of target concentration intervention is not optimal. Bayesian estimation of individual pharmacokinetic parameters (e.g. CL/F) may aid in prediction of the required individual tacrolimus dose to achieve a target concentration [46]. The presented population model should be suitable to provide a prior distribution for this purpose. The estimate of CL/F (18.8 L/h for a male CYP3A5 non-expresser with fat free mass of 60 kg and hematocrit of 45 %) was somewhat lower than estimated by others (26.8 L/h [10], 23.6 L/h [39]). This was expected because the parameters were standardized to a higher hematocrit value than the typical value in kidney transplant recipients. Studies reporting hematocrit found similar clearances (19.2 L/h [35] and 16.5 L/h [34]) when standardized to 45 %. Random variability of 17 % in bioavailability between dosing occasions (BOV) was the main source of variability in tacrolimus concentrations within patients as also previously described [6, 8]. Quantification of BOV is important, because it describes the variability that cannot be controlled by target concentration intervention [24].

The major limitation of the present study was a high proportion (67 %) of trough concentrations. Other limitations included absence of homozygote CYP3A5 *1/*1 expressers, a high frequency (30 %) of missing values for some covariates and only one patient with high hematocrit levels (> 45 %). Hematocrit-related bias in tacrolimus concentrations determined by analytical immunoassays should however not be a relevant limitation because CMIA is not affected by hematocrit [22] and all samples analyzed with microparticle enzyme immunoassay (MEIA) had corresponding hematocrit levels not associated with bias (25-45 %) [47].

A relationship between tacrolimus whole blood concentrations and clinical effects is not yet established, and there is lack of consensus on the optimal whole blood target concentration [22]. Unbound drug concentrations are generally more closely related to clinical effects. To date, no studies have been performed on the concentration-effect relationship of tacrolimus using unbound concentrations. However, one study used radiolabeling to estimate the unbound tacrolimus concentration and found it significantly lower in liver transplant patients experiencing acute rejection compared with stable patients ($p<0.001$), without a significant difference in whole blood concentration ($p=0.5$) [48]. Some studies on

cyclosporine, which have similar binding properties to tacrolimus in blood, found stronger inhibition of calcineurin at low hematocrit values [49], and others found that unbound concentrations predicted rejection rate [50]. Thus, variable results of reports using tacrolimus whole blood concentrations to characterize the concentration-effect relationship might partly result from not accounting for the confounding effect of hematocrit variability. In the absence of specialized analytical equipment to measure unbound concentrations, standardization of total concentrations to a normal hematocrit value provides a simple method to obtain concentrations that more accurately reflect the unbound concentrations [29]. Relating hematocrit-standardized tacrolimus concentrations to clinical effects may potentially extend the current understanding of the optimal target concentration of tacrolimus.

CONCLUSION

A population pharmacokinetic model for tacrolimus was developed using data from 69 kidney transplanted adults. The results suggest increasing initial tacrolimus dose by 2 in CYP3A5 expressers, by 1/3 in females and to scale pharmacokinetic parameters allometrically to fat free mass. Hematocrit appears as an important factor both for calculation of more accurate initial doses and to predict changes in whole blood concentrations of tacrolimus with time after kidney transplantation. By applying the presented population model in clinical dose decisions, individualized therapy with tacrolimus may be optimized, potentially improving long-term graft survival. A prospective study must be performed to evaluate the clinical utility of the model before application in clinical practice. The relationship between hematocrit-standardized whole blood concentrations of tacrolimus and clinical effects should be investigated.

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TABLES

TABLE 1. Characteristics of pharmacokinetic studies of tacrolimus

Study and ref.	Concs, n	Patients[a] (n)	Assay type	LLOQ (µg/L)	Assay CV (%)	Sampling times	Excluded patients from original dataset (reason)
1 [20]	134	5	MEIA[b]	3.0	13 at 5 µg/L 7 at 23 µg/L	0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 12 hours after administration	1 (use of carbamazepine)
2 [21]	164	7	LC-MS/MS[c]	1.1	≤ 5.2	0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hours after administration	1 (not known *CYP3A5* genotype)
3 [22]	216	19	CMIA[d]	1.0	< 6 at 2.3 µg/L < 9 at 7.0 µg/L	0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 23 and 24 hours after administration	1 (food intake before tacrolimus administration)
4 NP	1032	44	CMIA [d]	1.0	< 6 at 2.3 µg/L < 9 at 7.0 µg/L	Predose (Trough concentrations)	

Ref, reference; Conc, concentration; n, number; LLOQ, lower limit of quantification; CV, between series coefficient of variation; MEIA II, microparticle enzyme immunoassay; LC-MS/MS, liquid chromatography/tandem mass spectrometry; CMIA, chemoluminescent microparticle immunoassay; NP, not previously published data

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

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

[c] Converted to corresponding immunoassay concentrations CM = (LC – 0.19) / 0.80, see main text for details

[d] Analyzed on the Architect® instrument (Abbott Laboratories, Abbott Park, IL, USA)

TABLE 2. Demographic and clinical data

	Number	Mean	(SD)	Change in TDM Group after transplantation Day 1 Mean(SD)	Day 70 Mean(SD)
Patients (Intensive group)	29[b]				
Male / Female	21 / 8				
CYP3A5 genotype (*1/*1, *1/*3, *3/*3)	0 / 3 / 26				
Tacrolimus concentrations (below LLOQ)	546 (0)				
Tacrolimus samples per patient		18	(9)		
Tacrolimus concentration (µg/L)		11.3	(6.0)		
Patients (TDM group)	44[b]				
Male / Female	32 / 12				
CYP3A5 genotype (*1/*1, *1/*3, *3/*3)	0 / 8 / 36				
Tacrolimus concentrations (below LLOQ)	1038 (2)				
Tacrolimus samples per patient		23	(5)		
Tacrolimus concentration (µg/L)		7.0	(2.5)[b]		
High-risk patients	7				
Patients (all)[a]	69				
CYP3A5 genotype (*1/*1, *1/*3, *3/*3)	0 / 10 / 59				
Male / Female	50 / 19				
Age (years)		43	(14)		
Height (cm)		176	(11)		
Total body weight (kg)		81	(21)	83(22) to	80(21)
Predicted fat free mass (kg)		59	(12)	60(12) to	55(10)
Hematocrit (%)		36	(5)	31(5) to	38(3)
Serum creatinine (µmol/L)		147	(73)	409(262) to	120(40)
CRP (mg/L		7	(8)	12(11) to	3(5)
Serum albumin (g/L)		42	(4)	37(5) to	45(4)
Total serum bilirubin (µmol/L)		7	(3)	5(3) to	6(4)
ASAT (IU/L)		23	(7)	35(26) to	20(5)
ALAT (IU/L)		31	(20)	50(45) to	22(11)
ALP (IU/L)		66	(21)	60(19) to	70 (22)

CYP3A5, cytochrome P450 3A5; LLOQ, lower limit of quantification; TDM, therapeutic drug monitoring; CRP, C-reactive protein; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; ALP, alkaline phosphatase

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

[b] Four patients contributed data to both groups

[c] Trough concentration

TABLE 3. Results from backward elimination of covariates

Parameter	Covariate	Number of parameters	Δ OFV	p
F	Hematocrit	0	+ 52.2	< 0.001
F	Function for early time effect	3	+ 77.0	< 0.001
F	Function for late time effect	4	+ 80.2	< 0.001
F	*CYP3A5* *1/*3 genotype	1	+ 32.7	< 0.001
F	Sex	1	+ 6.4	0.01
V_1/F, V_2/F	Allometric scaling to fat free mass	0	+ 6.6	-
CL/F, Q/F	Allometric scaling to fat free mass	0	+ 3.2	-

OFV, Objective function value; F, bioavailability; CYP3A5, cytochrome P450 3A5; CL/F, apparent clearance; V_1/F, apparent central volume of distribution; Q/F, apparent intercompartmental clearance; V_2/F, apparent peripheral volume of distribution

TABLE 4. Final model parameter estimates and bootstrap results

Parameter		Model estimate [a]	BS mean	BS 95 % CI [b]
CL/F	(L/h)	18.6	19.2	16.3-22.5
V_1/F	(L)	104	97	48-138
Q/F	(L/h)	29.6	32.3	21.5-48.5
V_2/F	(L)	411	424	297-583
K_a	(h^{-1})	1.21	1.16	0.57-1.88
$K_{astudy\ 2}$	(h^{-1})	0.41	0.37	0.25-0.52
Lag time	(h)	0.21	0.22	0.20-0.36
Lag $time_{study\ 2}$	(h)	0.81	0.81	0-38-0.90
Covariates on F				
CYP3A5 *1/*3	Factor	0.50	0.51	0.38-0.65
Female sex	Factor	0.77	0.76	0.61-0.94
Time, early				
$Fmax_{early}$		0.95	1.22	0.60-2.98
$F_{early\ 50}$	days	2.6	2.4	1.6-2.9
$F_{early\ Hil}$		10.0[c]	8.7	3.2-10.0[c]
Time, late				
$Fmax_{late}$		0.28	0.31	0.16-0.53
$F_{late\ 50}$	days	29.6	30.2	21.7-40.8
$F_{late\ Hil}$		2.4	2.4	1.5-4.6
BSV				
CL/F	(CV %)	30	30	19-40
V_1/F	(CV %)	24	27	0.2-99
Q/F	(CV %)	88	75	32-105
V_2/F	(CV %)	58	56	36-76
$F_{late\ Hill}$	(CV %)	105	104	72-139
Correlation				
CL/F ~ Q/F		0.58	0.58	0.03-0.92
BOV				
Relative F	(CV %)	17	16	13-20
k_a	(CV %)	67	60	29-88
Residual variability				
Proportional error	(%)	16.7	17.0	15.3-18.5
Study 2	Factor	0.57	0.56	0.42-0.70
Study 3	Factor	0.72	0.71	0.55-0.87

BS, bootstrap; CI, confidence interval; CL/F, apparent clearance; V_1/F, apparent central volume of distribution; Q/F, apparent intercompartmental clearance; V_2/F, apparent peripheral volume of distribution; F, bioavailability; k_a, absorption rate constant; CYP3A5, cytochrome P450 3A5; $Fmax_{early}$, the maximum increase in F immediately after transplantation; $F_{early50}$, the day with half maximum early effect on F, $Hill_{Fearly}$; shape coeffiecient at early time; F_{late50}, the day with half maximum later effect on F; $Hill_{Flate.}$, shape coefficient at later time; $Fmax_{late}$,maximum value of the late change in F; BSV, between subject variability; BOV, between occasion variability

[a]Standardized to male, CYP3A5 nonexpresser with fat free mass of 60 kg and hematocrit of 45 %, at a time point with the lowest relative bioavailability (e. g. day 4 post transplant).

[b]2.5-97.5 percentile obtained from 500 bootstraps replicates

[c]Upper bound

The final model was parameterized as follows:

$CL/F = 18.8\times (FFM / 60)^{3/4}$ L/h, $V_1/F = 104 \times (FFM / 60)$ L, $Q/F = 29.6\times (FFM / 60)^{3/4}$ L/h, $V_2/F = 411 \times (FFM / 60)$ L

$F = (1 + 0.95 / (1 + (TXT/2.6)^{10})) \times (1 + 0.28 / (TXT/29.6)^{-2.4}) \times 0.50$ (if CYP3A5 expresser) × 0.77 (if female)

FIGURES

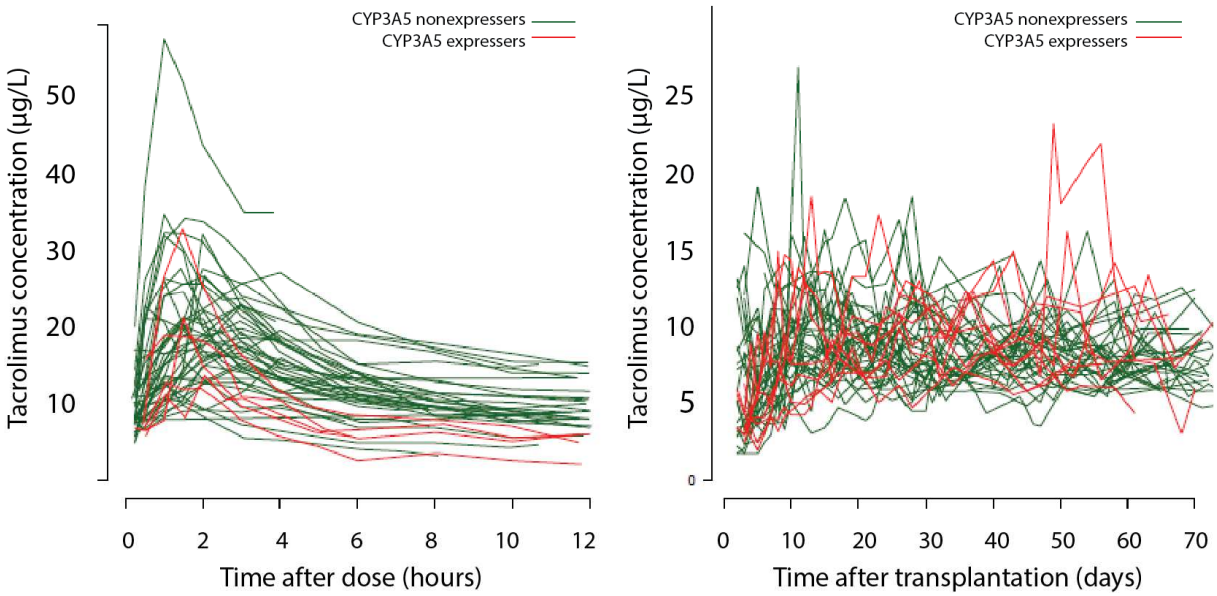


Figure 1. Observed tacrolimus whole blood concentrations during a) a dosing interval (12 hours) at steady state; and b) during the first seventy days after transplantation (only trough concentrations). Both figures are stratified by cytochrome P450 3A5 (*CYP3A5)* genotype.

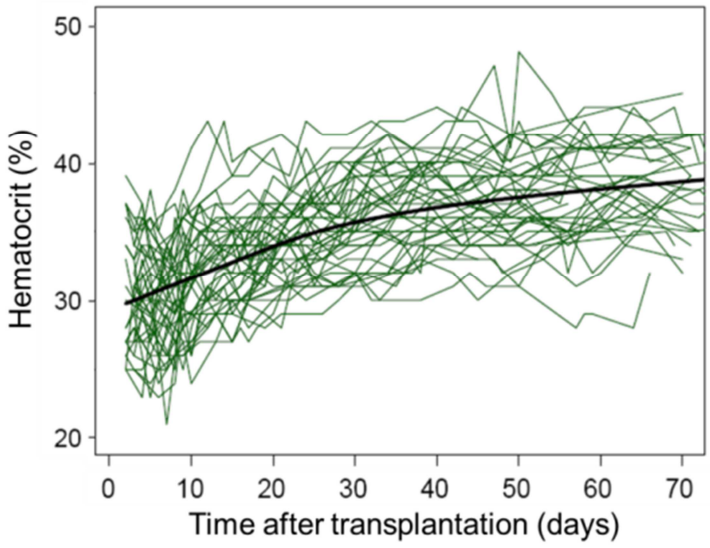


Figure 2. Hematocrit as a function of time after transplantation (n=44, thin lines). A smoother is added to visualize the trend.

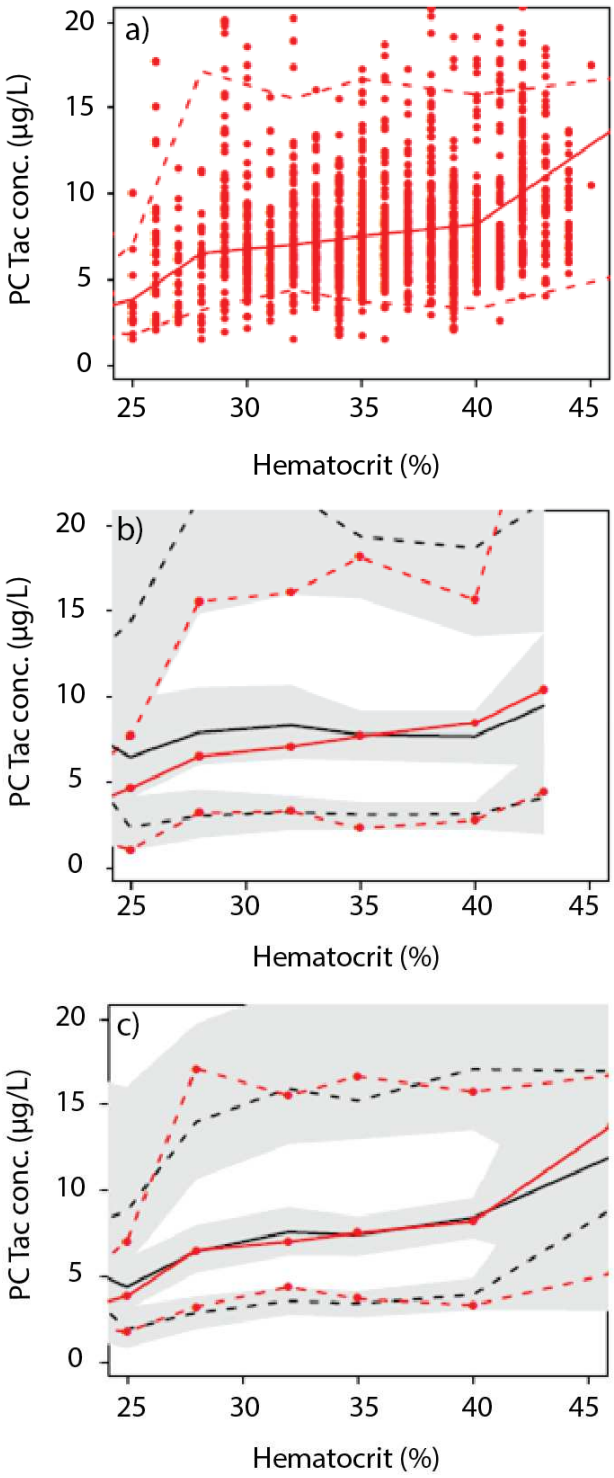


Figure 3. Prediction corrected visual predictive checks over the range of hematocrit. a) Observed whole blood concentrations over the range of hematocrit values; b) Prediction corrected visual predictive check over the range of hematocrit using the base model; c) Prediction corrected visual predictive check over the range of hematocrit using the base model with hematocrit standardized concentrations. PC Tac conc, Prediction corrected tacrolimus whole blood concentration. Solid gray line is median observed blood concentration, dashed red lines are 90% observation interval, solid black line is median predicted blood concentrations, dashed black lines are the 90 % prediction interval. Gray shaded area represents 95 % confidence interval of each prediction interval.

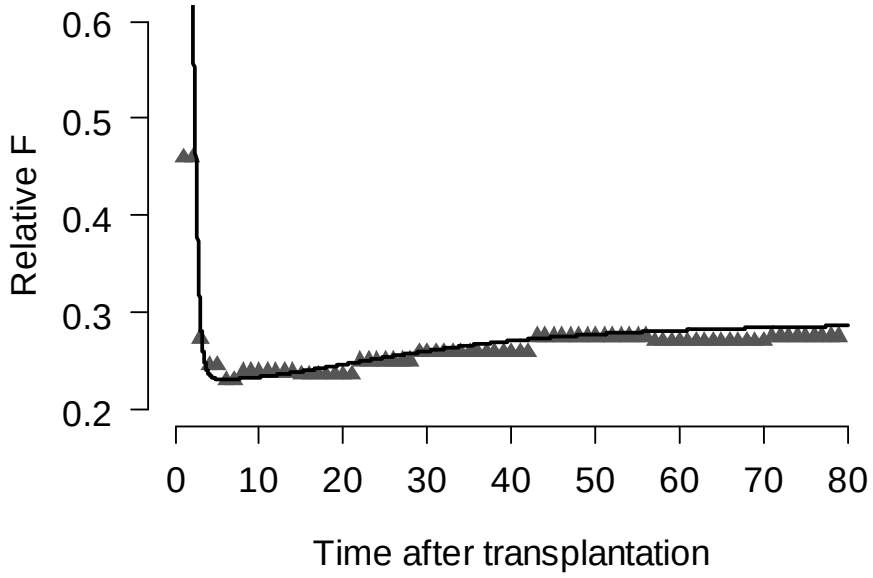
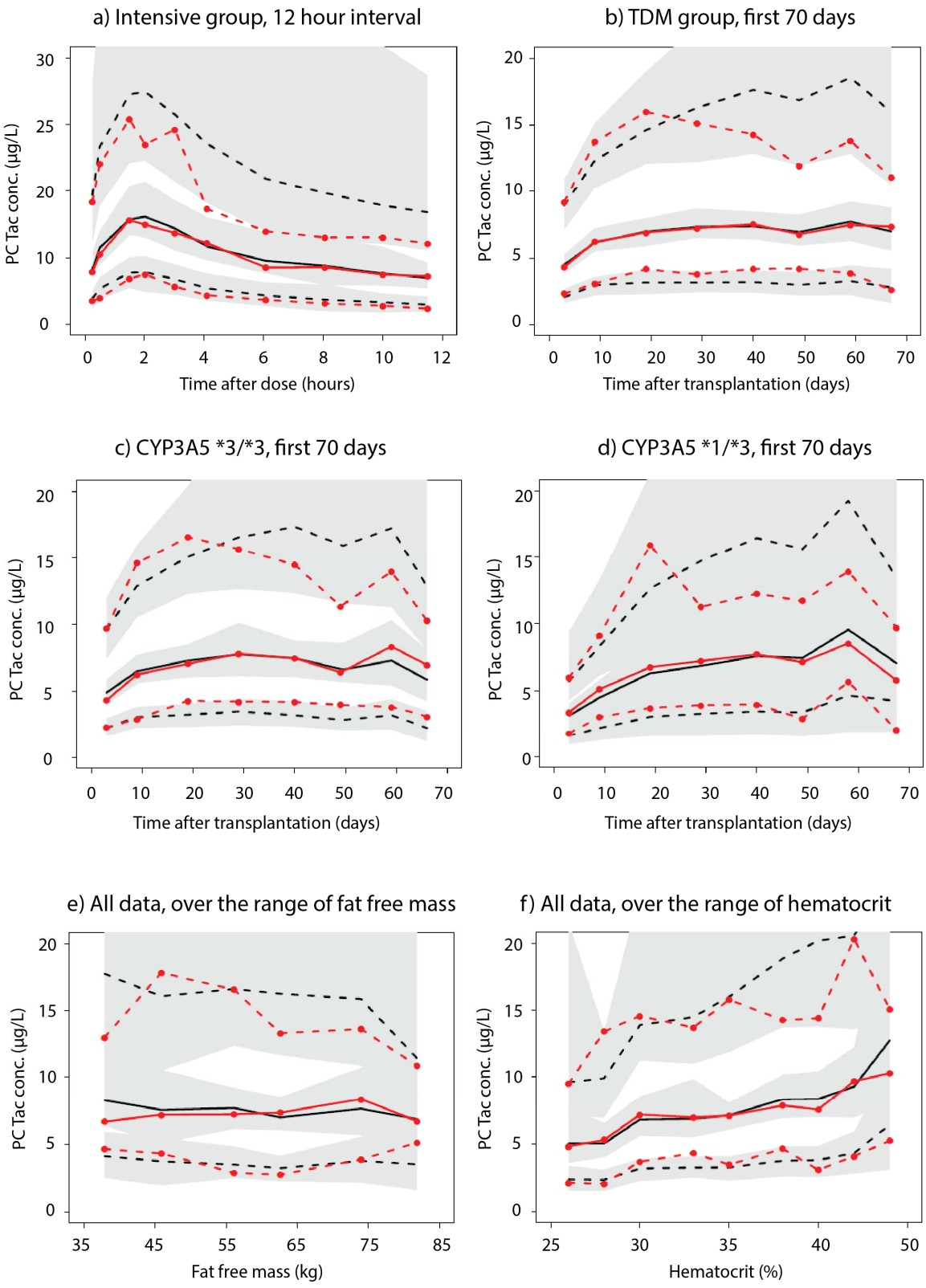


Figure 4. Estimated time course of relative bioavailability (F) after transplantation. Time interval specific means in relative bioavailability are illustrated by triangles. Each triangle represents one day. Baseline bioavailability is set to the literature value of 0.23 [6], and other values of bioavailability are relative to this baseline value. The length of the symbols at the same level represent one pre-defined time-interval. The superposed line represents the model estimated time course of relative bioavailability described by two separate sigmoidal functions (Eq. 8).


a) Intensive group, 12 hour interval
PC Tac conc. (µg/L)
Time after dose (hours)
b) TDM group, first 70 days
PC Tac conc. (µg/L)
Time after transplantation (days)
c) CYP3A5 *3/*3, first 70 days
PC Tac conc. (µg/L)
Time after transplantation (days)
d) CYP3A5 *1/*3, first 70 days
PC Tac conc. (µg/L)
Time after transplantation (days)
e) All data, over the range of fat free mass
PC Tac conc. (µg/L)
Fat free mass (kg)
f) All data, over the range of hematocrit
PC Tac conc. (µg/L)
Hematocrit (%)

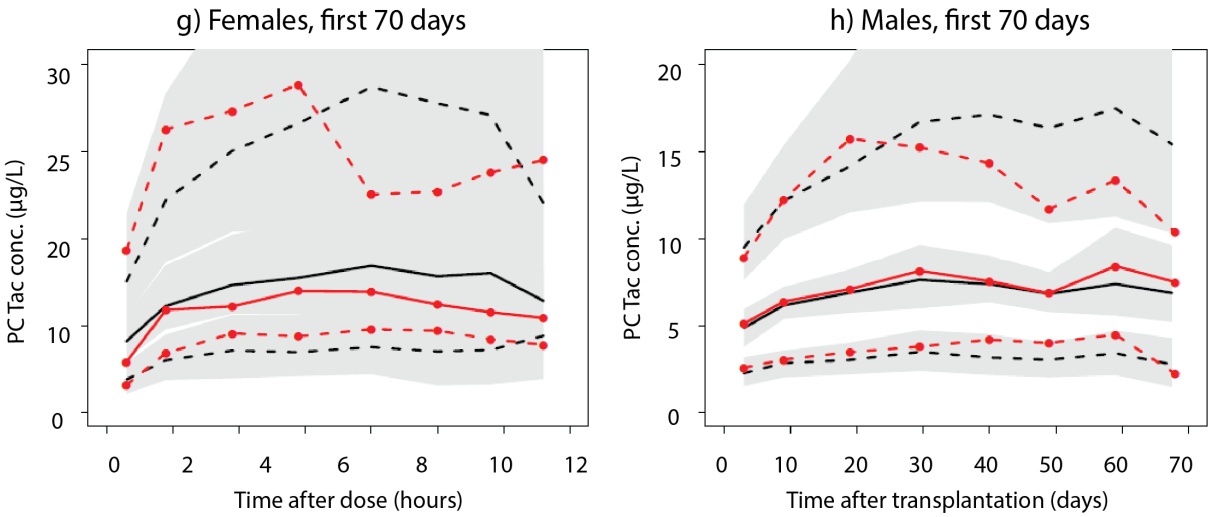


Figure 5. Prediction corrected visual predictive checks of the final model; a) within a 12 hour dose interval of tacrolimus at steady state; b) over the first seventy days after transplantation; c) over the first seventy days after transplantation in CYP3A5 *3/*3 expressers; d) over the first seventy days after transplantation in CYP3A5 *1/*3 expressers; e) in all patients over the range of fat free mass; f) in all patients over the range of hematocrit; g) over the first seventy days after transplantation in females; h) over the first seventy days after transplantation in males. PC Tac conc, Prediction corrected tacrolimus whole blood concentration. Solid gray line is median observed blood concentration, dashed red lines are 90% observation interval, solid black line is median predicted blood concentrations based on 100 simulated datasets, dashed black lines are the 90 % prediction interval based on 100 simulated datasets. Gray shaded area represents 95 % confidence interval of each prediction interval.

3 EXTENDED DISCUSSION ON METHODOLOGICAL CONSIDERATIONS

3.1 STUDY DESIGN

One of the strengths of the nonlinear mixed effects modeling approach is its ability to handle sparse and unbalanced data from different sources. If only trough concentrations had been available, clearance would be the only identifiable parameter [68]. Prior to the study, it was hypothesized that pharmacokinetic information from the twelve-hour pharmacokinetic profiles (Intensive group) would stabilize the sparsely sampled data to be fit to a two-compartment model. The stability of the final model indicated that this was successful. There was, however, a high degree of uncertainty in some of the parameter estimates (Table 4 in Research Article). Whereas CL/F was the most precisely estimated parameter (CV 9 %), the lower precision of the remaining pharmacokinetic parameters (CV 18-26 %) probably reflected the high proportion of trough concentrations [68]. It has been demonstrated in studies evaluating different study designs that trough concentrations are the least informative concentration time point [69], both because they are late in the dose interval and because there is uncertainty in the time passed since dose administration. Thus, although the population approach allows analysis of sparsely sampled data, the results will still suffer from data associated with little information or uncertainty [4].

Another aspect of the study design was the range the covariates were represented at. Body weight was represented at a wide range (44 - 155 kg). In contrast, the distribution of hematocrit values did not cover the upper range, and except from one patient with mean hematocrit of 56 %, mean hematocrit was below 45 % in all patients. In addition, there were relatively few older patients (43 ± 14 years) because cyclosporine A was the preferred calcineurin inhibitor in elderly patients at the time patients were included in the study [34]. Females were also underrepresented (19 out of 69 patients). Extrapolating a model to patients with covariate values outside the range of those evaluated during model development should generally be done with caution [70]. Future studies should particularly include more females, elderly and patients with hematocrit > 45 %.

3.2 HANDLING MISSING DATA

Tacrolimus blood concentrations below quantification limit (BQL) of the analytical assay were discarded. BQL data are left-censored, which means that they are not missing at random. Omitting data not missing at random may lead to biased parameter estimates [71]. Other methods exist for more appropriate handling of such data [72]. However, the methodology is complex and not further discussed here. Only two out of 1548 concentration measurements were BQL. Therefore, one can assume that discarding them had little effect on overall results.

The method for handling missing covariates (last value carried forward/backward) was chosen for its simplicity and because it is commonly used in population pharmacokinetic analyses [7, 73]. In this study, albumin, ASAT, ALAT, ALP and bilirubin were frequently missing, and the method was therefore not optimal. Linear extrapolation of the covariate between the last known value and the next known value or predicting the most likely covariate value from other known covariates would probably be more appropriate to impute missing values for these covariates [74]. If a similar situation is encountered in a future study it should be considered using another method for covariate imputation to increase the probability of establishing true covariate relationships.

3.3 ASSUMPTIONS AND LIMITATIONS IN NONLINEAR MIXED EFFECTS MODELING

Global minimum

Several assumptions are made when population modeling is based on maximum likelihood estimation [29]. One assumption is that the model has found its global minimum (the lowest possible objective function value for that set of parameters) during minimization of "*-2 × log likelihood*". To check whether the final parameter estimates were sensitive to the initial user-defined parameter estimates, the model parameters were re-estimated after increasing and decreasing the initial estimates by 25 % [29]. The parameter estimates were similar in both cases with identical OFV. This was a strong indication that the proposed model was at global minimum [7]. However, this is never guaranteed.

Residual error model

The residual errors (predicted concentrations subtracted from observed concentration) were assumed to be normally distributed with mean zero [29]. To assess this assumption, a histogram of the conditional weighted residuals (CWRES) was created (Figure 3-1). The plot indicated that the residuals were normally distributed and that the selected residual error model was appropriate. CWRES did not systematically change over time after transplantation (Figure 3-2, left). However, CWRES versus predicted concentrations showed a slight bias towards overprediction at predicted concentrations above 10-15 µg/L (Figure 3-2, right). It appeared from a closer look that the trend was driven by about 20 lower tacrolimus concentrations observed mainly in two patients with unexplained higher values of individual clearance (Patient 216 and 313). Therefore, this slight bias does not seem to reflect a major model misspecification.

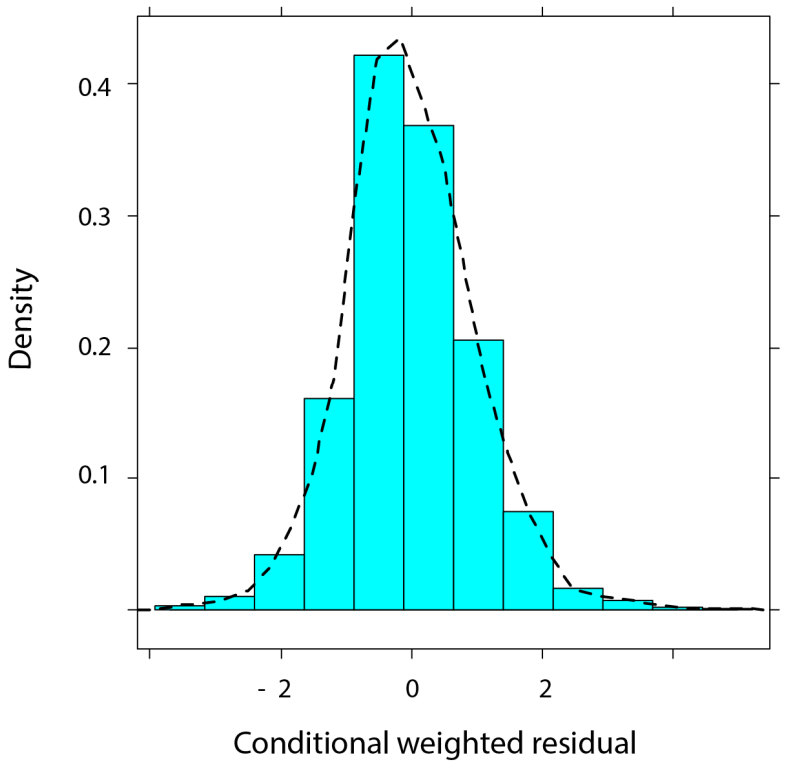


Figure 3-1. Distribution of conditional weighted residuals, superimposed with a dashed line showing the normal distribution.

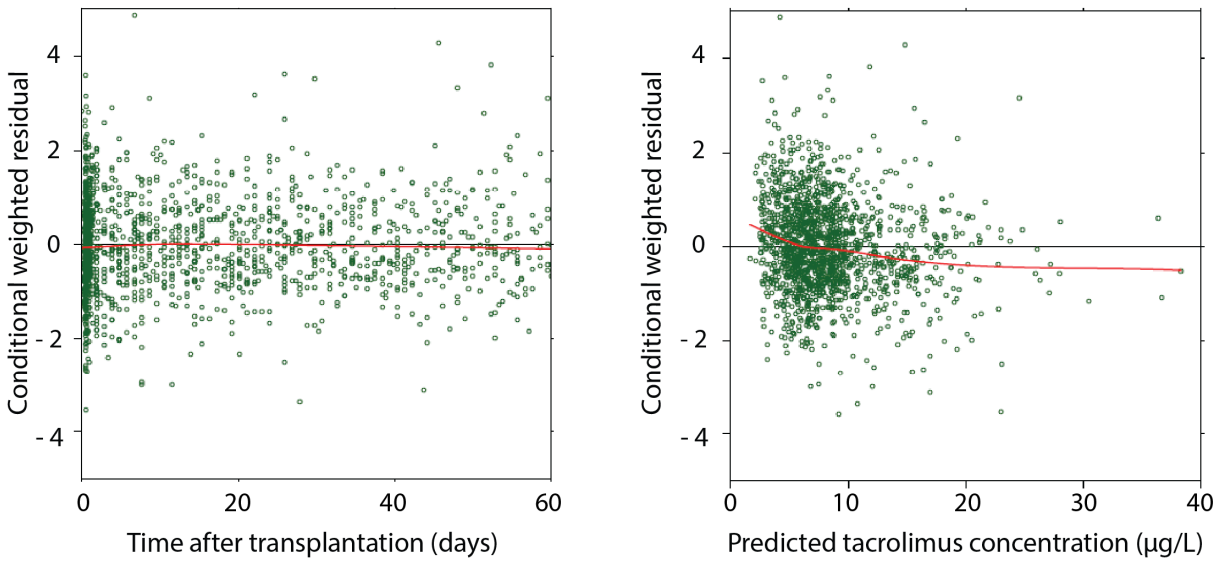


Figure 3-2. Residual error plots. *Left panel*: Conditional weighted residuals versus time. *Right panel*: Conditional weighted residuals versus predicted tacrolimus concentrations.

Between subject variability model

The distribution of individual pharmacokinetic parameters were assumed to be log normally distributed with mean zero. Frequency distributions of the Bayesian estimates of the individual pharmacokinetic parameters (expressed as η's) were generated in order to test this assumption (Figure 3-3) [29]. In addition, the means of these estimates and the associated p-value for the probability that the mean was different from zero were calculated by NONMEM [12]. All the parameter means were close to zero ($< \pm 0.02$) with associated p-values > 0.05, and the parameters were symmetrically distributed around the value of zero, indicating that the assumption held.

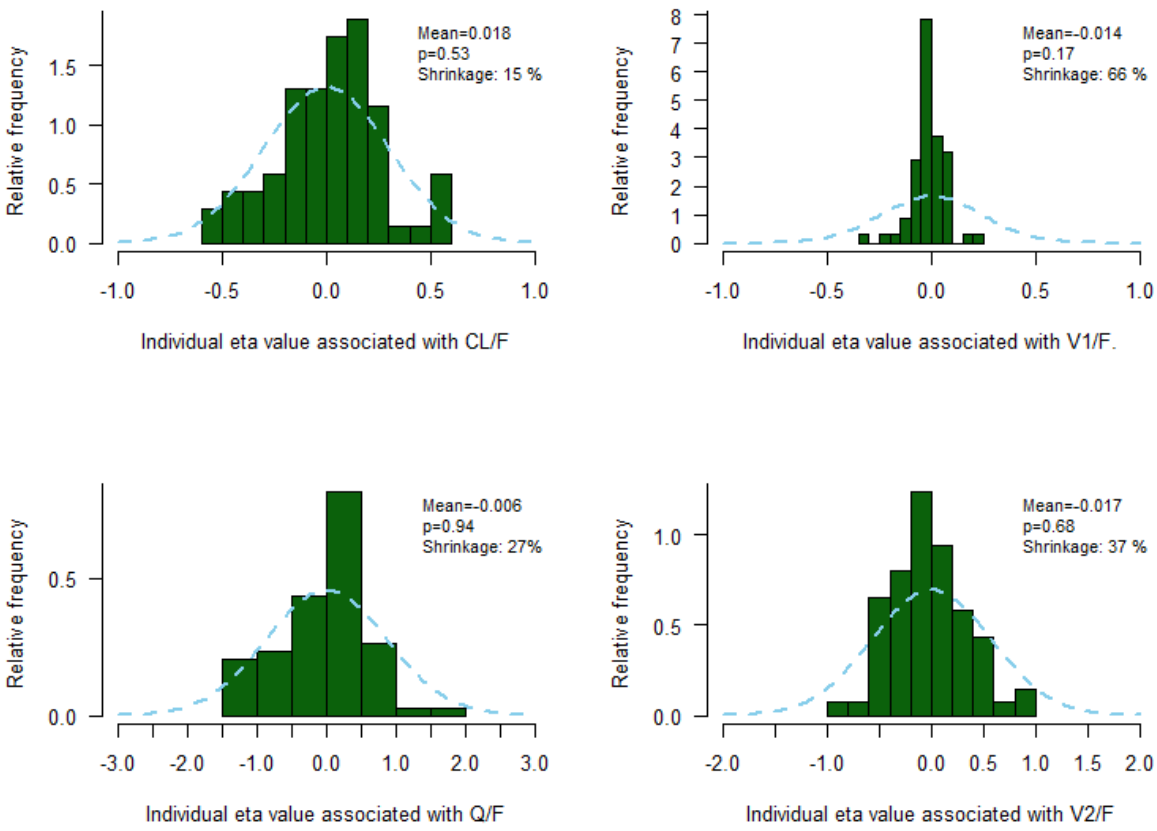


Figure 3-3. Frequency distribution of the individual eta values of the Bayesian individual estimates of the disposition parameters: Apparent clearance (CL/F), apparent central volume of distribution (V1/F), apparent intercompartmental clearance (Q/F) and apparent peripheral volume of distribution (V2/F), respectively (green bars). Blue dashed lines are superimposed and show the estimated normal distribution of the corresponding etas in the population according to the final model. Shrinkage is calculated by dividing the standard deviation of the EBEs by the estimated population standard deviation (these values are also given by NONMEM). Means are expressed in each plot with an associated p-value. The p-value is calculated by NONMEM and is given for the null hypothesis that the true value is different from 0.

3.4 RELIABILITY OF THE INDIVIDUAL PHARMACOKINETIC PARAMETER ESTIMATES

The greatest advantage of using the Bayesian method to estimate the individual pharmacokinetic parameters is that samples may be drawn at any time point, without being restricted to for example exactly two hours after dose [75]. This allows flexibility in a clinical setting. In addition, the number of required samples for therapeutic drug monitoring are normally reduced when Bayesian Estimation is applied, which leads to lower costs and less discomfort for patients [64]. However, not all sampling time points contain equal amounts of pharmacokinetic information as feedback [76]. A great limitation of Bayesian estimation is

shrinkage of the individual estimates towards the population mean values when the concentrations are sparse or measured at uninformative time points [19]. In Figure 3-3 it is illustrated that the standard deviations for the distributions of the individual parameter estimates were smaller than the standard deviation for the estimated population variability. The amount of shrinkage was quantified by calculating the ratio between these standard deviations [7]. CL/F was the parameter with lowest degree of shrinkage (15 %). Accurate estimation of CL/F is important because CL/F determines the required dosing rate to achieve a steady state target concentration [3]. In contrast, considerable shrinkage (66 %) was seen for V_1/F, probably reflecting the lack of information about V_1/F in samples drawn late in the dosage interval. Shrinkage > 25 % in the remaining disposition parameters (Q/F, V_2/F) is also considered high [19]. These individual pharmacokinetic parameter estimates are therefore not reliable. The optimal sampling time points after dose to obtain higher quality of the Bayesian individual parameter estimates should be investigated [76].

3.5 CHOICE OF COVARIATES IN THE FINAL MODEL

Type I error rate

Type I error rate describes to the risk of including a covariate in the model when the covariate is in fact not a true covariate [70]. The least significant covariate in the final model was the effect of sex on bioavailability (p=0.012, Table 3 in Research Article). One of the main limitations of the likelihood ratio test is that ΔOFV is only approximately χ^2-distributed. Significance levels obtained by the likelihood ratio test are therefore not exact and are called nominal significance levels [77]. The p-value is defined as the probability of finding a relationship, given that the null hypothesis (no relationship with the covariate) is true [7]. If ΔOFV is large and the associated p-value very small, the approximate results of the likelihood ratio test are sufficient. However, the p-value for the effect of sex on bioavailability in the presented model is questionable. A randomization test was performed to calculate the actual p-value. For this test, 1000 data subsets were generated. These were identical except from a random permutation of the sex characteristics between the individuals in each subset to remove the potential covariate effect. The model control stream allowing an effect of sex was fit for each data subset and ΔOFV was calculated. Subsequently, all the results were ranked in the order of ΔOFV [77]. The two original nested models had a difference in OFV of 6.4. This value was fund associated with a percentile in the ranked ΔOFVs. The results showed that

ΔOFV was greater than 6.4 in 25 of the 1000 runs, corresponding to p=0.025, slightly higher than the nominal value (Figure 3-4).

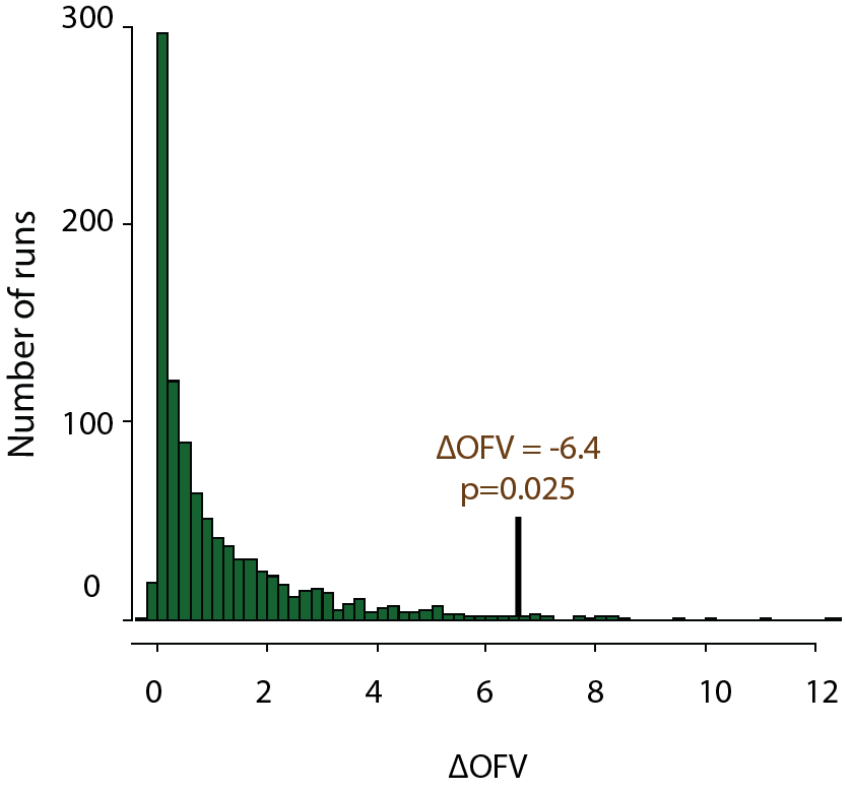


Figure 3-4. Randomization test for the effect of sex on bioavailability. Distribution of the difference in objective function from 1000 simulated datasets that were fit to the final model and compared with the model without a sex effect on bioavailability. The original difference of 6.4 was associated with an actual significance level of p=0.025

To reduce the risk of including false covariates, a more stringent significance criterion of p<0.01 has been suggested during backward elimination of covariates [7]. If the more stringent significance criterion was selected in this study, sex would be statistically rejected as a covariate. However, biological plausibility and improvement in plots of goodness of fit supported retaining sex as a covariate in the final model, and bias was evident over the range of weight without it (appendix E.6). This emphasizes the importance of not relying solely on statistical significance in model selection and also demonstrates how model selection is affected by subjective judgment. It was apparent from the visual predictive check (Figure 5g in Research Article) that tacrolimus concentrations in females were still overpredicted. This unexplained overprediction seems to be the greatest weakness of the model. More females should be included in a future modeling dataset to investigate further the true effect of sex on tacrolimus pharmacokinetics.

Type II error rate

Type II error rate describes the risk of rejecting a true covariate [70]. Prednisolone, albumin concentration and liver function tests are examples of rejected covariates that are biologically plausible and were identified as covariates in literature [45, 55, 61, 78]. If these covariates are true covariates, some factors may have contributed to failure of establishing a relationship. First, when the effect of a covariate on a pharmacokinetic parameter is expected to occur after a time delay, the traditional methods for covariate inclusion will not capture the relationship well [12]. Covariates such as corticosteroids or cytokines are potential covariates by induction of CYP3A4 transcription, which is a delayed process. Although methods exist to take such delays into account [79], this was not attempted in the present study due to the complexity of such methodology. In addition, prednisolone pharmacokinetics vary between individuals [80], and the interaction potential is probably not reflected well by dose. All patients received similar prednisolone dosage regimens. To distinguish between the relative underlying effects of prednisolone and other factors varying with time after transplantation, patients on corticosteroid-free immunosuppressive regimens should be included in future studies.

Although the liver function markers (serum albumin, serum bilirubin, ASAT, ALAT, ALP) tend to increase in liver disease, they are affected differently dependent on type of liver disease and are not good measures of the liver's drug metabolizing capacity [81]. In a previous study, patients with moderate to severe reduced liver function were identified with lower clearance of tacrolimus [82]. In this study, it was attempted to model liver disease as a dichotomous covariate by defining a liver function test value above 1.5 times the upper normal limit as '1' and otherwise '0'. This approach was used in a model for nevirapine which is a drug similar to tacrolimus with respect to pharmacokinetic properties [83]. However, no relationship was revealed. Importantly, these results are not evidence of that tacrolimus metabolism is not altered during liver disease. An overview of all evaluated covariate relationships is shown in appendix F.

Error in covariates

Covariates were treated as if they are measured without error. However, manual transfer of data from patient charts to a computer might introduce errors. In addition, some covariates may be measured imprecisely, such as laboratory values or genotype [29]. Moreover, fat free mass was not measured, but predicted from total body weight, height and sex (appendix A)

[84]. Although the employed equation is acknowledged to perform accurate predictions of fat free mass, it has not been evaluated in transplant patients. During the initial year following kidney transplantation, the proportion of fat in patients increases (fat mass content 25.8 to 31.2 %, $p<0.001$) [85]. Determining fat free mass more accurately is based on advanced techniques using bone mineral density, which are less available in clinical practice [86]. Nevertheless, small errors in covariates are not expected to contribute to false covariate selection and probably contribute little to the overall variability.

Factors not accounted for

Although some of the prior unexplained variability was explained by covariates, the unexplained variability was large also subsequent to covariate inclusion. For example, BSV in the asymptotic value of individual bioavailability was 104 %. Some factors are known to contribute to the overall variability, but cannot be easily quantified. For example, when patients experience a diarrhea episode, tacrolimus concentrations are observed to increase, probably due to damaged enterocytes and decreased presystemic metabolism [87]. Diarrhea episodes occur frequently in kidney transplant patients as adverse reactions to tacrolimus or mycophenolate mofetil [87]. However, such episodes are usually not reported by the patient or recorded by the clinician and diarrhea is therefore difficult to assess as a covariate. In addition, bioavailability may decrease in presence of food [88] or antacids [89]. Although patients are informed to fast, eating habits are normally not controlled in the outpatient setting. Finally, dosing history errors in the dataset due to non-compliant patients is expected to be an important contributor to the residual error [7].

Practical aspects

The applicability of presented model is limited by fact that not all transplant centers have the opportunity to perform CYP3A5 genotyping. Sex, height, total body weight and hematocrit on the other hand are factors that are normally readily available. All the model components seem to be clinically relevant, because each covariate leads to dose alterations of at least 30 % within the expected covariate range. It is however questionable whether it is clinically relevant to account for the early time effects in the model. In fact, it may be beneficial that a higher extent of the dose is absorbed during the first days after transplantation. In practice, it

would be similar to administration of a loading dose. These aspects should be further considered before clinical application.

3.6 EXAMPLE OF INDIVIDUAL GOODNESS OF FIT

The patient with the most inaccurate population predicted concentrations was a male CYP3A5 nonexpresser. The concentration-time profile of this patient is shown in Figure 3-5 (subsequent to the initial five dose intervals, the predictions within the dose intervals are omitted for easier interpretation). Observed tacrolimus concentrations during the first week after transplantation varied between 1.5 and 2.5 µg/L and were consistently below the acceptable concentration range (3-7 µg/L). The clinicians did not establish the individual dose requirement of 16 mg daily before one week had passed. The Bayesian estimate of individual CL/F was 33 L/h, almost twice as high as the population predicted value of CL/F in this patient. If this estimate of individual CL/F had been available to the clinicians, it would encourage a more rapid increase in dosage and potentially prevented consistent underexposure during the critical initial post-transplant week. This figure emphasizes the importance of revising the individual parameters when tacrolimus concentrations become available as feedback to the model. Additional examples of individual goodness of fit plots are provided in appendix D.

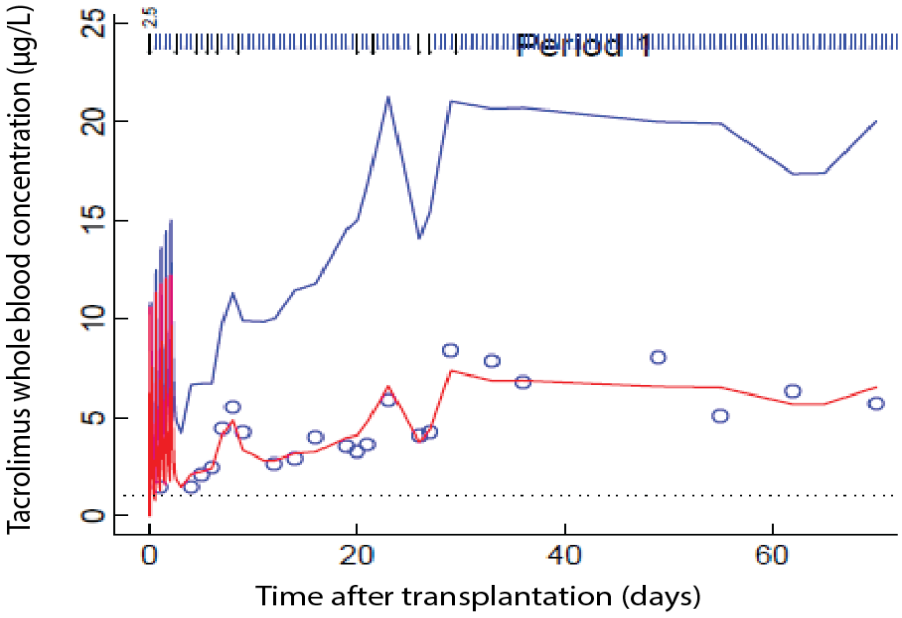


Figure 3-5. Example of individual goodness of fit. ID 44 is a 47 year old male CYP3A5 nonexpresser receiving 2.5 mg of tacrolimus twice daily as initial dose. The individual dose requirement was 8 mg twice daily. Circles represent measured tacrolimus concentrations. The blue line reflects the population predicted concentrations. The red line reflects the individually predicted concentrations. The arrows on top represent administered doses.

3.7 SUBJECTIVITY IN MODEL DEVELOPMENT

A number of subjective considerations and choices were made during model development. Both evaluation of goodness of fit plots and biological plausibility are highly subjective processes. The main results from the modeling process are presented in appendix E. All models are wrong, but some are useful [7]. Several models could probably be developed from the dataset in the present study, and several models may be useful for the purpose of dose individualization of tacrolimus. The presented work suggests one of these.

4 FUTURE DIRECTIONS

Individual dosing based on Bayesian estimation of individual pharmacokinetics has been found superior to traditional therapeutic drug monitoring for several drugs with narrow therapeutic windows, including vancomycin [90], aminoglycosides [91], antiretroviral drugs [92], phenytoin [93] and cyclosporine [94]. However, before the present model can be clinically applied for tacrolimus, an external validation procedure must be performed [9]. Information on hematocrit, *CYP3A5* genotype, fat free mass, sex, dosing history and measured drug concentrations must be collected in a group of new patients to generate an external validation dataset. The model's ability to predict the initial concentrations should be compared with the predictions of the current initial dose algorithm based on total body weight. The model control stream and an example of the dataset structure are provided in appendix G and H, respectively. Next, to evaluate the usefulness of the model as a dose adjustment tool, a randomized prospective study should be performed [94]. For example, one group may receive tacrolimus doses guided by traditional therapeutic drug monitoring while the other group receives doses as predicted by the model. Comparison of the observed tacrolimus concentrations between the groups will demonstrate whether patients receiving model-based individual dosing are reaching the target concentration more rapidly and whether the target concentration is more precisely maintained. If so, the model may be clinically applied to improve individual dosing of tacrolimus.

To date, eleven population pharmacokinetic models have been published for tacrolimus in kidney transplanted adults. In the end, pharmacokinetics by itself is of little value if pharmacodynamics and clinical effects are poorly reflected by the pharmacokinetics. Monitoring of biomarkers reflecting the immunosuppressive effect has been proposed as a next step towards individualized treatment, although not currently clinically available [95]. Future population modeling of tacrolimus should aim to integrate pharmacokinetic and pharmacodynamic biomarkers in a joint pharmacokinetic and pharmacodynamic (PK/PD) model. The goal of all modeling is to improve patient care. Developing a population pharmacokinetic model is an enjoyable process and might lead to a useful dose adjustment tool. We are, however, not successful before the population model leads to observable clinical effects in terms of fewer rejections, longer survival times and healthier patients.

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APPENDICES

APPENDIX A. Algorithm for prediction of fat free mass

Fat free mass (FFM, Janmahasatian et al. [84])

$$FFM, male\ (kg) = \frac{9\ 270\ \times Total\ body\ weight\ (kg)}{6\ 680 + 216\ \times BMI}$$

$$FFM, female\ (kg) = \frac{9\ 270\ \times Total\ body\ weight\ (kg)}{8\ 780 + 244\ \times BMI}$$

Body mass index (BMI)

$$BMI\ \left(\frac{kg}{m^2}\right) = \frac{Total\ body\ weight\ (kg)}{Height(m^2)}$$

APPENDIX B. Frequency distribution of bootstrap parameter estimates

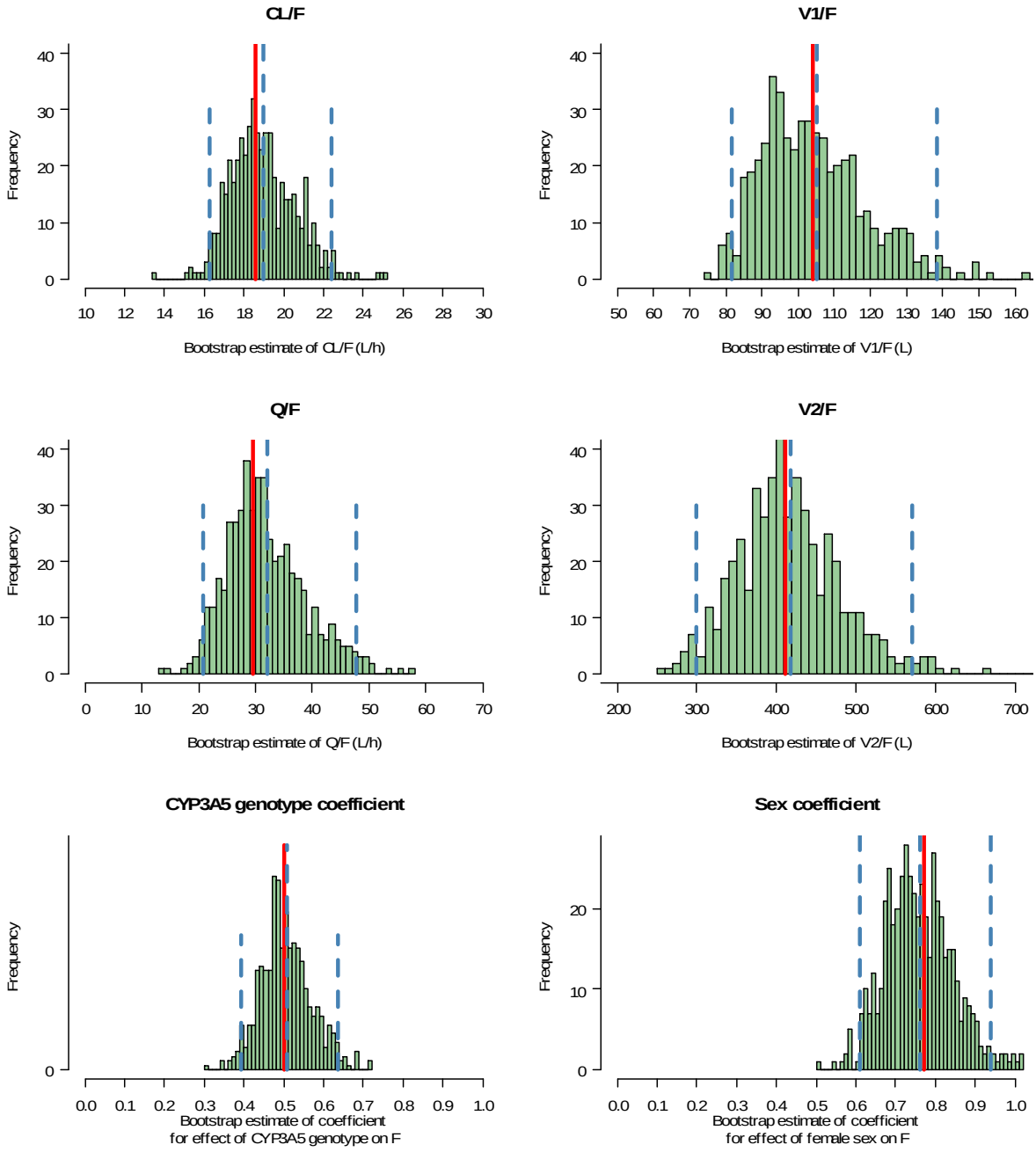


Figure B-1. Frequency distribution of the results from the bootstrap procedure of 500 dataset replications, showing the distribution of the typical estimates of apparent clearance (CL/F), apparent central volume of distribution (V_1/F), apparent intercompartmental clearance (Q/F) and apparent peripheral volume of distribution (V_2/F) and the covariate coefficients of the effect of CYP3A5 genotype and sex on bioavailability (F). The 2.5th, 97.5th percentiles and the mean are shown in blue dashed lines. Red solid line marks the parameter estimate of the final model.

APPENDIX C. Traditional goodness of fit plots

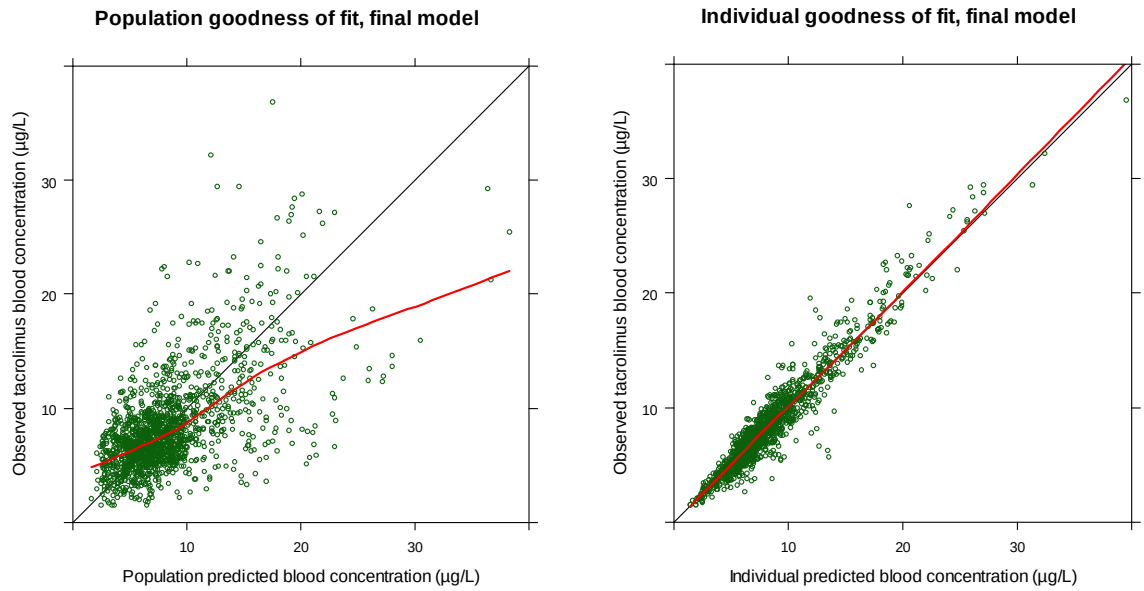
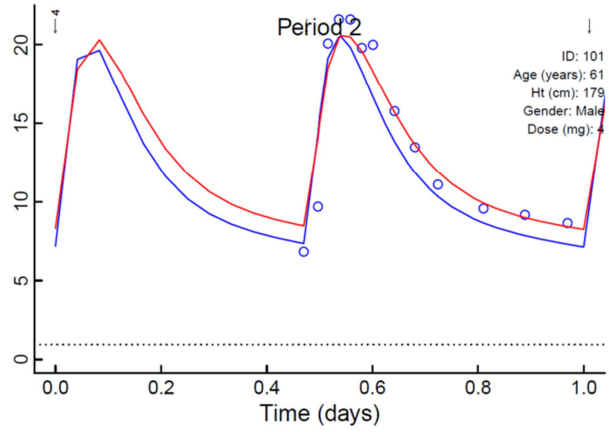
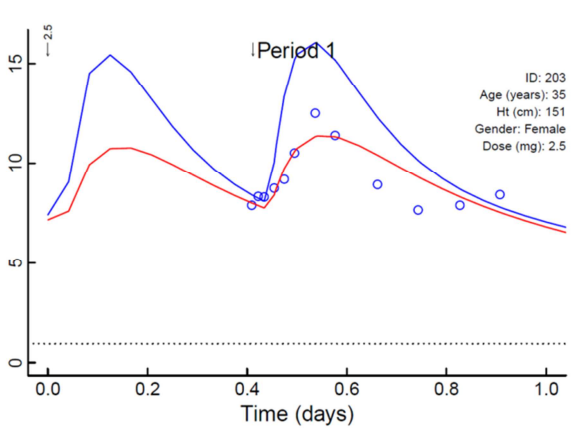


Figure C-1. Left panel: Population predicted concentration versus observed concentration. Right panel: Individual predicted concentration versus observed concentration. Green circles represent a measured tacrolimus whole blood concentration. The diagonal line is the line of unity. The red line is a smoother to show the general trend around the line of unity.

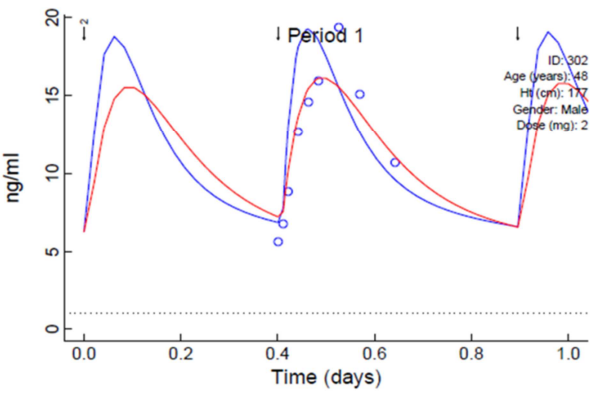
APPENDIX D. Individual goodness of fit in selected individuals



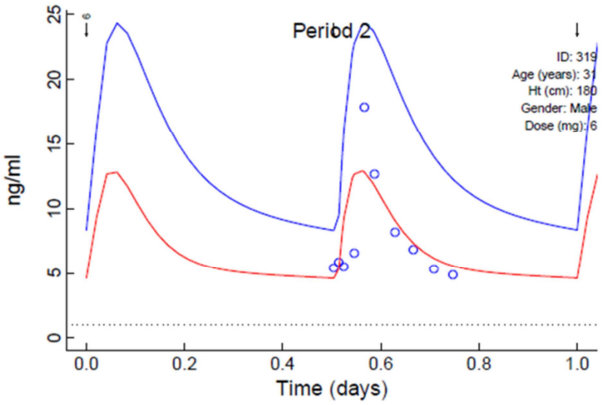
ID 101. 61 year-old male CYP3A5 nonexpresser receiving 4 mg of tacrolimus twice daily



ID 203. 35 year-old female CYP3A5 nonexpresser receiving 2.5 mg of tacrolimus twice daily

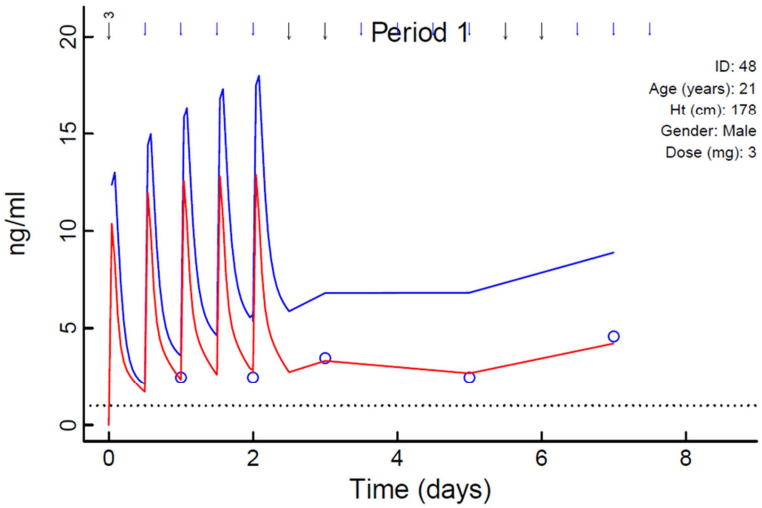


ID 302. 48 year-old male CYP3A5 nonexpresser receiving 2 mg of tacrolimus twice daily

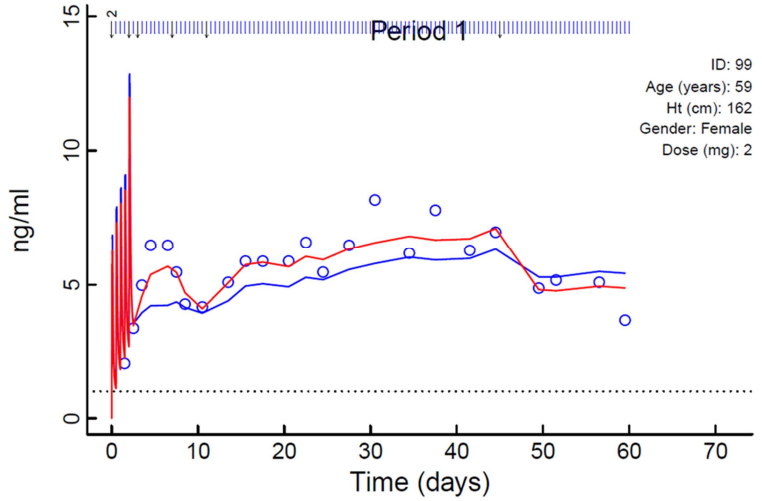


ID 319. 31 year-old male CYP3A5 expresser receiving 6 mg of tacrolimus twice daily

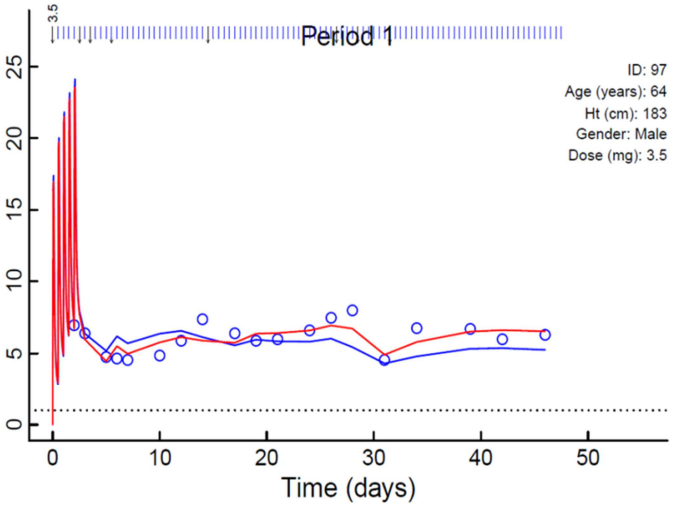
Figure D-1. Examples on goodness of fit at the individual level, represented by the first individual in each sub-study from which full pharmacokinetic profiles were obtained. One CYP3A5 expresser from sub-study 3 is also shown to represent an individual with poorly population predicted concentrations, emphasizing that some patients are different on the individual level compared to the group level due to unexplained variability. Circles represent observed tacrolimus concentrations. Blue line represents the group prediction using population pharmacokinetic parameters and covariates. Red line represents the individual prediction using individual parameters.



ID 48. 21 year-old male CYP3A5 nonexpresser receiving 3 mg of tacrolimus twice daily as initial dose



ID 97. 59 year-old female CYP3A5 expresser receiving 2 mg of tacrolimus twice daily as initial dose



ID 97. 64 year old male CYP3A5 expresser receiving 3.5 mg of tacrolimus twice daily as initial dose

Figure D-2. Examples showing the individual fit, represented by randomly selected subjects from sub-study 4 (TDM group) representing males, females, CYP3A5 expressers and nonexpressers at different age groups and tacrolimus dose requirements. The predictions during the first five dose intervals are fully illustrated, followed by only prediction of trough concentration. In ID 48 the predictions during the first post-transplant week is shown. ID 97 and 99 are examples of that in some patients the the trough concentrations are predicted well by both using population parameters and individual parameters.

APPENDIX E. Supplemental material for decision-making during model development

E.1. Development of the structural model

Table E.1 shows the results from the development of the structural model. Only key models referred to in the research article are provided.

TABLE E.1. Structural model development summary.

Model nr.	Compart-ment model	Absorption model	Random effects	OFV	Compared to model nr.	Number of parameters removed	ΔOFV
Absorption model							
1	2 comp.	First order absorption with *lag time*	*BOV*: All *BSV*: All	3148.9			0
2	2 comp.	First order absorption	*BOV*: All *BSV*: All	3343.4	1	+ 1	+ 194.5
3	2 comp.	Zero order absorption	*BOV*: All *BSV*: All	3530.0	1		+ 381.1
Number of compartments							
4	1 comp	First order absorption with lag time	*BOV*: All *BSV*: All	3620.2	1	- 6	+ 471.4
Random effects reduction and correlation matrix							
5	2 comp.	First order absorption with lag time	*BOV*: K_a, F, *BSV*: All	3156. 2	1	- 4	+ 7.3
6	2 comp.	First order absorption with lag time	*BOV*: K_a, F, *BSV*: CL/F, V1/F, Q/F, V2/F, F	3159.6	5	- 1	+ 3.4
7 Struct-ural model	**2 comp.**	**First order absorption with lag time**	***BOV*: K_a, F, *BSV*: CL/F, V1/F, Q/F, V2/F Correlation matrix between CL/F, V1/F, Q/F, V2/F**	**3133.0**	**6**	**- 1 +4**	**- 26.6**

Comp, compartments; OFV, Objective function value; CL/F, apparent clearance; V_1/F, apparent central volume of distribution; Q/F, apparent intercompartmental clearance; V_2/F, apparent peripheral volume of distribution; F, bioavailability; k_a, absorption rate constant; BSV, between subject variability; BOV, between occasion variability

E.2. Parameter estimates and goodness of fit for the structural model

Table E.2 shows the parameter estimates of the structural model and Figure E-2.1 and E-2.2 show prediction corrected visual predictive check of the structural model over the range of time after transplantation and hematocrit, respectively.

TABLE E.2. Parameter estimates, bootstraps means and 95 % confidence intervals of the parameter of the base model

Parameter		Model estimate	BS mean	BS 95 % CI[a]
CL/F	(L/h)	20.4	20.6	18.0-23.2
V_1/F	(L)	177	184	142-249
Q/F	(L/h)	18.3	17.9	13.6-23.8
V_2/F	(L)	1820	2022	1190-3242
K_a	(h^{-1})	1.41	1.42	0.91-2.12
$K_{astudy\ 2}$	(h^{-1})	0.47	0.81	0.34-2.32
Lagtime	(h)	0.22	0.23	0.20-0.25
$Lagtime_{study\ 2}$	(h)	0.80	0.82	0.73-0.89
BSV				
CL/F	(%)	48	47	38-56
V_1/F	(%)	65	64	46-82
Q/F	(%)	82	81	60-98
V_2/F	(%)	138	134	90-175
Correlations				
CL/F ~ V_1/F		0.77	0.65	0.03-0.92
CL/F ~ Q/F		0.50	0.53	0.41-0.82
CL/F ~ V_2/F		0.38	0.26	-0.18-0.59
V_1/F ~ Q/F		-0.11	-0.16	-0.50-0.27
V_1/F ~ V_2/F		0.54	0.73	0.22-0.96
Q/F ~ V_2/F		0.17	-0.15	-0.52-0.33
BOV				
Relative F	(%)	17	18	14-23
k_a	(%)	81	89	65-131
Residual variability				
Proportional error	(%)	16.4	16.1	13.4-19.0
Additive error	µg/L	0.30	0.40	0.05-0.57
Study 2	Factor	0.53	0.55	0.41-0.73
Study 3	Factor	0.68	0.66	0.53-0.83
Study 4	Factor	1.00	1.00	0.84-1.18
BSV	(%)	13.6	10.5	0.001-0.22

BS, bootstrap; CI, confidence interval; CL/F, apparent clearance; V_1/F, apparent central volume of distribution; Q/F, apparent intercompartmental clearance; V_2/F, apparent peripheral volume of distribution; F, bioavailability; k_a, absorption rate constant; BSV, between subject variability; BOV, between occasion variability

[a]2.5-97.5 percentile obtained from 500 bootstraps replicates

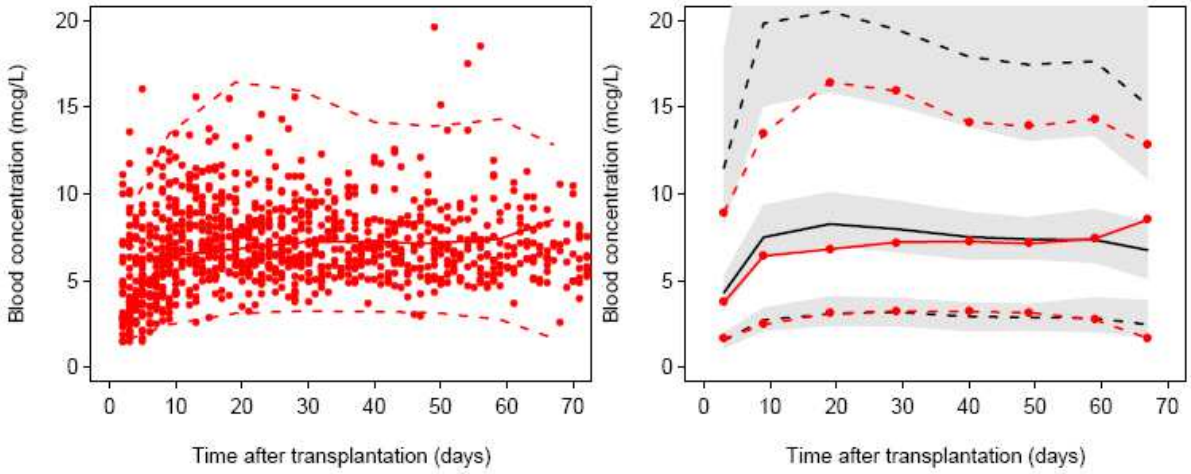


Figure E-2.1. Prediction corrected visual predictive checks of the structural model. The concentrations are systematically overpredicted during the first 40 days. *Left panel*: All observed tacrolimus concentrations over time after transplantation. Solid red line is median observation in each time interval, consisting of 10 days. Dashed red lines are the 90 % observation interval. *Right panel*: Red solid and dashed line are identical to left panel. Observations are excluded for easier visual interpretation. Solid black line is median predicted concentration, dashed black lines are the 90 % prediction interval. Gray shaded area represents 95 % confidence interval of each prediction interval.

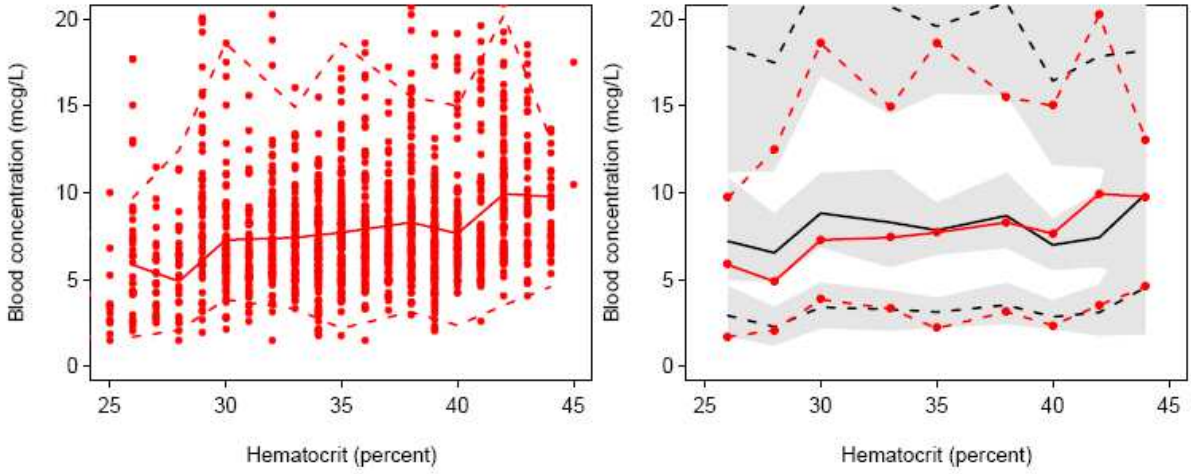
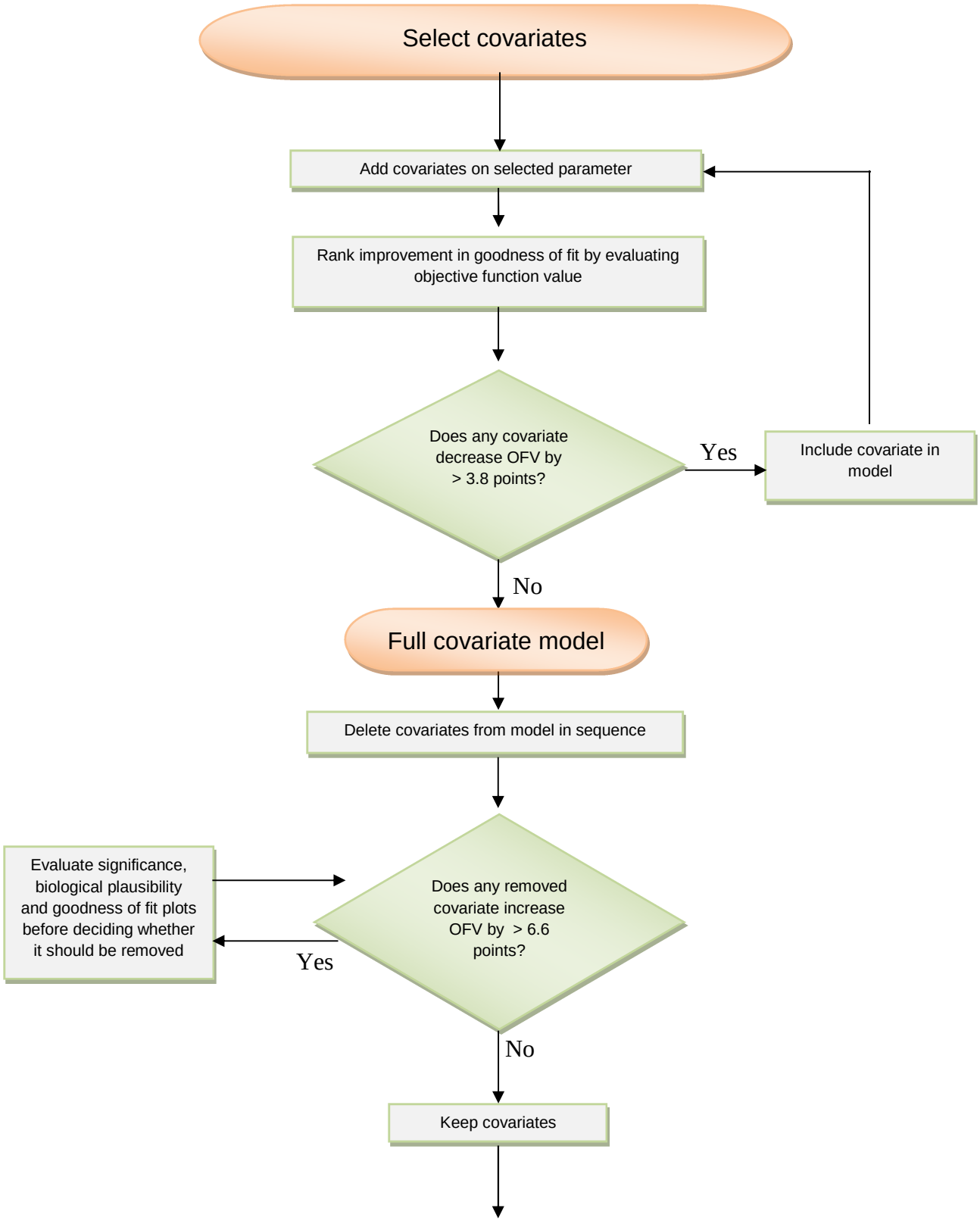


Figure E-2.2. Prediction corrected visual predictive check of the structural model. The concentrations are systematically overpredicted at low hematocrit (< 35 %) and high hematocrit (>40 %) values. For graphical interpretation, see Figure E.2-1.

E.3. Flowchart of covariate model development



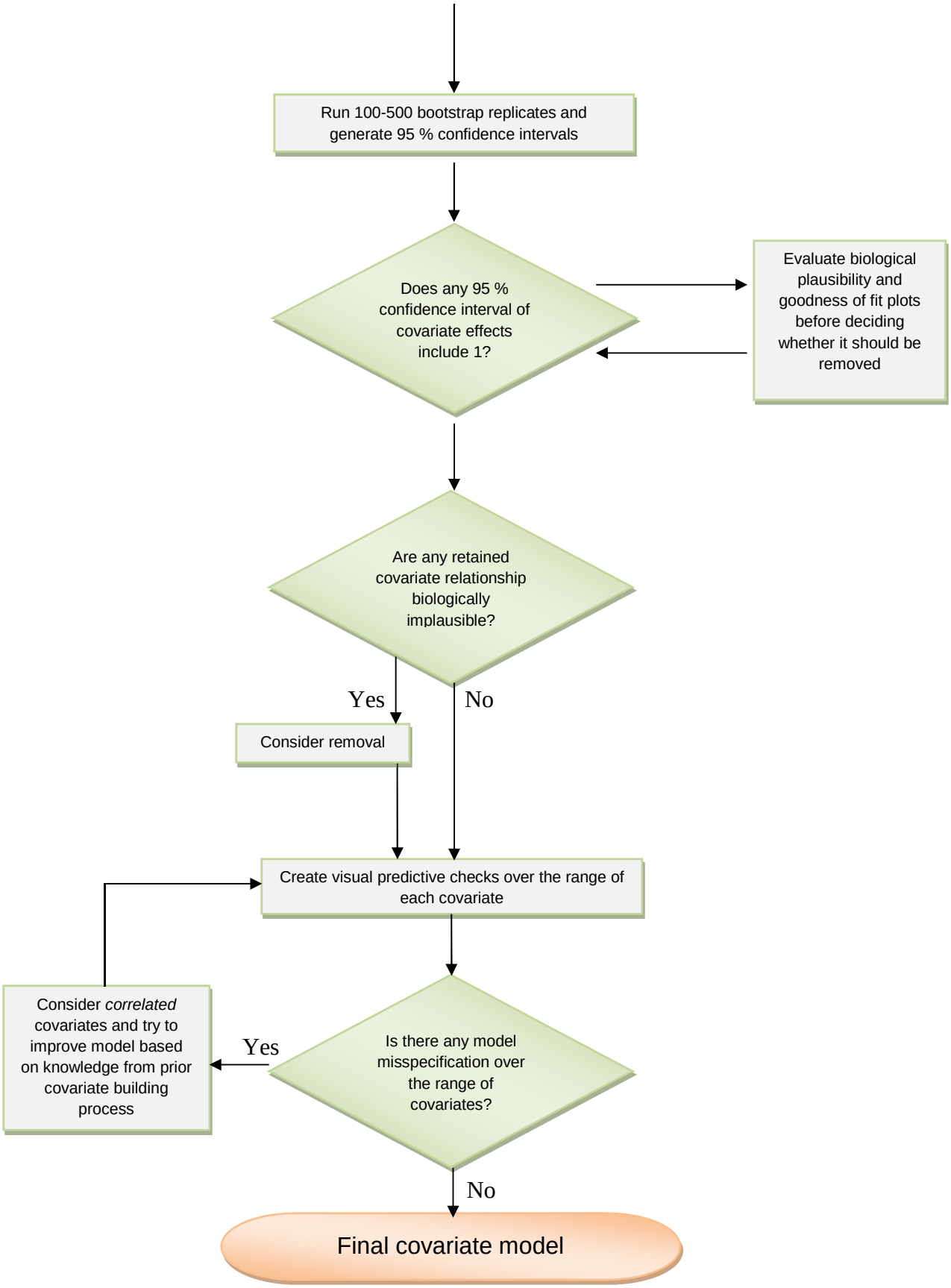


Figure E-3.1. Flowchart of the main steps during the covariate modeling process. Several of the choices made are subjective. This is only a general overview. The modeling process will typically move upwards and downwards in the flowchart depending on observations and experience gained.

E.4 Covariate inclusion

Table E.4 gives an overview of the main steps during covariate inclusion. Only a short summary is provided, excluding evaluated models not leading to model improvement. See appendix F for a qualitative overview of all evaluated models.

TABLE E.4. Results from covariate inclusion

Model number	Parameter	**Covariate**	**Implemented as**	**OFV**	**LRT**	**Added parameters**	**Compared to**	**p**
8	Conc.	Hematocrit	Linear with parameter	3052.7	-80.4	1	7	<0.001
9	Conc.	Hematocrit	Nonlinear with power parameter	3054.2	-78.8	1	7	<0.001
10	Conc.	Hematocrit	Nonlinear, Smax	3055.8	- 77.3	2	7	<0.001
11	Conc.	Hematocrit	Nonlinear, Bmax	3059.0	-74.0	1	7	<0.001
12	**Conc.**	**Hematocrit**	**Linear without parameter**	**3054.6**	**-78.4**	**0**	**7**	**<0.001**
13	**CL/F**	**CYP3A5**	**Proportional**	**3035.3**	**-19.3**	**1**	**12**	**<0.001**
14	**F**	**CYP3A5**	**Proportional**	**3020.4**	**-14.9**	**1**	**13**	**<0.001**
15	F	Time	Time intervals	2953.9	-66.5	12	14	<0.001
16	CL/F	Time	Time intervals	2967.7	-52.7	12	14	<0.001
17	F	Time	Sigmoidal (early)	2967.5	-52.9	3	14	<0.001
18	F	Time	Sigmoidal (late)	2955.8	-11.7	3	17	<0.001
19	F	BSV in F_time	Exponential	2908.7	-47.1	1	18	<0.001
20	CL/F,V1/F, Q/F, V2/F	Fat free mass	Allometric scaling	2908.9	+0.2	0	19	-
21	F	Sex	Proportional	2907.9	-0.75	1	19	-
22*	**CL/F,V1/F Q/F, V2/F** **F**	**Fat free mass** **and** **Sex**	**Allometric scaling** **Proportional**	**2902.5**	**-6.2**	**1**	**19**	**0.01**

OFV, Objective function value; LRT, Likelihood ratio test; CL/F, apparent clearance; V_1/F, apparent central volume of distribution; Q/F, apparent intercompartmental clearance; V_2/F, apparent peripheral volume of distribution ; F, bioavailability; Smax, Empirical sigmoidal function; Bmax, Saturation function using values for Bmax (maximal binding constant) and Kd (affinity constant) obtained from literature (see details in Appendix F); CYP3A5, Cytochrome P450 3A5
***** Full covariate model

E.5. Parameter estimates of the full covariate model

Table E.5 shows the parameter estimates of the full covariate model.

TABLE E.5. Typical values, bootstraps mean and 95 % confidence intervals of the parameters of the full covariate model (model nr. 22). Gray marked parameters were removed in reduction round 1.

Parameter		Estimate	BS mean	BS 95 % CI[a]
CL/F	(L/h)	18.9	19.2	16.2 – 22.5
V_1/F	(L)	112	103	56.7 – 154
Q/F	(L/h)	31.9	32.8	18.3 – 48.5
V_2/F	(L)	385	393	270 – 568
K_a	(h^{-1})	1.22	1.2	0.62 – 1.8
$K_{astudy\ 2}$	(h^{-1})	0.39	0.36	0.22 – 0.55
Lagtime	(h)	0.22	0.22	0.19 – 0.24
$Lagtime_{study\ 2}$	(h)	0.84	0.84	0.78 – 0.90
Covariates on relative CL/F				
CYP3A5 *1/*3	Factor	1.38	1.30	0.73 – 1.82
Covariates on relative F				
CYP3A5 *1/*3	Factor	0.76	0.73	0.37 – 1.39
Female sex	Factor	0.75	0.76	0.60 – 0.94
Time, early				
F_{early}		1.21	1.51	0.65 – 2.98
$F_{early\ 50}$	days	2.19	2.2	1.6 – 2.8
$F_{early\ Hil}$		6.85	8.0	3.6 – 10.0[b]
Time, late				
F_{late}		0.28	0.29	0.14 – 0.50
$F_{late\ 50}$	days	29.9	30.6	22.6 – 46.1
$F_{late\ Hil}$		2.17	2.4	1.5 – 3.9
BSV				
CL/F	(%)	38	36	25 – 49
V_1/F	(%)	44	58	29 – 116
Q/F	(%)	84	77	35 – 113
V_2/F	(%)	74	72	52 - 98
$F_{late\ Hill}$	(%)	109	108	76 - 149
Correlations				
CL/F ~ V_1/F		0.63	0.55	-0.16 – 0.98
CL/F ~ Q/F		0.66	0.57	0.12 – 0.93
CL/F ~ V_2/F		0.40	0.35	-0.12 – 0.71
V_1/F ~ Q/F		-0.08	-0.17	-0.97 – 0.81
V_1/F ~ V_2/F		0.43	0.09	-0.69 – 0.80
Q/F ~ V_2/F		0.50	0.60	- 0.04 – 0.99
BOV				
Relative F	(%)	14	14	9 - 18
k_a	(%)	68	58	25 - 85
Residual variability				
Proportional error	(%)	16.8	17.0	13.7 – 20.6
Additive error	µg/L	0.28	0.40	0.003 – 0.53
Study 2	Factor	0.50	0.55	0.41 – 0.68
Study 3	Factor	0.62	0.66	0.47 – 0.78
Study 4	Factor	0.90	1.00	0.73 – 1.11
BSV	(%)	16.9	10.5	0.002 – 0.27

BS, bootstrap; CI, confidence interval; CL/F, apparent clearance; V_1/F, apparent central volume of distribution; Q/F, apparent intercompartmental clearance; V_2/F, apparent peripheral volume of distribution; F, bioavailability; k_a, absorption rate constant; BSV, between subject variability; BOV, between occasion variability

[a] 2.5-97.5 percentile obtained from 200 bootstraps replicates

[b] 10.0 was upper bound

E.6. Visual predictive check without and with the effect of sex on bioavailability

The following pcVPCs (Figure E.6-1) using fat free mass on the X-axis guided the inclusion of sex on bioavailability to reduce the systematic bias over the range of fat free mass. The effect of these covariates masked each other, and only improved the model when added simultaneously (ΔOFV =-6.2, p=0.01).

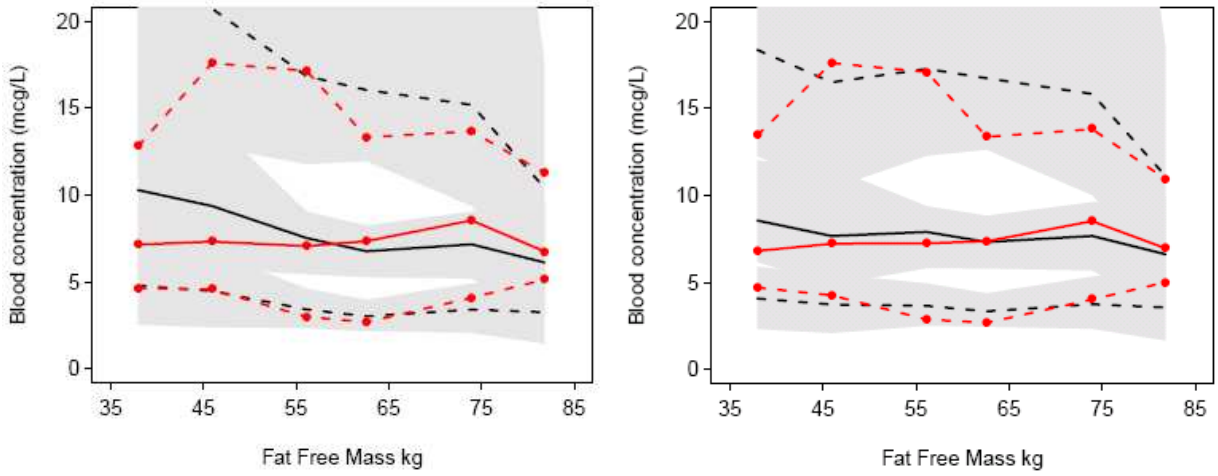


Figure E.6-1. Prediction corrected visual predictive check over the range of fat free mass using model nr. 20 (without sex on bioavailability, *left*) and nr. 22 (with sex on bioavailability, *right*). For graphical interpretation, see figure E-2.1.

APPENDIX F. Overview of evaluated models

Function	Comment on models not mentioned in main text
Compartmental models	
1 compartment model 2 compartment model	
Absorption process models	
Zero order absorption	
No lag time	
Singe k_a for all patients	+ More sensible to describe the absorption profile uniformly across patients — Unstable model and worsens the fit in group 2 considerably. There is clearly a different type of absorption profiles in study 2, but the reason for this is unknown.
Mixture model for k_a	+ Flexible — Long model run times — Models not stable
Random effects models	
BSV in lagtime	+ Biologically plausible that lag time differs between patients — Not enough data from absorption phase to support BSV in both lag time and k_a
BSV in ka only for intensively sampled group	
Both BSV and BOV in k_a	
Occasions for TDM data divided by day or week	— Violates the assumption of random distribution of the random effects described by BOV.
Covariate models	

Hematocrit on whole blood concentration
 	
<u>General model</u>
`CBSTD=F`
`CONC = CBSTD * Function of hematocrit`
`Y = CONC + error`
	In all following models, CBSTD means standardized whole blood concentration and equal the model predicted standardized concentrations (F).
	CONC are the non-standardized predicted concentrations, and Y are the measured concentrations. The *function* for the relationship between CBSTD and CONC were defined using a different approaches:

Exponential hematocrit model `CONC=CBSTD*EXP(THETA(X)*(HCT-45))`	

Power model for hematocrit allowing saturation `CONC=CBSTD*(HCT/45)**(THETA (1))`	Allows nonlinear relationship

Saturation model for hematocrit estimating plasma based pharmacokinetic paramters
 	
`CP=CSTD/55 ;Standardized for a BPR of 55:1`
`BMAX=418`
`KD=3.8`
`BPR=1+(HCT/100)*BMAX/(KD+CP)`
`CONC=CP*BPR`
	Estimating plasma based pharmacokinetic parameters using literature values of saturable binding to erythrocytes (Jusko et al. 1995)

Empirical saturation model for hematocrit
`IF (CBSTD.LE.0) THEN`
`CONC=0`
`ELSE`
 	
`CONC=HCT/45*((CBSTD**THETA(1))*THETA(2))/`
`((CBSTD**THETA(1))+(THETA(3)**THETA(1)))`
`ENDIF`
	Estimating erythrocyte saturation using empirical Smax function for the relationship between whole blood concentrations and hematocrit
	
	Unstable

Random effects in the standardization to hematocrit `CONC=CBSTD*(HCT/45)**(1*EXP(ETA(X)))`	Account for different binding affinity across patients
Hematocrit on clearance and volume of distribution Linear function Power function	Not superior to hematocrit on concentration
***CYP3A5* genotype** Proportional on only clearance Proportional on both clearance and bioavailability	Not superior to CYP3A5 on only F
Total body weight, BMI, BSA on clearance and volume of distribution Linear function Allometric function Power function Sigmoidal function Estimating the effect of fat mass:	Not superior to allometric scaling to fat free mass
Time after transplantation on clearance and bioavailability Linear function Exploratory model using twelve time intervals	
Prednisolone on clearance and bioavailability Linear function Power function Sigmoidal Treated as binary covariate with cut-off values at daily doses of 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 mg. Treated as categorical covariate with different parameter for each discrete dose size	Significant improvement, but not superior to the use of time after transplantation as covariate
Prednisolone/kg on clearance and bioavailability Linear function Power function	Significant improvement, but not superior to the use of time after transplantation as covariate

Age on clearance and volume of distribution Linear function Power function	No improvement
Height on clearance and volume of distribution Linear function Power function	No improvement
Sex Binary effect on clearance Binary effect on volume of distribution	Not superior to effect on bioavailability Tendency towards model improvement, significant during covariate inclusions but not during backward elimination
Albumin on clearance and volume of distribution Linear function Power function	No improvement
Serum creatinine Linear function on clearance Linear function on volume of distribution	No improvement Decreased OFV by 6 points, but not retained due to lack of biological plausibility
C-reactive protein on clearance and bioavailability Linear function Power function Treated as categorical covariate with cut-off values at C-reactive protein=5, 10, 20, 40, 50 mg/L	No improvement. Difficult to model and should account for a delay in a possible induction process
ASAT on clearance Linear function Power function Linear function using log ASAT Power function using log ASAT Cutoff value: 1.5 * upper normal value	No improvement
ALAT on clearance Linear function Power function Linear function using log ALAT Power function using log ALAT Cutoff value: 1.5 * upper normal value	No improvement
ALP on clearance Linear function Power function Linear function using log ALP Power function using log ALP Cutoff value: ALP=200	No improvement
Bilirubin on clearance Linear function Power function Linear function using log bilirubin Power function using log bilirubin Cutoff value: 1.5 * upper normal value	No improvement
Acute rejection episode on clearance and bioavailability Binary effect Account for delayed effect by assigning different clearance during 3,4,5,7,10 days after a rejection episode	No improvement

Methylprednisolone on clearance and bioavailability Distinct values for CL and F at different time intervals after administration of a methylpednisolone dose (in conjunction to surgical procedure and rejection episodes)	No improvement
Lansoprazole on clearance and bioavailability Binary effect (use/not use) not accounting for different dose strengths	No improvement
Nifedipine on clearance and bioavailability Binary effect (use/not use) not accounting for different dose strengths	No improvement
Cinacalcet on clearance and bioavailability Binary effect (use/not use) not accounting for different dose strengths	No improvement

APPENDIX G. Final model control stream

CODE	Comment
;---------- ; Final control strem ; Elisabet 21.09 ;---------- ; BSV: CL, V1, Q, V2, Flate ; BOV: F, Ka ; Covariates: ; Sex on F ; CYP3A5 on F ; Two separate time effects on F ; Concentrations: Hematocrit standardized ;----------	The symbol ";" comments out and are not read by NONMEM. This allows specifications in the control stream for the user Description
$SIZES LVR=46 $SIZES PL=15 $PROBLEM Final model $INPUT ID STU TIME EVID DV AMT II ADDL SS STER CYP LAN NIF CRP TXT HCT SCR BILI ASAT ALAT ALP ALB M0F1 AGE TBWKG HTCM FFMKG FATKG BMI BSA XOCC RICH OCC	$SIZES is used to increase the number of allowed parameters $PROBLEM is user-defined model name $INPUT refers to each column in the data set. NONMEM-defined column names are underlined.
$DATA DATA\FULL_input.csv IGNORE=#	$DATA tells NONMEM where to find the input file (dataset) and instructs NONMEM to ignore the rows starting with # (header row)
$ESTIMATION METHOD=COND INTER MAX=9990 NSIG=3 NOABORT PRINT=1 $COV PRINT=E	$ESTIMATION is user-defined to instruct NONMEM on which estimation method to use. METHOD=COND INTER means *Use the conditional method (FOCE) with interaction.* MAX=9990 means *Iterate max 9990 times, if not successfully converged at this time, please stop.* NSIG=3 means *Aim for 3 significant digits in the parameters before converging* PRINT=1 means *print each iteration in NONMEM output* $COV PRINT=E means *Estimate covariance matrix and print associated eigenvalues*
$THETA (0.1,18,) ; CLNR_STD L/H (0.1,104,) ; VC_STD L (0.1,29.5,) ; Q_STD L/H (0.1,411,) ; VT_STD L (0,0.213,) ; LAG1_STUDY1 (0,0.81,) ; LAG2_STUDY2 (0,1.20,) ; KA1_STUDY1	$THETA is used for initial estimates of the vector of fixed effects (typical values, pharmacokinetic parameters and covariates). First number = Lower bound (optional) Middle number = Initial estimate Last number = Upper bound (optional)

```
(0,0.407,)          ; KA2_STUDY2


;CYP3A5 ON F
(0,0.498,3)         ; F_CYP

;TIME FUNCTIONS
(0.1,0.949,3)       ; TXT_F   EXTENT OF INCREASE IN F
(0.1,2.48,100)      ; TXT50    DAY OF 50 % CHANGE
(0.1,9.,10)         ; TXTHILL

;LATE TIME ON F
(0,0.281,3)        ; TIME_F INCREASE IN F FROM BASELINE
(1,29.6,70)        ; TIME50 DAY OF 50 % CHANGE
(1,2.35,10)        ; TIMEHILL

;SEX ON F
(0,0.773,)         ; FSEX_FEM

;RESIDUAL ERROR
(0,0.569,)         ; Residual STUDY 2
(0,0.725,)         ; Residual STUDY 3
(0,0.167,)         ; Proportional residual error
```

FIX means that the estimate is not varied during minimization. Name of each theta is given after semicolon. This is not read by NONMEM but helps the user keep track of the thetas.
Although not stated, thetas are counted in the sequence they are stated.

```
$OMEGA BLOCK(1)
0.0585 ; BSV_VC
$OMEGA BLOCK(2)
0.0918 ; BSV_CL
0.153 0.767 ; BSV_Q

$OMEGA BLOCK(1)
0.332 ;BSV_VT
```

$OMEGA is the matrix of random effects.
BLOCK matrices allow estimating the correlation between two random effects. Here, a correlation is estimated between clearance and intercompartmental clearance (Q) (the underlined number).

```
$OMEGA BLOCK(1)
0.0283 ; BOV_F_1 ; BOV FOR RICH AND SPARSE DATA
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
```

When between occasion variability (BOV) is estimated, an $OMEGA must be defined for each defined occasion, and “SAME” gives NONMEM information that the estimated value should be equal for all omegas.
This is a “trick” to force NONMEM to estimate BOV, because an easier method is not currently available in NONMEM.

```
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
```

22 $OMEGAs are given because there are 22 occasions defined.

```
$OMEGA BLOCK(1)
0.437 ; BOV_KA1
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
```

BOV in KA is only estimated from the intensively sampled profiles at maximum 3 occasions.

```
$OMEGA BLOCK(1)
1.12 ; BSV_FTIME
```

```
$SIGMA 1. FIX ; EPS1
```

$SIGMA denotes the initial estimate of epsilon. However, oftentimes this is fixed to 1, and thetas are used in the residual error instead. This is done because modelers have found it to be more stable in addition to allow printing of the residual error in the tables, which is not allowed for sigma. It is a matter of what the modeler prefers. Using the $SIGMA would give similar results.

```
$SUBROUTINE ADVAN4 TRANS4
```

Subroutines are library built-in codes in NONMEM. ADVAN4 instructs NONMEM to use a two-compartment model with first order absorption and elimination.

```
$PK
;GROUP PK PARAMETERS
   TVCL  = THETA(1) *  (FFMKG/60)**(3/4) ; L/H
   TVV1  = THETA(2) *  (FFMKG/60)**(1/1) ; L
   TVQ   = THETA(3) *  (FFMKG/60)**(3/4) ; L/H
   TVV2  = THETA(4) *  (FFMKG/60)**(1/1) ; L

   IF (STU.EQ.2) THEN
      TVLAG = THETA(6)
      TVKA  = THETA(8)
   ELSE
      TVLAG = THETA(5)
      TVKA  = THETA(7)
   ENDIF
```

$PK is the block where the user defines the pharmacokinetic model properties. Typical values/group values of all pharmacokinetic parameters are defined and associated with the covariates.

```
IF (M0F1.EQ.1) THEN
      FFSEX=THETA(16)
   ELSE
      FFSEX=1
ENDIF
```

For example, this block states that if M0F1 (a column in the dataset for sex) is 1 (female) the user-defined name FFSEX is equal to the estimable theta. If gender is male, FFSEX is 1. Later, when typical value of F is declared, it is multiplied with FFSEX. There are also other methods to obtain the same result.

`FFTXT=1+THETA(10)/(1+(TXT/THETA(11))**(THETA(12)))`	This is a sigmoidal function for the early time function
`INDTIME_F=THETA(13)*EXP(ETA(30))`	An eta (random effect) is added to the individual change in F over time

`IF (TXT.GT.3) THEN`
`  FFTIME=1+(INDTIME_F)/(1+(TXT/THETA(14))**(-THETA(15)))`
`ELSE`
`  FFTIME=1`
`ENDIF`
	Sigmoidal function for the late time function

`IF (CYP.EQ.1) THEN`
`  FFCYP=THETA(9)`
`ELSE`
`  FFCYP=1`
`ENDIF`
	The effect of *CYP3A5* genotype on F

`TVF=1*FFCYP*FFTXT*FFTIME*FFSEX`	Here, all above introduced covariates on F are associated with typical value of F (TVF).

`IF(OCC.EQ.1) THEN`
`  BOVKA=ETA(27)`
`  BOVF=ETA(5)`
`ENDIF`
`IF(OCC.EQ.2) THEN`
`  BOVKA=ETA(28)`
`  BOVF=ETA(6)`
`ENDIF`
`IF(OCC.EQ.3) THEN`
`  BOVKA=ETA(29)`
`  BOVF=ETA(7)`
`ENDIF`
`IF(OCC.EQ.4) THEN`
`  BOVF=ETA(8)`
`ENDIF`
`IF(OCC.EQ.5) THEN`
`  BOVF=ETA(9)`
`ENDIF`
`IF(OCC.EQ.6) THEN`
`  BOVF=ETA(10)`
`ENDIF`
`IF(OCC.EQ.7) THEN`
`  BOVF=ETA(11)`
`ENDIF`
`IF(OCC.EQ.8) THEN`
`  BOVF=ETA(12)`
`ENDIF`
`IF(OCC.EQ.9) THEN`
	This is a necessary block of statements to allow between occasion variability (BOV) in k_a and F. OCC is referring to occasions defined in the dataset.

```
   BOVF=ETA(13)
ENDIF
IF(OCC.EQ.10) THEN
   BOVF=ETA(14)
ENDIF
IF(OCC.EQ.11) THEN
   BOVF=ETA(15)
ENDIF
IF(OCC.EQ.12) THEN
   BOVF=ETA(16)
ENDIF
IF(OCC.EQ.13) THEN
   BOVF=ETA(17)
ENDIF
IF(OCC.EQ.14) THEN
   BOVF=ETA(18)
ENDIF
IF(OCC.EQ.15) THEN
   BOVF=ETA(19)
ENDIF
IF(OCC.EQ.16) THEN
   BOVF=ETA(20)
ENDIF
IF(OCC.EQ.17) THEN
   BOVF=ETA(21)
ENDIF
IF(OCC.EQ.18) THEN
   BOVF=ETA(22)
ENDIF
IF(OCC.EQ.19) THEN
   BOVF=ETA(23)
ENDIF
IF(OCC.EQ.20) THEN
   BOVF=ETA(24)
ENDIF
IF(OCC.EQ.21) THEN
   BOVF=ETA(25)
ENDIF
IF(OCC.EQ.22) THEN
   BOVF=ETA(26)
ENDIF
```

```
;INDIVIDUAL PK PARAMETERS

CL=TVCL*EXP(ETA(2))
V2=TVV1*EXP(ETA(1))
Q =TVQ *EXP(ETA(3))
V3=TVV2*EXP(ETA(4))
KA=TVKA*EXP(BOVKA)
ALAG1=TVLAG
```

Finally, individual parameters are defined and associated with random effects.

`F1=TVF*EXP(BOVF)`	
`; Scale to central compartment` `S2=V2`	The scale factor is important to convert mass unites and to define which compartment blood samples are drawn from. V2=Central volume of distribution in ADVAN4.

`$ERROR`
`; Standardize`
`IPRED=F*(HCT/45)`
	$ERROR is the block for defining the residual error model.
	F is the model prediction (NONMEM-specific name)

`IF (STU.EQ.2) THEN`
`  FSDYRUV=THETA(17) ; STUDY 2`
`ELSE IF (STU.EQ.3) THEN`
`  FSDYRUV=THETA(18) ; STUDY 3`
`ELSE`
`  FSDYRUV=1`
`ENDIF`
`PROP=IPRED*THETA(19)`
`W=FSDYRUV*SQRT(PROP*PROP)`
	Study-specific residual errors are introduced.
	
	Proportional residual error defined by a theta.

`Y = IPRED + W*EPS(1)`	Measured concentrations (Y) = individual predicted concentration (IPRED) + residual error (W). EPS(1) is epsilon, which is fixed to 1, but is required by NONMEM to run.

`$TABLE ID DV TIME PRED IPRED RES CWRES NOPRINT`
`ONEHEADER FILE=sdtab295`
`$TABLE ID DV TIME IPRED CL V2 Q V3 KA ALAG1 NOPRINT`
`NOAPPEND ONEHEADER FILE=patab295`
`$TABLE ID DV STER CRP TXT HCT SCR BILI ASAT ALAT ALP`
`ALB AGE TBWKG HTCM FFMKG FATKG BMI`
`BSA NOPRINT ONEHEADER NOAPPEND FILE=cotab295`
`$TABLE ID DV CYP LAN NIF M0F1 NOPRINT ONEHEADER`
`NOAPPEND FILE=catab295`
	Finally, the user can ask NONMEM for to create tables of interest.
	Create a table of the parameters and residuals
	Create a table of individual parameter estimates
	Create a table of continuous covariates
	Create a table of categorical covariates

APPENDIX H. Example of dataset structure

#ID	STU	TIME	EVID	DV	AMT	II	ADDL	SS	OCC	STER	TXT	CYP	LAN	NIF	CIN	AGE	WT	HEIG	SEX	FFM	CRP	HCT	SCR	BILI	ASAT	ALAT	ALP	ALB
101	1	0	1	0	4000	12	0	1	1	20	25	0	0	0	0	61	104	179	0	70.4	26	29	269	7	20	47	51	36
101	1	10.88	0	13.8	0	0	0	0	1	20	26	0	0	0	0	61	104	179	0	70.4	26	29	269	7	20	47	51	36
101	1	11.47	1	0	4000	0	0	0	1	20	26	0	0	0	0	61	104	179	0	70.4	26	29	269	7	20	47	51	36
101	1	11.98	0	17.3	0	0	0	0	1	20	26	0	0	0	0	61	104	179	0	70.4	26	29	269	7	20	47	51	36
101	1	12.5	0	26.4	0	0	0	0	1	20	26	0	0	0	0	61	104	179	0	70.4	26	29	269	7	20	47	51	36
101	1	13.02	0	28.8	0	0	0	0	1	20	26	0	0	0	0	61	104	179	0	70.4	26	29	269	7	20	47	51	36
101	1	13.5	0	28.4	0	0	0	0	1	20	26	0	0	0	0	61	104	179	0	70.4	26	29	269	7	20	47	51	36
101	1	14.03	0	26.7	0	0	0	0	1	20	26	0	0	0	0	61	104	179	0	70.4	26	29	269	7	20	47	51	36
101	1	14.5	0	24.6	0	0	0	0	1	20	26	0	0	0	0	61	104	179	0	70.4	26	29	269	7	20	47	51	36
101	1	15.53	0	19.3	0	0	0	0	1	20	26	0	0	0	0	61	104	179	0	70.4	26	29	269	7	20	47	51	36
101	1	16.52	0	14.5	0	0	0	0	1	20	26	0	0	0	0	61	104	179	0	70.4	26	29	269	7	20	47	51	36
101	1	17.47	0	12.8	0	0	0	0	1	20	26	0	0	0	0	61	104	179	0	70.4	26	29	269	7	20	47	51	36
101	1	19.5	0	10.8	0	0	0	0	1	20	26	0	0	0	0	61	104	179	0	70.4	26	29	269	7	20	47	51	36
101	1	21.48	0	10	0	0	0	0	1	20	26	0	0	0	0	61	104	179	0	70.4	26	29	269	7	20	47	51	36
101	1	23.47	0	9.8	0	0	0	0	1	20	26	0	0	0	0	61	104	179	0	70.4	26	29	269	7	20	47	51	36
101	1	0	4	0	4000	12	0	1	2	15	31	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	11.28	0	6.9	0	0	0	0	2	15	32	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	11.4	1	0	4000	0	0	0	2	15	32	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	11.93	0	9.7	0	0	0	0	2	15	32	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	12.38	0	20.1	0	0	0	0	2	15	32	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	12.88	0	21.6	0	0	0	0	2	15	32	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	13.4	0	21.6	0	0	0	0	2	15	32	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	13.92	0	19.8	0	0	0	0	2	15	32	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	14.42	0	20	0	0	0	0	2	15	32	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	15.4	0	15.8	0	0	0	0	2	15	32	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	16.33	0	13.5	0	0	0	0	2	15	32	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	17.38	0	11.1	0	0	0	0	2	15	32	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	19.45	0	9.6	0	0	0	0	2	15	32	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	21.33	0	9.2	0	0	0	0	2	15	32	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	23.27	0	8.7	0	0	0	0	2	15	32	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	24.25	1	0	4000	0	0	0	2	15	32	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	35	0	10.8	0	0	0	0	2	15	33	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
101	1	35.75	0	9.1	0	0	0	0	2	15	33	0	0	0	1	61	99	179	0	68.7	12	29	256	9	23	42	53	36
102	1	0	1	0	2000	12	0	1	1	25	55	0	0	1	0	52	77	186	0	62.1	1	30	99	12	20	30	100	38
102	1	11.08	0	5	0	0	0	0	1	25	56	0	0	1	0	52	77	186	0	62.1	1	30	99	12	20	30	100	38
102	1	11.17	1	0	2000	0	0	0	1	25	56	0	0	1	0	52	77	186	0	62.1	1	30	99	12	20	30	100	38
102	1	11.68	0	7.1	0	0	0	0	1	25	56	0	0	1	0	52	77	186	0	62.1	1	30	99	12	20	30	100	38
102	1	12.17	0	12.9	0	0	0	0	1	25	56	0	0	1	0	52	77	186	0	62.1	1	30	99	12	20	30	100	38
1	4	0	1	0	2500	12	2	0	1	20	1	0	0	0	0	42	80	171	0	58.9	27	35	344	6	87	155	58	40
1	4	36	1	0	3000	12	3	0	2	20	2	0	0	0	0	42	81	171	0	59.3	27	34	259	6	87	155	58	40
1	4	48	0	4.1	0	0	0	0	2	20	3	0	0	0	0	42	84	171	0	60.4	29	31	167	6	87	155	58	40
1	4	72	0	3.8	0	0	0	0	2	20	4	0	0	0	0	42	84	171	0	60.4	21	30	129	6	87	155	58	40
1	4	84	1	0	4000	12	22	0	3	20	4	0	0	0	0	42	84	171	0	60.4	21	30	129	6	87	155	58	40
1	4	96	0	4.8	0	0	0	0	3	20	5	0	0	0	0	42	84	171	0	60.4	9	30	112	6	87	155	58	40

#ID	STU	TIME	EVID	DV	AMT	II	ADDL	SS	OCC	STER	TXT	CYP	LAN	NIF	CIN	AGE	WT	HEIG	SEX	FFM	CRP	HCT	SCR	BILI	ASAT	ALAT	ALP	ALB
1	4	144	0	6.2	0	0	0	0	3	20	7	0	0	0	0	42	79	171	0	58.5	4	33	108	6	87	155	58	40
1	4	168	2	0	0	0	0	0	3	20	8	0	0	0	0	42	78	171	0	58.1	4	33	108	6	87	155	58	40
1	4	192	0	7.6	0	0	0	0	3	20	9	0	0	0	0	42	77	171	0	57.7	2	36	107	5	77	180	66	45
1	4	240	0	8.5	0	0	0	0	3	20	11	0	0	0	0	42	78	171	0	58.1	2	35	100	5	77	180	66	45
1	4	312	0	8.9	0	0	0	0	3	20	14	0	0	0	0	42	78	171	0	58.1	1	35	114	7	33	92	49	43
1	4	360	0	9.9	0	0	0	0	3	20	16	0	0	0	0	42	79	171	0	58.5	1	35	124	7	33	92	49	43
1	4	360	1	0	3000	0	0	0	4	20	16	0	0	0	0	42	79	171	0	58.5	1	35	124	7	33	92	49	43
1	4	372	1	0	2000	12	120	0	5	20	16	0	0	0	0	42	79	171	0	58.5	1	35	124	7	33	92	49	43
1	4	408	0	5.6	0	0	0	0	5	15	18	0	0	0	0	42	78	171	0	58.1	1	36	113	7	33	92	49	43
1	4	480	0	4.3	0	0	0	0	5	15	21	0	0	0	0	42	78	171	0	58.1	0	35	109	5	32	64	44	44
1	4	528	0	4.8	0	0	0	0	5	15	23	0	0	0	0	42	78	171	0	58.1	1	34	113	5	32	64	44	44
1	4	576	0	4.4	0	0	0	0	5	15	25	0	0	0	0	42	78	171	0	58.1	1	35	107	5	32	64	44	44
1	4	648	0	4.8	0	0	0	0	5	15	28	0	0	0	0	42	79	171	0	58.5	2	35	109	5	30	67	45	44
1	4	696	0	5	0	0	0	0	5	15	30	0	0	0	0	42	78	171	0	58.1	3	35	113	5	30	67	45	44
1	4	744	0	4.9	0	0	0	0	5	15	32	0	0	0	0	42	79	171	0	58.5	3	37	109	5	30	67	45	44
1	4	840	0	4.9	0	0	0	0	5	10	36	0	0	0	0	42	79	171	0	58.5	7	36	100	6	41	82	55	46
1	4	912	0	5.1	0	0	0	0	5	10	39	0	0	0	0	42	78	171	0	58.1	5	35	111	6	41	82	55	46
1	4	984	0	4.9	0	0	0	0	5	10	42	0	0	0	0	42	79	171	0	58.5	6	35	102	8	37	81	59	43
1	4	1056	0	5.4	0	0	0	0	5	10	45	0	0	0	0	42	79	171	0	58.5	7	36	112	8	37	81	59	43
1	4	1152	0	5.1	0	0	0	0	5	10	49	0	0	0	0	42	79	171	0	58.5	8	35	104	5	51	85	66	43
1	4	1200	0	5.4	0	0	0	0	5	10	51	0	0	0	0	42	77	171	0	57.7	4	36	107	6	60	107	70	44
1	4	1320	0	7.4	0	0	0	0	5	10	56	0	0	0	0	42	78	171	0	58.1	1	35	94	5	52	84	56	42
1	4	1392	0	8.9	0	0	0	0	5	10	59	0	0	0	0	42	78	171	0	58.1	1	38	98	5	52	84	56	42
1	4	1488	0	7	0	0	0	0	5	10	63	0	0	0	0	42	79	171	0	58.5	1	37	99	6	30	60	50	42
1	4	1560	0	6.4	0	0	0	0	5	10	66	0	0	0	0	42	79	171	0	58.5	1	36	103	6	30	60	50	42
1	4	1704	0	5.8	0	0	0	0	5	10	72	0	0	0	0	42	79	171	0	58.5	1	35	102	6	31	43	43	45
2	4	0	1	0	4000	12	4	0	1	20	1	1	0	0	0	24	107	206	0	81.8	17	30	755	6	139	134	46	37
2	4	48	0	3.7	0	0	0	0	1	20	3	1	0	0	0	24	108	206	0	82.2	17	29	453	6	139	134	46	37

ID, patient ID; STU, study number; TIME, time relative to zero; EVID, Event ID (1=Dosing record, 0=Observation record, 4=Reset system and introduce new dose; DV, Dependent variable (Tacrolimus concentration); Amt, Dose amount in micrograms; II, dose interval in hours; ADDL, Additional doses; SS, steady state is assumed if 1; OCC, Occasion; STER, steroid dose; TXT, Time after transplantation; CYP, CYP3A5 expresser=1, non-expresser=0; LAN, lansoprazole; NIF, nifedipine; CIN, cinacalcet; AGE, patient age; WT, Total body weight in kg, HEIG, height in cm; SEX, sex (0=male, 1=female); FFM, predicted fat free mass; CRP, C-reactive protein, HCT, hematocrit (%), SCR, Serum creatinine; BILI, bilirubin; ASAT, Aspartate aminotransferase; ALAT, Alanine aminotransferase; ALP, Alkaline phosphatase; ALB, albumin