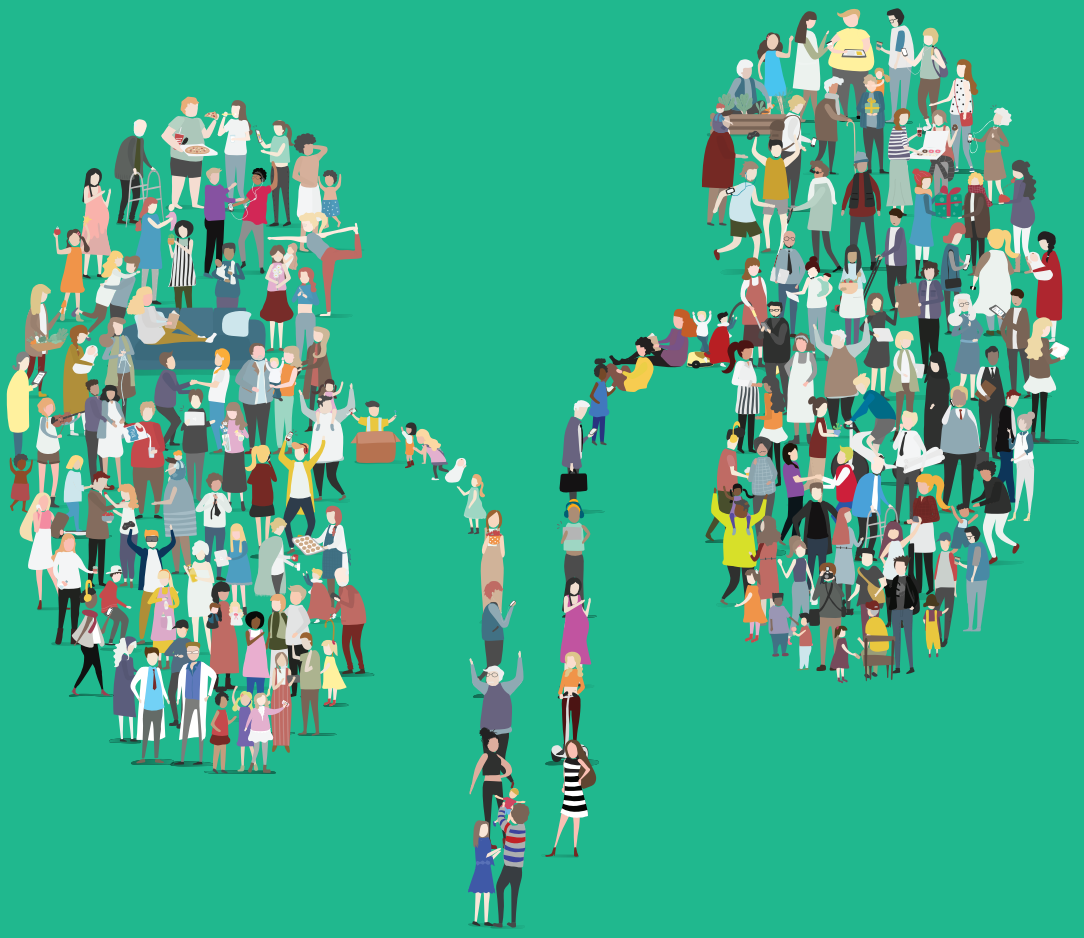
MODEL BASED DOSING OF TACROLIMUS AFTER RENAL TRANSPLANTATION



Louise Andrews

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Model gestuurd doseren van tacrolimus na niertransplantatie

MODEL BASED DOSING OF TACROLIMUS AFTER RENAL TRANSPLANTATION

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Voor mijn moeder

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Expert Opin Drug Metab Toxicol. 2015 Jun;11(6):921-36.

ABSTRACT

Introduction

Starting doses of tacrolimus and ciclosporin are typically chosen on a calculated mg/kg bodyweight basis. After initiation of treatment, doses are adjusted with therapeutic drug monitoring (TDM). This trial and error approach has been accepted by most physicians and pharmacists involved in the care of transplanted patients.

Areas covered

Only fairly recently, dosing algorithms have been proposed to better individualize the starting dose. This review provides an overview of all the currently available dosing algorithms in adult and children for the starting dose of ciclosporin, tacrolimus and mycophenolic acid. In these algorithms, multiple other co-variates influencing the starting dose, such as age, hematocrit, co-medication and genotype are taken into account. After selecting the starting dose with an algorithm and after initiation of treatment, TDM will however, remain necessary. Whether or not implementation of such algorithms will improve clinical outcome remains to be demonstrated.

Expert opinion

First of all an algorithm needs to be validated, against an independent dataset. Second, in a prospective study the algorithm should prove to reduce the time to reach the target concentration, and to reduce the number of patients with drug concentrations (far) outside the therapeutic window. Finally, a clinical trial demonstrating a benefit on clinical outcome will be crucial in achieving broad acceptance of calculating starting dose using individualized dosing algorithms.

ARTICLE HIGHLIGHTS

- Starting doses of tacrolimus and ciclosporin are typically based on bodyweight, although bodyweight is a poor predictor for clearance of these drugs in adults
- Dosing algorithms have been proposed to better individualize the starting dose of these immunosuppressants but it remains to be demonstrated if using these algorithms will improve clinical outcome
- Algorithms need to be validated, and subsequently tested in a prospective clinical trial to investigate if they reduce the time to reach target concentration and reduce the number of patients with drug concentrations (far) outside the therapeutic window. Ultimately, a clinical trial demonstrating a benefit on clinical outcome will be crucial in achieving broad acceptance of calculating starting dose with an algorithm.

INTRODUCTION

Current immunosuppressive drug treatment after solid organ transplantation

Nowadays, immunosuppressive treatment for the prevention of acute rejection after solid organ transplantation mostly consists of the combination of a calcineurin inhibitor (CNI; either ciclosporin or tacrolimus), in combination with mycophenolic acid (MPA), with or without glucocorticoids and induction therapy with an interleukin (IL)-2 receptor blocker or a T-lymphocyte depleting agent[1-4].

The most important challenge associated with the clinical use of CNIs is the narrow therapeutic range between efficacy and toxicity, and the influence of co-medication. The associated adverse events include nephrotoxicity, neurotoxicity, diarrhea, hypertension, cosmetic adverse events and disturbances in lipid and glucose metabolism[5]. Tacrolimus is generally preferred over ciclosporin because it is associated with decreased acute rejection rates and similar overall costs. It is also better tolerated by patients[6, 7]. In most protocols for immunosuppression after solid organ transplantation, tacrolimus is the CNI of first choice.

MPA´s use has been associated with gastrointestinal adverse effects, especially diarrhea, and hematological disorders. Furthermore, changes in MPA clearance in the first 3 months post-transplantation and the influence of co-medication are additional factors that complicate the assessment of pharmacokinetics[8-11].

Therapeutic Drug Monitoring

Therapeutic drug monitoring (TDM) is performed for drugs with a narrow therapeutic index and marked pharmacokinetic variability[12].

CNI: There is a relationship between CNI concentrations and drug effects, both in terms of efficacy and safety. Higher exposure to CNIs has been associated with increased risk of toxicity[13-15]. In addition, there is evidence that low predose blood tacrolimus concentrations correlate with increased risk of rejection, although this has recently been debated[16, 17]. For toxicity the concentration – effect relationship is less well established.

The concentration-effect relationship, combined with high inter-patient variability in pharmacokinetics underlines the necessity of individualizing tacrolimus dosages and to perform therapeutic drug monitoring (TDM) as an indicator of drug exposure[18].

MPA: Although there is a rationale for MPA TDM, it remains a matter of ongoing debate whether concentration-controlled dosing of mycophenolate mofetil (MMF) is superior to using a fixed drug dose[5, 19]. Upon oral administration, MMF is hydrolyzed to the active agent MPA.

The large inter-patient variability in MPA pharmacokinetics at fixed-dose and the observation that the risk for acute rejection increases with lower MPA plasma concentrations suggest that a strategy of TDM would improve outcome, and reduce the risk of treatment failure and acute rejection in renal allograft recipients without an increase in adverse events and without adding any extra costs[8, 20]. It is especially during the first week that MPA exposure is highly predictive of the incidence of acute rejection[21].

Research has shown that the incidence of rejection is increased in high-risk renal transplant patients with a total MPA-AUC below 30 mg·hr/L. However, a difference in acute rejection incidence in low-risk patients was not observed[22] . A French study concluded that after 12 months the concentration-controlled group had fewer treatment failures (a composite endpoint consisting of death, graft loss, acute rejection and MMF discontinuation) and acute rejection episodes than the fixed-dose group[23]. In addition, it was recently demonstrated that each 1 mg·hr/L increase of the total MPA AUC was associated with a 4% decreased risk of acute rejection, graft loss or death[16]. Interestingly, CNI AUC was not significantly associated with these events[16]. In contrast to the French study, two other large randomized studies (the so-called FDCC and Opticept trials) attempting to improve outcome using TDM for MPA, failed to demonstrate a benefit[19, 24].

Enteric-coated mycophenolate sodium (EC-MPS) has unpredictable pharmacokinetics. Especially the time to C-max may differ substantially between patients, in contrast to MMF that has a more predictable C-max occurring one hour after oral intake. It is therefore challenging to obtain a reliable estimation of MPA exposure after EC-MPS intake. It has proven to be difficult to use limited sampling strategies for the estimation of MPA AUC after ingestion of EC-MPS[25].

Starting dose for CNI and MPA

Tacrolimus is usually administered at an oral starting dose of 0.1 mg/kg twice daily[26]. Recommended TDM schemes require blood sampling and dose adjustments to reach the therapeutic window. The specific target concentrations may differ depending on the transplanted organ, the perceived risk of rejection and the immunosuppressive co-treatment. Obviously in CNI minimization protocols, target concentrations are lower, and thus starting doses need to be adjusted. As an example, in the Symphony study[27] (kidney transplantation) a target range of 3-7 ng/mL was used, and in the 3C study[28] (kidney transplantation) a slightly more narrow target range of 5-7 ng/mL.

Ciclosporin: The initial dosing recommendation in the Symphony study was a standard dose of 3-5 mg/kg twice daily or a lower dose of 1-2 mg/kg twice daily. The corresponding target range was 150-300 ng/mL for 3 months, followed by 100-200 ng/mL[27]. In a multi-center study, the mean ciclosporin dose was 3.6 mg/kg daily. The dose was adjusted to maintain predose whole blood ciclosporin concentrations within the therapeutic range established for each center. Mean predose levels were 175 ng/mL[29].

MMF: For adult kidney recipients who were on tacrolimus therapy, a starting dosage of 2 g/d MMF guaranteed that 76.2% of patients achieved the target therapeutic range of 30 to 60 mg * h/L by day 3, whereas, for ciclosporin treated patients, only 51.2% of the treated patients reached the target concentration[30]. For overcoming this early MPA underexposure, intensified MPA dosing in the early postoperative period is a potential strategy to consider in patients receiving ciclosporin: 3 or 4 g/d MMF or EC-MPS equivalent dose[30, 31]. In pediatric patients, MMF is dosed by body surface area (BSA). This overcomes under-dosing in younger children if a standard mg/kg dose would be used, as younger children require higher mg/kg doses to reach therapeutic levels. An initial dose of 600 mg/m^2/dose twice daily is recommended, with a maximum of 2 gram per day[32].

After initiation of standard treatment, doses are corrected with TDM. This trial and error approach has been accepted by most physicians and pharmacists involved in the care of transplanted patients. Only fairly recently individualized dosing algorithms, based on relevant co-variates have been proposed to better individualize the starting dose.

In this paper we aim to review current dosing algorithms for ciclosporin, tacrolimus and MMF in both adults and children. First, covariates influencing drug clearance will be reviewed, and subsequently the dosing algorithms incorporating one or more of these covariates will be discussed. Glucocorticoids are only rarely subjected to TDM approaches, and will not be discussed. mTOR inhibitors are only used in a minority of patients as *de novo* therapy and are also not discussed in this paper[33]. A literature search was performed using Medline (OvidSP), Embase, PubMed publisher, Web-of-Science, Scopus, Cochrane and Google scholar with the following key words: ''cytochrome P450'', ''pharmacogenetics'', "pharmacokinetics", ''tacrolimus'', ''ciclosporin'', "mycophenolic acid", ''P-glycoprotein'', ''P450 oxidoreductase'', ''kidney transplantation'', ''liver transplantation'', "dosing algorithm", "dosing equation", "dose...", "concentration", "drug dosing requirements". Only articles published in English were included. Reference lists of relevant articles were also reviewed manually to identify any additional papers of interest.

VARIABLES AFFECTING TACROLIMUS, CICLOSPORIN AND MYCOPHENOLIC ACID CLEARANCE

CNIs and MMF display considerable between- and within-subject pharmacokinetic variability[34]. Covariates reported to influence the clearance of these drugs, include hepatic and renal function, body size, age (in pediatrics), hematocrit, genetics, co-medication, time post-transplant, and transplant type. A large number of studies described covariates affecting CL in the immediate post-transplant period. A summary of the main conclusions of these studies is presented.

Tacrolimus

Tacrolimus is a ABCB1 substrate and the absorption from the intestinal tract is incomplete and variable. It is approximately 99% bound to plasma protein[35]. The metabolism is hepatic, primarily by CYP3A4. Less than 1% of the dose administered is excreted unchanged in urine. The disposition of tacrolimus is largely impacted by variation in absorption, distribution metabolism and excretion. An important role in these processes is played by drug metabolizing enzymes (DME) and protein transporters. Genetic polymorphisms in the encoding genes for these DMEs and transporters have been implicated in the variation in tacrolimus disposition: *CYP3A5*, *CYP3A4*, P450 oxidoreductase (POR), *CYP3A7*, Pregnane X Receptor (PXR), ABCB1 (P-gp) and UDP-glucuronosyltransferase (UGT). In particular, a highly prevalent single nucleotide polymorphism (SNP) in *CYP3A5* is an important factor affecting tacrolimus clearance[36]. PharmGKB has stated that there is evidence to support an interaction between tacrolimus and CYP3A5, however, there are no dosing recommendations at this time[37].

In the adult kidney transplant population, Press *et al*. found that *CYP3A5*1/*3*, PXR A+7635G GG, and prednisolone comedication in a dose over 10 mg per day were the most important factors affecting the early tacrolimus exposure[38]. Patients with *CYP3A5*1/*3* genotype require a higher

initial dose compared with *CYP3A5*3/*3* to reach adequate target tacrolimus exposure early post transplantation[26].

As tacrolimus binds to erythrocytes, hematocrit was studied as a covariate in a renal transplant population. Low hematocrit values result in more unbound tacrolimus available for distribution in peripheral tissues with consequences such as neuro- or liver toxicity and delayed graft function[39]. Hematocrit has also been mentioned as a variable that may be of influence regarding analytical aspects[40].

Co-medication is known to affect tacrolimus exposure. Many drugs inhibiting CYP3A enzyme activity, including azoles, prednisolone and calcium channel blockers, may increase the tacrolimus exposure. Drugs inducing CYP3A enzyme activity, including anti-epileptics, may decrease the exposure[41, 42].

Co-medication is most pronounced in patients receiving human immunodeficiency virus protease inhibitors such as ritonavir, saquinavir, nelfinavir and indinavir[43]. Protease inhibitors' competition for binding to cytochrome P450 isoenzyme system CYP3A induce extreme inhibition of tacrolimus metabolism and such patients may be exposed to extremely high tacrolimus levels if not anticipated on time. Care should be exercised when these drugs are administered concomitantly and the doses should be adjusted even before the measurement of the first blood concentrations[44, 45].

In the pediatric kidney transplant population, tacrolimus disposition is also highly influenced by the presence (*CYP3A5*1*) or absence (*CYP3A5*3*) of *CYP3A5* expression[46]. Tacrolimus exposition was lower in recipients with *CYP3A5*1/*3*, increased in older patients during the first month after transplantation and decreased with concomitant treatment with glucocorticoids[42, 47, 48]. Age and body weight are also important covariates in clearance variability[49, 50].

Early after liver transplantation, tacrolimus pharmacokinetics vary due to variable activity of intestinal CYP3A4 and ABCB1, resuscitation and gradual recovery of liver function. Many studies reported that indicators of liver function are the main factors involved in the interindividual variability of tacrolimus clearance after liver transplantation[18, 51, 52]. The genotype of CYP3A and ABCB1 of the donor and the recipient may differ in liver transplantation, and may both have an impact. The most commonly found factors of variability in tacrolimus clearance after liver transplantation are type of graft, activity of aspartate aminotransferase and the time elapsed since transplantation[53].

In pediatric liver allograft recipients during the first year post transplantation, Guy-Viterbo *et al.* also described hematocrit levels and time after transplantation as factors that influence tacrolimus clearance[54]. ABCB1 mRNA level and *CYP3A5*1* in the graft liver affects interindividual variability in the clearance of tacrolimus, especially during the first 50 postoperative days[55]**.** In pediatric liver recipients, variation in tacrolimus disposition appears related to *ABCB1* genotype[50, 56].

Ciclosporin

Like tacrolimus, ciclosporin is also a ABCB1 substrate and the absorption from the gastrointestinal tract is incomplete and variable. It is approximately 90% protein-bound. The major route of elimination of ciclosporin is via the bile, primarily as metabolites of the drug. Patient's hematocrit and lipoprotein profile may affect ciclosporin distribution[57].

Factors affecting the metabolism of ciclosporin include liver disease, age, and concurrent drug therapy. CYP3A inhibitors such as diltiazem and azoles have the potential to decrease the clearance of ciclosporin[58-60]. Other clinical factors that significantly affected ciclosporin pharmacokinetics during the first year post liver transplantation also include prednisolone dose (in mg/day) and concurrent use of calcium channel blockers[61].

In one study, ciclosporin clearance in patients during the first week after transplantation was significantly higher (27%) compared to that in patients 6 months after kidney transplantation[62]. In contrast, Irtan *et al*. found a significant increase in CL/F with time after kidney transplantation, age, body weight, protein level in blood and serum creatinine[63].

In adult kidney transplant patients, Wu *et al*.[58], and Falck *et al*.[64] showed a significant decrease in clearance of ciclosporin with age. In children age is a determinant in systemic CL. In general, pediatric patients require higher body-weight corrected daily doses of ciclosporin (about 20-25%) to achieve target blood concentrations of the drug[57].

A more rapid apparent blood clearance was described in young children (2-5 years old) than in older (>10 years old)[65]. In their review, Han *et al*. discussed the effect of age in pediatric clearance of CNI. This effect may be masked by the influence of body size as it is difficult to distinguish the interindividual variability caused by age-factors from that caused by size-factors[66]. Other significant covariates such as hematocrit, plasma cholesterol and creatinine, were estimated to explain 20-30% of interindividual differences in pediatric clearance[67].

Genetic polymorphisms of the *CYP3A4* and *CYP3A5* genes do not fully explain the variability of ciclosporin pharmacokinetics. A slightly higher clearance was studied in carriers of a *CYP3A4*1B* variant allele in adult renal and heart transplant patients[68].

The presence of specific ABCB1 and Pregnane X Receptor (PXR) SNPs did significantly affect ciclosporin exposure during a kidney transplant patient's development from childhood to adulthood in a time-dependent fashion[69-71].

Elens *et al*. showed that the *POR*28* allele is associated with increased *in vivo* CYP3A5 activity for tacrolimus in CYP3A5 expressers, whereas *POR*28* homozygosity was associated with a significantly higher CYP3A activity in CYP3A5 nonexpressers for both tacrolimus and ciclosporin[72]. The *POR*28* allele influences tacrolimus exposure independent of the *CYP3A5*3* status. *CYP3A4*22* is a significant independent predictor of ciclosporin exposure[73].

Mycophenolate Mofetil

Mycophenolate mofetil is rapidly and completely absorbed, undergoing extensive presystemic de-esterification to mycophenolic acid (MPA). MPA is mainly glucuronidated to the inactive 7-O-mycophenolic acid glucuronide (MPAG) by UGT1A9, 1A8 and UGT2B7[74]. MPA binds for 97% and MPAG for 82% to plasma proteins[75]. Gender differences in MPA pharmacokinetics have been reported, possibly due to hormonal modification of glucuronidation activity[76]. Renal function and the plasma albumin concentration correlate with clearance of total MPA. MPA has complicated pharmacokinetics, including biliary excretion of MPAG, which after de-glucuronidation in the gut is restored as MPA. MPAG finally undergoes renal elimination and therefore accumulates in patients with impaired renal function. The accumulated MPAG concentrations result in increased recirculation of MPAG to MPA. Because of the extra recirculation, MPAG does not accumulate to

an extent where it can displace MPA from its protein binding sites. Therefore the MPA clearance seems to decrease. If these patients are cotreated with ciclosporin the recirculation of MPAG will be inhibited, resulting in even higher MPAG concentrations which can displace MPA from its binding sites. The increase of unbound MPA due to elevated MPAG concentrations or low albumin concentrations results in higher MPA CL[75, 77, 78].

It has been demonstrated that in the first week after pediatric kidney transplantation, low MPA AUC_{0-12} values were associated with young age, low serum albumin levels, and decreased renal transplant function[79].

The co-medication in immunosuppressive regimens has a strong impact on MPA exposure. It is well known that ciclosporin co-treatment results in lower exposure to MPA, as a result of an inhibition of enterohepatic recirculation of MPA[80]. In ciclosporin-treated patients, mycophenolate doses are higher compared to patients not on ciclosporin[81-83]. Also glucocorticoids may induce the clearance of MPA[84] . Van Hest *et al*. concluded that monitoring creatinine clearance, albumin concentration, hemoglobin and ciclosporin predose concentration, is useful in predicting changes in MPA exposure over time[85].

Polymorphisms in *UGT1A9* and *UGT1A8* may alter MPA pharmacokinetics in kidney transplantation[86-88]. The variants -275T/A and -2152C/T of the *UGT1A9* promoter region are associated with a higher hepatic expression of *UGT1A9* and an increase in glucuronidation activity to form the inactive metabolite (MPAG)[89]. Moreover, *UGT2B7* genotype contributes significantly to the interindividual variability of MPA disposition in pediatric renal-transplant patients: clearance was significantly lower in patients with *UGT2B7* 802 C/C genotype compared with patients with *UGT2B7* 802 C/T and 802T/T genotypes[90]. Possibly, the prevalence of gene polymorphisms within ethnic subgroups explains why Asian patients have higher MPA exposure at standard dosing compared to Caucasian or African American patients[91].

KIDNEY TRANSPLANTATION DOSING ALGORITHMS

Tacrolimus in adult transplant recipients

Table 1 gives an overview of the available dosing algorithms for the starting dose of tacrolimus in adult kidney transplant recipients. The table consists of readily available dosing algorithms in literature, but also algorithms that we calculated based on population-pharmacokinetic models. All algorithms are rewritten for a starting dose to achieve a tacrolimus predose concentration of 10 ng/mL or a ciclosporin predose concentration of 200 ng/mL. The used formula to create the required dose is: Cl/F (L/h) x target C_0 (ng/mL) x 24 hours, divided by 1000, as previously described[92, 93].

In 2011 Passey *et al*. created the first tacrolimus dosing algorithm using a combination of genetic information and clinical factors in adult kidney transplant recipients[93]. The algorithm was developed from a large tacrolimus pharmacogenetic study (DeKAF study), and showed that the clearance of tacrolimus was significantly influenced by *CYP3A5* genotype, days post-transplant, age, transplantation in a steroid sparing center, and the use of calcium channel blockers[93]. The dosing algorithm was later successfully validated in an independent cohort of 795 kidney transplant recipients[94]. A prospective validation, applying the algorithm in *de novo* kidney recipients, has however, not been done. Unfortunately, using the DeKAF algorithm, Boughton *et al*. were unable to predict the estimated tacrolimus clearance accurately based on real tacrolimus

Table 1. Tacrolimus starting dose algorithms for adult renal transplant recipients.

AUTHOR	N	COVARIATES USED IN ALGORITHM						FINAL ALGORITHM	TDD average patient[b]
		BW	HCT	CYP3A5	COMED	POD	OTHERS		
Passey *et al*[93]	681			X	X	X	Age	Dose = ((38.4 x [(1.69, if CYP3A5*1/*3 genotype) or (2.00, if CYP3A5*1/*1 genotype)] x (0.70, if receiving transplant at steroid sparing center) x ([age in years/50]$^{-0.4}$) x (0.94, if CCB is present)) x 0.24	9.2 mg
Chen *et al*[120]	120	X		X	X			Dose (mg/kg) = 10 / (128.82 – (52.2 if CYP3A5*1) + (24.93, if diltiazem is present))	5.4 mg
Storset *et al*[123]	242		X	X	X	X	FFM	Dose = ((21.7, if HCT 33%) or (16.1, if HCT 45%) x (FFM/60)$^{3/4}$ x 1.30 (if CYP3A5*1)) / ((1- (0.67 x prednisolone dose)/(35 mg + prednisolone dose)) x 2.68 (if first day post-transplant) x 0.82 (if CYP3A5*1)) x 0.24	3.1 mg
Zuo *et al*[97]	161		X	X			CYP3A4	Dose = (26.6 x (HCT/27.9)$^{-0.451}$ x CYP3A) x 0.24[a]	3.3 mg
Asberg *et al*[124]	69		X	X		X	FFM, BMI	CYP3A5*1: 26.7 / 0.63 x 0.24 CYP3A5*3: 21.2 / 1 x 0.24	5.1 mg
Golubovic *et al*[98]	105	X	X			X	TP, AST	Dose = (10.017 x (POD/47)$^{-0.0283}$ x (BW/68)$^{0.869}$ x (TP/63)$^{0.161}$ x (1 – 86/1000 x (AST – 15)) x (1 – 0.831 x (HCT – 0.31)) x 0.24	0.4 mg
Press *et al*[38]	31			X				CYP3A5*3/*3: 14 mg/day CYP3A5*1/*3: 20 mg/day	14 mg
Thervet *et al*[99]	236	X		X				CYP3A5*1/*3 or *1/*1: 0.30 mg/kg/day CYP3A5*3/*3: 0.15 mg/kg/day	10.5 mg
Bergmann *et al*[101]	173	X		X				CYP3A5*1: 0.115 mg/kg bid CYP3A5*3: 0.075 mg/kg bid	5.3 mg
Zhang *et al*[100]	76	X		X				CYP3A5*1/*3: 0.15 mg/kg daily CYP3A5*3/*3: 0.08 mg/kg daily	5.6 mg

BW: body weight, HCT: hematocrit, CYP3A5: CYP3A5 genotype, COMED: relevant comedications, POD: time post-transplant, CCB: calcium channel blockers, FFM: fat free mass, BMI: body mass index, TP: total protein, AST: aspartate aminotransferase, TDD: total daily dose.

[a] CYP3A5*1 and CYP3A4*1G: 1.21, CYP3A5*1 and CYP3A4*1: 0.982, CYP3A5*3/*3 and CYP3A4*1G: 0.770, CYP3A5*3/*3 and CYP3A4*1: 0.577.

[b] 50 years old, 175 cm, 70 kg, received prednisolone 25 mg bid, no calcium channel blockers, hematocrit 0.35 L/L, CYP3A5*3/*3, CYP3A4*1/*1, total protein 70 g/L, fat free mass 57 kg, AST 25 U/L.

doses and blood concentrations in their cohort of patients[95]. Recently Elens *et al*. improved the DeKaF algorithm by incorporating the *CYP3A4*22* SNP[96].

Zuo *et al*. also used a combination of genetic information and clinical factors[97]. Besides *CYP3A5* genotype, they took it one step further and also added *CYP3A4* to the formula. Most dosing algorithms incorporate genetic information. The only exception for adult kidney recipients is the study from Golubovic *et al*., who have created an algorithm based on bodyweight, hematocrit, days post-transplant, total protein and AST[98].

A different and more simplistic strategy is to base the starting dose only on the *CYP3A5* genotype and bodyweight like Thervet *et al*. and Zhang *et al*. did[99, 100]. Recent studies have suggested that fixed doses seem equal to doses based on bodyweight, for example Press *et al*. advise a fixed dose based solely on the *CYP3A5* genotype[38]. Interestingly, Bergmann *et al*. conclude that carriers of *CYP3A5*1* should receive either 0.115 mg/kg or a fixed dose of 10 mg twice daily. Noncarriers should be prescribed 0.075 mg/kg or a fixed dose of 6 mg twice daily[101].

Pre-transplant pharmacokinetic profiling is an alternative strategy. For example Campbell *et al*. guided their dosing by the results of preoperative assessment of tacrolimus pharmacokinetics[102]. Patients were randomized to receive either a single pre-transplant dose of tacrolimus 0.1 mg/kg, followed by a single 2-hour whole blood tacrolimus concentration assessment; or to receive a single preoperative dose of tacrolimus 0.1 mg/kg followed by standard care. If the tacrolimus 2-hour blood concentration was ≤ 20 ng/mL, a postoperative dose of 0.15 mg/kg bid was prescribed. If the concentration was between 21 and 59 ng/mL, the postoperative dose was 0.1 mg/kg bid, and if it was ≥ 60 ng/mL the postoperative dose was 0.05 mg/kg bid. The authors conclude that a pre-transplant tacrolimus 2-hour blood concentration analysis, does not significantly increase the proportion of subjects achieving 10 ng/mL tacrolimus concentrations by day 3 using routine protocols. However, it does lead to patients achieving a whole-blood concentration of ≥ 10 ng/mL sooner.

Tacrolimus in pediatric patients

Table 2 shows the available dosing algorithms for the starting dose of tacrolimus in pediatric renal transplant recipients.

All the dosing algorithms for the initial dose of tacrolimus in pediatric kidney recipients include bodyweight. There is a relationship between age, body size and the rate of tacrolimus overexposure within the first three weeks post-transplantation in pediatric renal transplant recipients when the starting dose is based on bodyweight[103]. Kausman *et al*. suggest basing the starting post-operative tacrolimus dose solely on bodyweight, however he does distinguish whether the child weighs more or less than 40 kg[103]. De Wildt *et al*. made this distinction based on age and bodyweight[50]. Both de Wildt *et al*. and Zhao *et al*. have designed an algorithm including *CYP3A5* genotype[50, 104].

Ciclosporin in adult transplant recipients

An overview of the available dosing algorithms for the starting dose of ciclosporin in kidney recipients is presented in table 3.

Table 2. Tacrolimus starting dose algorithms for pediatric renal transplant recipients.

			COVARIATES USED IN ALGORITHM						
AUTHOR	**N**	**AGE (years)[a]**	**BW**	**HCT**	**CYP3A5**	**COMED**	**POD**	**OTHERS**	**FINAL ALGORITHM**
Kausman *et al*[103]	63	13.3[b] (1.9-17.7)	X				X		<40 kg: 0.3 mg/kg/day >40 kg: 0.2 mg/kg/day, maximum 10 mg/dose
Zhao *et al*[104]	50	10 (2-18)	X	X	X				Dose = 13.9 x $(BW/70)^{0.75}$ x [(2.26, if CYP3A5*1 genotype) or (1.00, if CYP3A5*3 genotype)] + 7.11 x [(1.74(if HCT<33%) or (1.00, if HCT≥33%)] x 0.24
De Wildt *et al*[50]	48	11.5[b] (1.5-17.7)	X		X			Age	<5 years, CYP3A5*1/*3 or *1/*1: 0.19 mg/kg bid <5years CYP3A5*3/*3: 0.13 mg/kg bid >5 years, CYP3A5*1/*3 or *1/*1: 0.13 mg/kg bid >5 years CYP3A5*3/*3: 0.09 mg/kg bid

BW: body weight, HCT: hematocrit, CYP3A5: CYP3A5 genotype, COMED: relevant comedications, POD: time post-transplant.
[a] Mean (range)
[b] Median

Table 3. Ciclosporin starting dose algorithms for adult transplant recipients.

			COVARIATES USED IN ALGORITHM							
AUTHOR	**N**	**GRAFT**	**BW**	**HCT**	**CYP3A5**	**COMED**	**POD**	**OTHERS**	**FINAL ALGORITHM**	**TDD average patient[b]**
Wu *et al[58]*	120	Kidney	X	X		X	X	Age, TBIL	Dose = (28.5 -1.24 x POD - 0.252 x (TBIL - 11) + 0.188 x (BW - 58) - 0.191 x (AGE - 42) - 2.45 (if diltiazem or verapamil is present) - 0.212 x (HCT - 28)) x 4.8	129 mg
Song *et al*[107]	69	Kidney			X		X		Dose = (3.32 x $POD^{-0.00002}$ x [($e^{2.89}$, if CYP3A5*1) or ($e^{2.72}$, if CYP3A5*3/*3)] x 4.8	242 mg
Chen *et al*[105]	146	Kidney	X				X	TBIL, ABCB1, gender	Dose = (49.5 x $POD^{-0.18}$ x (1.09, if female) x $BW^{0.46}$ x $TBIL^{-0.11}$ x (1+ABCB1 x -0.053)) x 4.8[a]	1095 mg
Sun *et al*[119]	124	Liver		X		X		Duration of CSA therapy	Dose = (23.1– 0.07 x HCT + 0.04 x PR) x 4.8	97 mg

BW: body weight, HCT: hematocrit, CSA: ciclosporin, CYP3A5: CYP3A5 genotype, COMED: relevant comedications, POD: time post-transplant, TBIL: total bilirubin, ABCB1: ABCB1 genotype, PR: prednisone dose, TDD: total daily dose.

[a] ABCB1: 0=CGC/CGC, 1=CGC/other, 2=other/other, 3=CGC/TTT, 4=other/TTT, 5=TTT/TTT

[b] male, 50 years old, 175 cm, 70 kg, received prednisolone 25 mg bid, no calcium channel blockers, hematocrit 0.35 L/L, CYP3A5*3/*3, bilirubin 10 µmol/L, ABCB1 CGC/TTT.

Compared to tacrolimus, there are relatively few dosing algorithms available for the starting dose of ciclosporin. In 2005, such a dosing algorithm for ciclosporin was created by Wu *et al*. using a combination of clinical factors in adult kidney transplant recipients[58]. The algorithm showed that the clearance of ciclosporin was significantly influenced by bodyweight, hematocrit, the use of calcium channel blockers, days post-transplant, age and total bilirubin level. A few years later, Chen *et al*. also found bodyweight, days post-transplant and total bilirubin level to significantly influence the clearance of ciclosporin. Interestingly, also *ABCB1* genotype was a covariate of ciclosporin clearance in this algorithm[105].

Contrary to tacrolimus, Press *et al*. concluded that bodyweight is the most important covariate and explains 35% of the random inter-individual variability in ciclosporin clearance[106]. Song *et al*. designed an algorithm including *CYP3A5* genotype and days post-transplant[107].

Ciclosporin in pediatric patients

In Helsinki it is common practice since 1988 to perform a pre-transplant pharmacokinetic profile assessment for ciclosporin[67, 71, 108-111]. Pediatric transplant candidates are given an intravenous 4 hour infusion of 3 mg/kg ciclosporin and at least 24 hours later, an oral dose of 10 mg/kg. Twelve blood samples are obtained in the 28 hours after the intravenous dose, and 10 samples in the 24 hours following the oral dose. The ciclosporin levels pre-transplant are then used to calculate the appropriate starting dose of ciclosporin post-transplant. The purpose of pre-transplant pharmacokinetic profiling is to reach the target level of ciclosporin more rapidly after transplantation. This intensive pre-transplant pharmacokinetic test design is time consuming, expensive and demanding on the patients. Currently Fanta *et al*. are investigating ways to improve their design[109].

Besides pre-transplant PK profile assessment to individualize dosing, we could not identify any pediatric dosing algorithms for ciclosporin.

MPA

No dosing algorithms or equations were found for MPA in kidney transplant recipients. Although there is a rationale for MPA TDM, it remains a matter of ongoing debate whether concentration controlled dosing of its pro-drug mycophenolate mofetil (MMF) is superior to using a fixed drug dose[5]. Based upon the well-established drug-drug interaction between MPA and ciclosporin, one could argue that in ciclosporin treated patients the MMF/EC-MPS starting dose should be 30-50% higher compared to patients treated with tacrolimus[19]. There is no consensus regarding the impact of glucocorticoid use on MPA exposure. Probably, if there is such an effect, it is likely to be of minor clinical relevance. Therefore, implementing steroid-use or steroid-avoidance as a variable in deciding on the best MPA dose cannot be supported. Several studies have shown the importance of reaching MPA target concentrations within the first week post-transplant, and as a result implementing dosing algorithms for MPA may be clinically relevant, even in a setting where TDM is not performed[112].

There is no additional data available for dosing MMF in children.

LIVER TRANSPLANTATION DOSING ALGORITHMS

Tacrolimus in adult transplant recipients

Table 4 shows an overview of the available dosing algorithms for the starting dose of tacrolimus in hepatic transplant recipients.

The six presented dosing algorithms were all created using population PK modeling, with NONMEM.

Only two dosing algorithm take the *CYP3A5* genotype into consideration. Li *et al*. based the starting dose on *CYP3A5* genotype of both donor and recipient in addition to bilirubin[113]. Gerard *et al*. found bodyweight, hematocrit and *CYP3A5* donor genotype to be relevant covariates[53]. An alternative strategy is presented by Oteo *et al*. who base the starting dose on AST[114]. Patients with an AST of > 500 U/L or with a slow recovery of liver function, receive a lower starting dose.

Tacrolimus in pediatric patients

An overview of the available dosing algorithms for the starting dose of tacrolimus in pediatric liver transplant recipients is presented in table 5.

In line with the kidney transplant recipients, most dosing algorithms for liver transplant recipients include bodyweight and/or age. The only exceptions are from Yang *et al*. and Staatz *et al*.[115, 116].

From the algorithms in table 5 it can be concluded that days post-transplant significantly influence the clearance of tacrolimus, with the exception of Staatz *et al*[116]. A possible explanation for this is that in the study of Staatz *et al*, not all children received glucocorticoid therapy. Interestingly, Staatz *et al*. were the only ones to take transplant type (whole or partial liver) into account[116]. The algorithm designed by Abdel Jalil *et al*. is the only one to include both the recipient's genetic information and clinical factors[92]. Musuamba *et al*. produced the most elaborate dosing algorithm, including bodyweight, hematocrit, liver weight and days post-transplant[117]. A more basic approach is proposed by Wallin *et al*. who give a model for the early post-transplantation phase[118].

Ciclosporin in transplant recipients

An overview of the available dosing algorithms for the starting dose of ciclosporin in liver recipients is presented in table 3.

For hepatic transplant recipients, only one dosing algorithm for the starting dose was found. Sun *et al*. concluded that the clearance of ciclosporin was significantly influenced by hematocrit, duration of ciclosporin therapy and prednisone dose[119].

There are no dosing algorithms available for ciclosporin in pediatric liver transplant recipients.

MPA

No dosing algorithms or equations were found for MPA in liver recipients.

Table 4. Tacrolimus starting dose algorithms for adult hepatic transplant recipients.

AUTHOR	N	COVARIATES USED IN ALGORITHM						FINAL ALGORITHM	TDD average patient[b]
		BW	HCT	CYP3A5	COMED	POD	OTHERS		
Fukatsu *et al*[125]	35					X	TBIL, serum creatinine, HW	Dose = ((0.737 + 0.0134 x POD) x (0.728, if TBIL >2.5 mg/dl) x (0.809, if serum creatinine > 1 mg/dl) x (HW/600) / 0.0677) x 0.24	2.2 mg
Fukudo *et al*[126]	47					X	TBIL, serum creatinine, HW	Dose = ((0.743 + 0.0157 x POD) x (0.792 if TBIL >2.5 mg/dl) x (0.810, if serum creatinine > 1 mg/dl) x (HW/600) / 0.0732) x 0.24	2.1 mg
Gerard *et al*[53]	66	X	X	X				For HCT 19%, 29% or 43% respectively: CYP3A5*1/*1: 0.22, 0.16, or 0.10 mg/kg/day CYP3A5*1/*3: 0.16, 0.11, or 0.07 mg/kg/day CYP3A5*3/*3: 0.11, 0.07, or 0.05 mg/kg/day	4.9 mg
Oteo *et al*[114]	85					X	AST	Standard group: Dose = 11.10 x 0.24 AST > 500 U/L Slow recovery: Dose = 8.04 x 0.24	2.7 mg
Li *et al*[113]	104			X		X	TBIL, CYP3A5 donor	Dose = (15.9 – 1.88 TBIL + 7.65 (if donor CYP3A5*1) + 7.00 (if recipient CYP3A5*1)) x 0.24[a]	3.8 mg
Zahir *et al*[127]	67		X		X		ALB, fluconazole	Dose = (21.3 + 9.8 (if HCT <33%) + 3.4 (if ALB<3.5g/dL) - 2.1 (if diltiazem is coadministered) - 7.4 (if fluconazole is coadministered)) x 0.24	5.1 mg

BW: body weight, HCT: hematocrit, CYP3A5: CYP3A5 genotype, COMED: relevant comedications, POD: time post-transplant, TBIL: total bilirubin, HW: hepatic weight, AST: aspartate aminotransferase, ALB: albumin, TDD: total daily dose.

[a] Discrete values for TBIL should be used according to table 2 of the original article[113].

[b] 70 kg, no calcium channel blockers, no fluconazole, hematocrit 0.35 L/L, albumin 40 g/L, AST 25 U/L, CYP3A5*3/*3 for donor and recipient, bilirubin 10 µmol/L, hepatic weight 615 g, serum creatinine of 100 µmol/L.

Table 5. Tacrolimus starting dose algorithms for pediatric hepatic transplant recipients.

			COVARIATES USED IN ALGORITHM						
AUTHOR	**N**	**AGE (years)[a]**	**BW**	**HCT**	**CYP3A5**	**COMED**	**POD**	**OTHERS**	**FINAL ALGORITHM**
Abdel Jalil *et al*[92]	43	5 (0.7-17.6)	X		X		X		Dose = (12.9x (BW/13.2)$^{0.75}$x e$^{-0.00158 \times POD}$ x (e$^{0.428}$, if CYP3A5*1) x 0.24
Wallin *et al*[118]	73	3.5 (0.4-16.9)	X				X		Day 0 + 1: 0,1mg/kg bid Day 2: 0.04 mg/kg$^{0.75}$ bid Day 3: 0.06 mg/kg$^{0.75}$ bid
Musuamba *et al*[117]	82	1.0[b] (0.3-14.1)	X	X		X	X	Hepatic weight	Dose = (0.001 + (13.9 x POD/3.97 + POD) x (BW/60)$^{0.21}$ x (HCT/28%)$^{-0.04}$ x (HW/255)$^{0.18}$ x 0.82 (if nifedipine or fluconazole is present)) x 0.24
Yang *et al*[115]	52	1.8 (0.4-17.8)					X	ALT	Dose = (5.72 x POD$^{0.152}$ x (ALT/70)$^{-0.111}$) x 0.24
Guy-Viterbo *et al[54]*	42	1.4[b] (0.5-10.9)	X	X			X		Not specified in article
Fukudo *et al*[55]	100	1.2[b] (0.1-15)	X		X		X	AST, ABCB1	Dose = ((0.134 x (1.8, if ABCB1 mRNA >0.22 amol/µg total RNA) + 0.0181 x (2, if CYP3A5*1) x POD) x 8.6 x (BW/8.6)$^{0.341}$ x e$^{-0.0358 \times AST/53)}$) x 0.24
Staatz *et al[116]*	35	5.7 (0.5-16.6)						Graft type, AST, Age, GGT	Not specified in article
Sam *et al[128]*	16	3.7 (1.1-13.9)	X					Age, TBIL	Dose = (1.46 x [1 + 0.339 x (AGE – 2.25)] / (0.197 x [1 + 0.0887 x (BW – 11.4)] x 1.61 (if TBIL ≥ 200µmol/L)) x 0.01

BW: body weight, HCT: hematocrit, CYP3A5: CYP3A5 genotype, COMED: relevant comedications, POD: time post-transplant, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma glutamyl transferase, graft type: cut-down or full liver.
[a] Mean (range)
[b] Median

CONCLUSION

Starting doses of tacrolimus and ciclosporin are typically chosen on a calculated mg/kg bodyweight basis. This is remarkable as bodyweight is only one of many co-variates predicting the drug dose required to reach a certain target concentration, especially for tacrolimus. Nevertheless, almost all centers use a tacrolimus starting dose of 0.2 mg/kg in adults and 0.3 mg/kg in children, divided over two separate gifts. After initiation of treatment, doses are corrected with TDM. Drug concentrations are usually measured multiple times in the first few weeks after transplantation, and following a number of dose adjustments the target concentration is reached in the majority of patients. This trial and error approach has been accepted by most physicians and pharmacists involved in the care of transplanted patients.

Only fairly recently, a number of dosing algorithms have been proposed to better individualize the starting dose. In some of these algorithms, bodyweight is one of the co-variates taken into account, but multiple other co-variates influence the starting dose. As shown in tables 1-5 also hematocrit, co-medication and genotype are integrated in many of these algorithms. From a theoretical point of view, these algorithms provide a more rational selection of the starting dose. By reaching target concentrations more quickly, and by avoiding substantial deviations from the target concentrations (both above and below target) transplantation outcome might be improved. Potentially, acute rejection incidence would be lower, as under-exposure is avoided, and drug toxicity may also be reduced, by limiting the number of drug concentrations (way) above target.

Expert opinion

Although the concept of implementing algorithms to guide the starting dose of immunosuppressants is appealing, it is good to consider a number of concerns. First of all, many of these proposed algorithms have not been validated. Some investigators have developed an algorithm on a single dataset but only the algorithms designed by Passey et al. and Musuamba et al. were tested in an independent validation set[94, 117]. The algorithms designed by Thervet et al. and Chen et al. were prospectively tested in a randomized clinical trial [99, 120]. Zhang et al. prospectively tested their equation in 48 de novo renal transplant recipients [100]. In only few cases, the published algorithm has been tested by other independent investigators in completely new patient populations[95]. Importantly, if this was done then the performance of the algorithms was often considerably less. To show the potential impact of these dosing algorithms on the actual starting dose, we have tested the adult algorithms by calculating starting doses for a standard kidney or liver transplant recipient, of 50 years, 70 kilograms, and other baseline characteristics typical for a *de novo* transplant recipient. For this patient, the standard starting dose for tacrolimus would be 14 mg bid and for ciclosporin approximately 210 mg bid. In the tables we have added one column showing the outcome of the calculated starting dose for each of these algorithms. There are huge differences in the starting doses calculated with these different algorithms. In some cases these differences may be due to the fact that the algorithm was developed on tacrolimus concentration/dose data collected over a longer period of time, and not only within the immediate post/transplant period. Within the first months after transplantation there is an impressive change in the dosages required to reach the target concentrations. In this period of time a substantial

change in the ratio between concentration/dose occurs. Especially in the first 2 weeks a relatively high dose is needed, possibly because bioavailability is reduced, clearance is high, or since time to reach steady-state may take up to the first week.

A second concern is the possibility that by implementing the algorithm some patients may end up with drug concentrations that are far off from target. For example, expressers of the CYP3A5 enzyme would receive higher starting dose of 0.3 mg/kg. Possibly in some of these patients this higher dose may result in very high blood concentrations. None of the algorithms have been tested in a *de novo* transplant population, and no comparisons have been made between the drug concentrations of standard dosing, or algorithm-based dosing. Only *in silico* validations have been performed so far.

A third concern relates to the lack of evidence that clinical outcome will improve. By performing TDM we are quite effective in reaching target concentrations. We have previously shown that 10 days after transplantation the actual tacrolimus concentrations in renal transplant patients expressing CYP3A5, and those not expressing CYP3A5, were not different[121]. Tacrolimus dosages were substantially different, as a result of repetitive dose adjustments within these first 10 days. It is questionable if a few days' delay in reaching target will translate into impaired clinical outcome, such as more acute rejection episodes, or more delayed graft function, nephrotoxicity or neurotoxicity.

A fourth concern is that the newly developed algorithms are often published in pharmacokinetic journals, making them less accessible to physicians. Especially NONMEM papers can be difficult to interpret correctly, and tend to discourage most physicians and pharmacists. The newly developed algorithms should be published in clinical journals in an easy to read manner. For example, the algorithm should be written to determine the dose, not the CL/F. Another way to make the algorithm more accessible is to incorporate it in a secure website as shown by our colleagues in Limoges[122].

Based on the above, we propose that newly developed algorithms aiming to select the best starting dose need to be based on early drug concentration data only. They also need to be validated in an independent validation set and may be subjected to *in silico* simulations. Subsequently, the algorithm needs to be tested in newly transplanted patients. In a first exploratory study the pharmacokinetic end-points may prevail, investigating if the use of the algorithm reduces the time to reach the target concentration, and reduces the number of patients with a too high, or too low concentration. If this pharmacokinetic study shows that implementation of the algorithm results in more patients reaching the right target more quickly, then a larger clinical trial could be performed to show the benefit of the algorithm in terms of clinical outcome. Whether or not such trials will ever be performed is doubtful, as sample size will need to be large, and funding for such trials will be hard to find. What the primary endpoint for such trials should be is open for discussion, but incidence of acute rejection is an obvious possibility.

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2

AIMS AND OUTLINE OF THIS THESIS

AIMS AND OUTLINE OF THIS THESIS

The primary objective of this thesis is to investigate the pharmacokinetics of tacrolimus in the direct post-transplantation phase and to use this information to better predict the tacrolimus exposure and thus adjust the starting dose following kidney transplantation for each individual patient. The secondary objective is to prospectively test the performance of the new dosing algorithm in newly transplanted patients.

The aims of this thesis are:

1. To give an overview of the published dosing algorithms for the starting dose of tacrolimus
2. To describe the pharmacokinetics of tacrolimus in the first weeks after kidney transplantation in paediatric kidney transplant recipients
3. To study the pharmacokinetics of tacrolimus in obese patients
4. To develop and validate a dosing algorithm for the starting dose of tacrolimus in both paediatric and adult renal transplant recipients
5. To investigate prospectively if the developed algorithms lead to more patients within the prespecified target range first steady state following transplantation

This thesis includes ten chapters divided over four parts.

Part I contains the introduction to the thesis.

Chapter 1 describes the available dosing algorithms to initiate immunosuppressive drugs in paediatric and adult solid organ transplant recipients.

Part II focuses on predicting tacrolimus exposure in children after a kidney transplantation.

In chapter 3 retrospective data was used to describe the pharmacokinetics of tacrolimus the first six weeks following transplantation, and to develop and validate a dosing algorithm for the starting dose of tacrolimus in paediatric renal transplant recipients. In chapter 4 this dosing algorithm was used to determine the initial dose of tacrolimus in newly transplanted children in a prospective trial.

Part III focuses on predicting tacrolimus exposure in adult renal transplant recipients.

Pharmacogenetic aspects of the use of tacrolimus in renal transplantation are reviewed in chapter 5. Chapter 6 discusses the dose of tacrolimus in (extreme) overweight patients and the risks of overdosing these patients. A validated population pharmacokinetic model to describe the pharmacokinetics the first three months following transplantation and to predict the individual starting dose of tacrolimus in adult renal transplant recipients is discussed in chapter 7.

Part IV summarises and discusses the results of these studies in the context of available relevant literature, and speculates on areas of future research (chapter 8 and 9).

II

PREDICTING TACROLIMUS EXPOSURE IN PEDIATRIC RENAL TRANSPLANT RECIPIENTS

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3

A POPULATION PHARMACOKINETIC MODEL TO PREDICT THE INDIVIDUAL STARTING DOSE OF TACROLIMUS FOLLOWING PEDIATRIC RENAL TRANSPLANTATION

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ABSTRACT

Background and objective

Multiple clinical, demographic and genetic factors affect the pharmacokinetics of tacrolimus in children, yet in daily practice a uniform body-weight based starting dose is used. It can take weeks to reach the target tacrolimus predose concentration (C_0). To determine the pharmacokinetics of tacrolimus right after kidney transplantation and to find relevant parameters for dose individualization, a population pharmacokinetic analysis was performed.

Methods

A total of 722 blood samples were collected from 46 children treated with tacrolimus over the first six weeks after renal transplantation. Nonlinear mixed-effects modeling (NONMEM) was used to develop a population pharmacokinetic model and perform covariate analysis. Simulations were performed to determine the optimal starting dose and to develop dosing guidelines.

Results

The data were accurately described by a two compartment model with allometric scaling for bodyweight. Mean tacrolimus apparent clearance was 50.5 L/h, with an inter-patient variability of 25%. Higher bodyweight, lower estimated glomerular filtration rate and higher hematocrit levels resulted in lower total tacrolimus clearance. Cytochrome P450 (CYP) 3A5 expressers and recipients who received a kidney from a deceased donor had a significantly higher tacrolimus clearance. The model was successfully externally validated. In total, these covariates explained 41% of the variability in clearance. From the significant covariates, *CYP3A5* genotype, bodyweight and donor type were useful to adjust the starting dose to reach the target C_0. Dosing guidelines range from 0.27 mg/kg/day to 1.33 mg/kg/day.

Conclusion

During the first 6 weeks after transplantation, the tacrolimus weight-normalized starting dose should be higher in pediatric kidney transplant recipients with a lower bodyweight, who express CYP3A5 and those who receive a kidney from a deceased donor.

INTRODUCTION

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

Tacrolimus is a critical dose drug with a narrow therapeutic index for which therapeutic drug monitoring (TDM) is routinely performed. Multiple factors, including bodyweight [10, 11], age [12, 13], drug interactions [14], hematocrit [10, 15], ethnicity [16], treatment with glucocorticoids [17] and cytochrome P450 (CYP) 3A genotype [10] affect the pharmacokinetics of tacrolimus. This has been extensively investigated in adults, however data in children are limited. Children younger than 5 years old appear to need higher weight-normalized doses of tacrolimus than older children to reach the target range [12]. The reason for this age-related increased clearance (CL) is unknown. It has been demonstrated that CYP3A5 expressers (those with the *CYP3A5*1/1* or *CYP3A5*1/*3* genotype) require at least a 1.5-fold higher tacrolimus dose compared to CYP3A5 non-expressers *(CYP3A5*3/*3* genotype) [10, 12, 18-21]. *CYP3A4*22* is associated with lower tacrolimus dose requirements post renal transplantation [22-24]. The *CYP3A4*1G* allele is associated with a higher tacrolimus dose requirement, however its contribution to tacrolimus exposure is less than half of that of *CYP3A5* genotype [25]. This effect is thought to be independent of *CYP3A5* status [16]. In routine clinical practice these factors are not taken into consideration. The starting dose of tacrolimus is usually based on bodyweight and then adjusted by means of TDM. TDM limits the time a patient is exposed to sub- and supra-therapeutic tacrolimus concentrations, but it can take up to two weeks to reach the target exposure range [26].

The use of a population pharmacokinetic model may help in predicting an individual's response to tacrolimus and can be applied before the start of therapy. To date, four models have been developed for pediatric renal transplant recipients [20, 27-30]. Of these models, only one was developed using transplant recipients in the immediate post-transplant phase and could therefore be used to determine the starting tacrolimus dose [10]. However, this model was developed using children that were treated in 9 hospitals with different immunosuppressive regimens, had sparse tacrolimus sampling, important covariates such as ethnicity were not included, and the model was not externally validated.

The aim of the current study was to describe the population pharmacokinetics of twice-daily immediate-release tacrolimus in the first weeks after pediatric renal transplantation and to develop a dosing guideline for the starting dose. In contrast to previous studies, all children were treated with the same immunosuppressive regimen, had an abbreviated time profile measured

with additional extensive tacrolimus sampling, important covariates such as ethnicity and *CYP3A5* genotype were included, and the model was externally validated.

MATERIALS AND METHODS

Study Design

A retrospective analysis of pediatric transplant recipients who received a donor kidney between November 2009 and April 2016 was performed. Clinical and demographic data were retrieved from the medical records for the first six weeks after pediatric renal transplantation. Data was collected in the pediatric nephrology department at the Erasmus MC - Sophia Children's Hospital, Rotterdam, The Netherlands. Eligible for enrolment were patients younger than 18 years, who received a kidney from an ABO compatible living or a deceased donor, and were treated with tacrolimus as part of their initial immune suppressive regimen. All clinical values were collected from 24 hours before transplantation until 6 weeks post-transplantation.

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

The study was designed in accordance with the Declaration of Helsinki of 1975. For the model building dataset all laboratory analyses, were performed for routine clinical practice. The Ethics Review Board (ERB) of the Erasmus MC decided that the rules laid down in the Medical Research Involving Human Subjects Act, do not apply to this study (Medical Ethical Review Board number 2017-092). The extra genotyping was approved by the ERB of the Erasmus MC (number 2010-219). For the validation dataset, the ERB of the RadboudUMC approved the genotyping for *CYP3A5* in leftover material (number 2014-1282). The parents or legal caregivers of all participants signed an informed consent prior to DNA collection.

Immunosuppression

All patients were treated according to the TWIST protocol with basiliximab, tacrolimus, mycophenolic acid, and a five-day course of glucocorticoids [31]. Both the twice-daily formulations Prograft® (Astellas Pharma, Leiden, The Netherlands), and Modigraf® (Astellas Pharma, Leiden, The Netherlands) granules for suspension were used. An extemporaneously prepared suspension from the Prograft® capsules in an oral suspending vehicle from our Good Manufacturing Practice certified pharmacy was used by some children. All children received an initial tacrolimus dose of 0.3 mg/kg/day divided into two doses every 12 hours. The subsequent doses were adjusted using TDM. In our hospital it is common to measure the tacrolimus predose concentration for the first time after 4-5 dosages of tacrolimus, *i.e.* approximately 3 days after transplantation. As this is a study with data obtained in routine clinical practice, not all patients had their first tacrolimus predose concentration measurement on day 3. This depended on multiple factors, including clinical factors (e.g. signs of tacrolimus toxicity or rejection), as well as logistic factors (tacrolimus concentrations are not routinely measured during the weekend in our hospital). In the first three weeks post-transplantation the target C_0 was 10-15 ng/mL, from then onwards the target C_0 was 7-12 ng/mL.

Approximately two weeks after transplantation, an abbreviated four hour tacrolimus concentration *versus* time profile was obtained. For these profiles, blood samples were taken before tacrolimus administration, and 10, 30, 90, 120 and 240 minutes post ingestion. The dose of immunosuppression and other co-medication was recorded in the electronic prescribing system during the entire study period. Tacrolimus sample collection times and time of latest ingestion were also recorded.

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

Laboratory Analysis

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

Population Pharmacokinetic Modelling

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

Base Model Development

Based on visual inspection of the data and a review of the literature, one and two compartment models were considered to describe the concentration-time data. Typical values for lag-time (t_{lag}), absorption rate constant (k_a), volume of distribution (V_d), CL and inter-compartmental clearance (Q) were estimated. Bioavailability could not be quantified, therefore certain parameters were estimated as ratios: CL/F, Q/F and V_d/F. Inter-individual variability (IIV) and inter-occasion variability (IOV) were modeled for each pharmacokinetic parameter using an exponential model, and residual variability was incorporated as an additive and proportional error for each analytical

method. Allometric scaling was used to account for variability in pharmacokinetic parameters due to differences in bodyweight. Shrinkage was calculated for all model parameters for which IIV was estimated. A shrinkage value below 20% was considered acceptable [35]. Model selection was based on minimum objective function values (OFVs), parameter precision, error estimates, shrinkage values and visual inspection of the GOF plots.

Covariate Model Development

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

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

Model Evaluation

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

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

Table 1. Patient Characteristics the first six weeks after transplantation

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

Table 1. (continued)

	Model building group (n = 46)	Model validation group (n = 23)
Donor type		
Living	36 (78.3%)	19 (82.6%)
Deceased	10 (21.7%)	4 (17.4%)
Mismatch		
0	3 (6.5%)	Unknown
1	8 (17.4%)	Unknown
2	12 (26.1%)	Unknown
3	23 (50.0%)	Unknown
Tacrolimus initial daily dose (mg)*	0.29 (0.23-0.39)	0.31 (0.24-0.54)
First available tacrolimus C_0 (ng/mL)	8.7 (2.0-51.4)	8.8 (1.7-29.6)
Route of administration		
Suspension	13 (28.3%)	Unknown
Capsule	45 (97.8%)	Unknown
Co-medication		
Calcium channel blockers		
Amlodipine	15 (32.6%)	6 (24.0%)
Nifedipine	13 (28.3%)	0 (0%)
Antibiotics		
Erythromycin	1 (2.2%)	0 (0%)
Antimycotics		
Fluconazole	2 (4.3%)	Unknown
Voriconazole	1 (2.2%)	0 (0%)
Glucocorticoids (prednisolone equivalents) dose/day (mg/kg)	0.8 (0.1-17.8)	Unknown

ASAT aspartate aminotransferase, C0 predose concentration, CAKUT congenital anomalies of the kidney and the urinary tract, CYP cytochrome P450, eGFR estimated glomerular filtration rate, RRT renal replacement therapy
* Presented as median and range for continuous variables.

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

RESULTS

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

Base Model

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

Covariate Analysis

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

$$CL/F = 50.5 * \left(\frac{weight}{70}\right)^{0.75} * [(1.0, if\ CYP3A5*3/*3\ or\ unknown)\ or\ (2.0, if\ CYP3A5*1/*3\ or\ CYP3A5*1/*1)]$$

$$* (0.74, if\ living\ donor) * \left(\frac{eGFR}{69}\right)^{0.19} * \left[\left(\frac{hematocrit}{0.3}\right)^{-0.44}, if\ hematocrit < 0.3\right]$$

Evaluation of the Final Model

All estimates were within the limits, given the criteria as defined under "Materials and Methods", except for shrinkage on V_2 which was 27%. GOF plots of the final model showed the population predictions and individual predictions were evenly distributed around the line of unity, and the conditional weighted residuals were normally distributed (Figure 1).

A bootstrap analysis with 500 bootstrap replicates was performed to obtain 95% CIs for all pharmacokinetic parameters. Due to minimization and boundary errors, the bootstrap results were recomputed without filtering these samples. Results of the bootstrap are shown in Table 2. VPC showed that the median and the variability fell within the corresponding simulations (Figure 2A). This demonstrates the good predictive performance of the final model in the internal validation. Evaluation of the predictive performance by NPDE analysis showed adequate predictive ability, with distribution of the NPDEs not significantly deviating from a normal distribution and the majority of the values were between -2 and 2. Evaluation of the individual's influence on change in OFV by sharkplot showed 70% of the included children had a decrease in OFV with the final model *versus* the base model. In the external validation the median and variability were adequately described, confirming the validation of the model (Figure 2B). There was an insufficient number of *CYP3A5* expressers in this validation cohort to validate the algorithm for this subgroup.

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

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

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

Simulations

Based on the final model, CYP3A5 expressers had a 2 times higher apparent oral clearance (CL/F). An increase in eGFR from 30 to 90 ml/min resulted in 19% higher CL/F, whereas a decrease in hematocrit levels from 0.3 to 0.25 L/L corresponded with a 20% higher tacrolimus CL/F. Deceased donor was associated with a 35% higher tacrolimus CL/F than living donor. The effect of *CYP3A5* genotype, hematocrit, eGFR, donor and bodyweight on CL/F are shown in Figure 3. In total, these covariates explained 41% of the variability in CL/F.

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

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

CYP cytochrome P450, *CL* clearance, *eGFR* estimated glomerular filtration, k_a absorption rate constant, *OFV* objective function value, V_2 peripheral compartment for tacrolimus.
[a] Caucasian, Asian and Black ethnicity respectively compared with subjects from other ethnicities.
[b] CYP3A5 non-expresser and expresser respectively compared with unknown genotype.
[c] Living donor compared with a deceased donor.
[d] Age > 7 years was chosen after visual inspection of the data. Age as a continuous variable was not a significant covariate.
[e] No mismatches compared with one or more mismatches.
[f] *CYP3A4*1/*1*, *CYP3A4*1/*1G* and *CYP3A4*1G/*1G* respectively compared with unknown genotype.

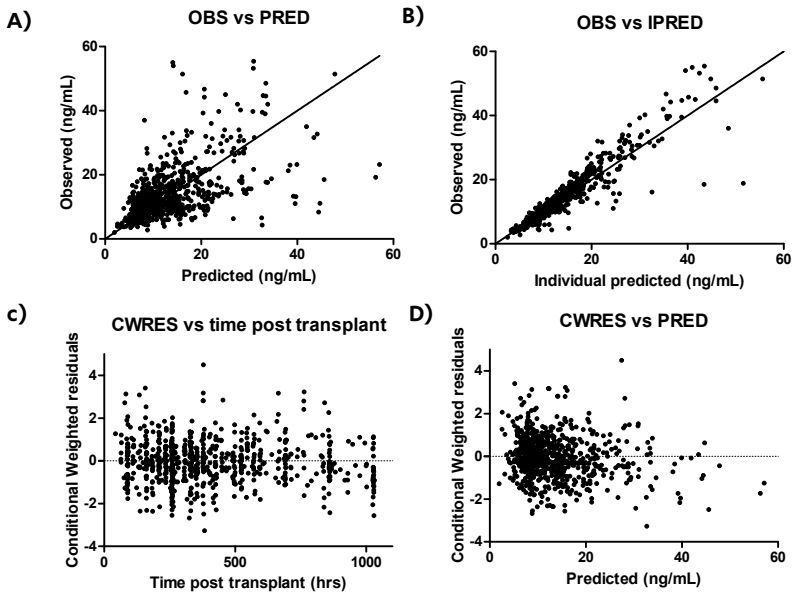

Figure 1. Goodness-of-fit plots of the final model. **A** DV plotted against PRED. **B** DV plotted against IPRED. **C** The correlation of CWRES with the time after the tacrolimus dose. **D** The correlation of CWRES with PRED. The line represents the line of identity. *CWRES* conditional weighted residuals, *DV* observed concentrations, *IPRED* individual predicted concentration, *OBS* observed concentration, *PRED* predicted concentration.

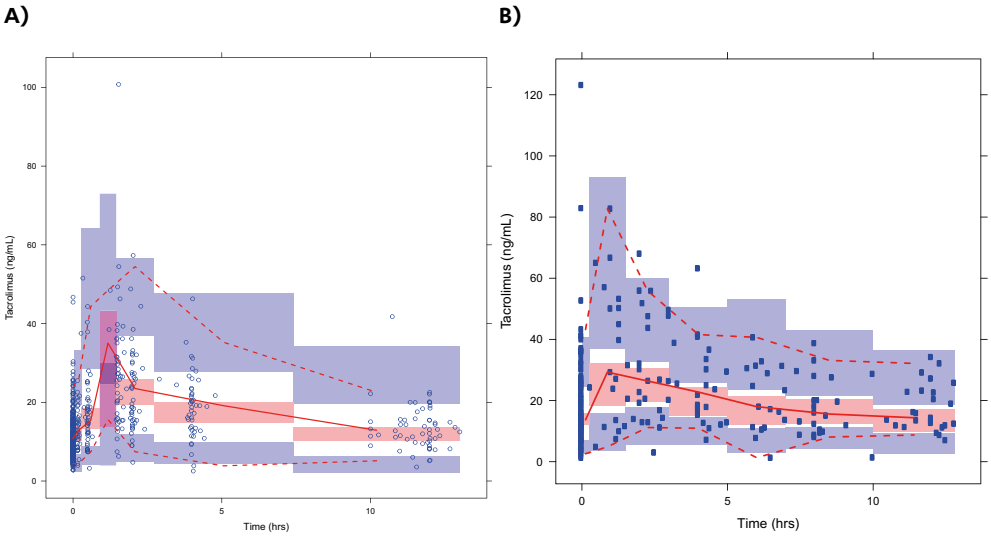

Figure 2. Prediction corrected visual predictive check showing how well the average trend of the observations (solid line) and how well the variability of the observed data (two dashed lines) fall within the model simulated average trend (red shaded area) and the model simulated variability (blue shaded areas) represented as 95% CI. The average and the variability of the observed data both fall within the corresponding simulations. **A** internal VPC of the final model. **B** external VPC of the final model. *VPC* visual predictive check.

Dosing guidelines

The model was used to design a dosing algorithm for the starting dose of tacrolimus after pediatric kidney transplantation. The last measured eGFR and hematocrit before transplantation did not significantly influence the CL/F and because these parameters change tremendously post transplantation, were not incorporated in the algorithm for the starting dose. The following equation described the estimation of tacrolimus CL/F (L/hr) right after transplantation:

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

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

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

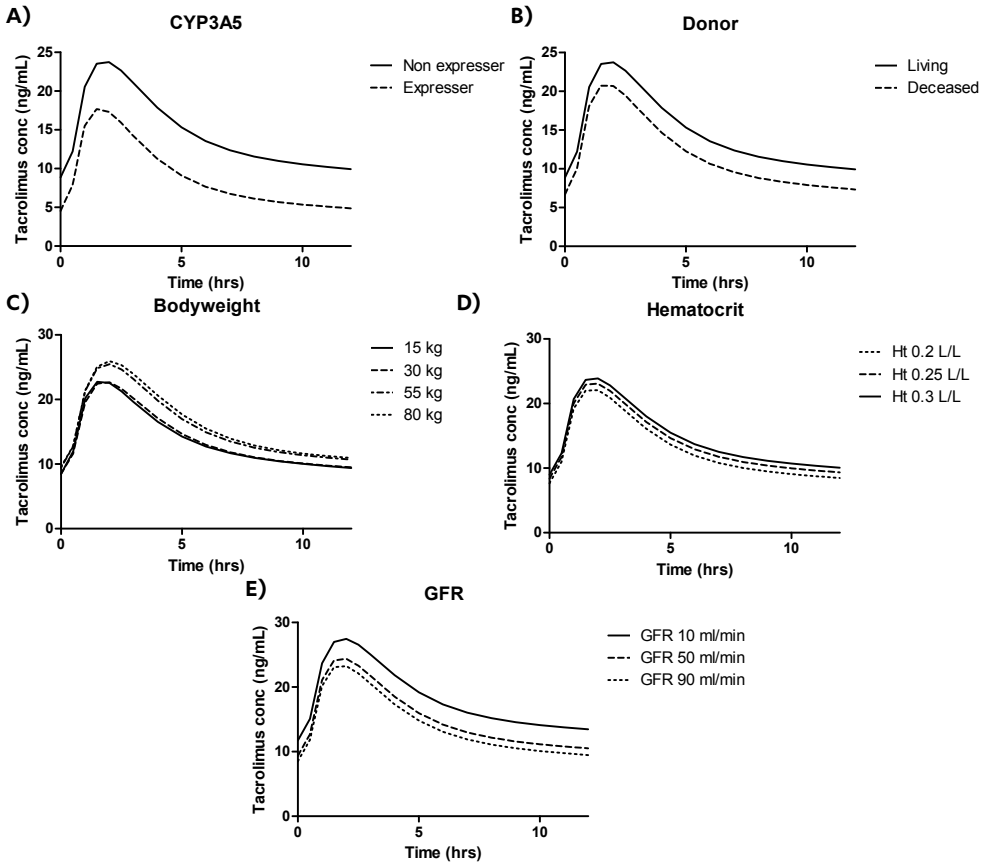

Figure 3. Simulated plasma profiles of tacrolimus at first steady state after transplantation. **A** Simulated plasma profiles of tacrolimus for CYP3A5 non-expressers (*CYP3A5*3/*3)* and CYP3A5 expressers (*CYP3A5*1/*1* or *CYP3A5*1/**3). **B** Simulated plasma profiles of tacrolimus for patients receiving a kidney from a living or deceased donor. **C** Simulated plasma profiles of tacrolimus for patients with a bodyweight of 15, 30, 55 or 80 kg. **D** Simulated plasma profiles of tacrolimus for patients with plasma hematocrit levels of 0.2, 0.25 and 0.3 L/L. **E** Simulated plasma profiles of tacrolimus for patients with an eGFR of 10, 50 or 90 ml/min.*CYP* cytochrome P450, e*GFR* estimated glomerular filtration rate.

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

Table 4. Dosing guidelines for the starting dose of tacrolimus with a target C_0 of 10-15 ng/mL

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

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

DISCUSSION

This is the second population pharmacokinetic study of tacrolimus in pediatric renal transplant recipients covering the first six weeks after transplantation. Our results showed that a two compartment model with first-order absorption, lag time, allometric scaling, IIV on CL, V_1, V_2 and k_a, and IOV on CL and V_2 was optimal for data modelling. Besides bodyweight, we also demonstrated that *CYP3A5* genotype, hematocrit, eGFR and donor type all significantly influence the tacrolimus CL. Together these covariates explained 41% of the variability in tacrolimus CL. Calculation of tacrolimus dosing guidelines for the initial dose after transplantation showed a factor 4 difference in daily dose, ranging from 0.27 mg/kg to 1.33 mg/kg. This is vastly different from the 0.3 mg/kg/day currently used in clinical practice [40]. At first steady state only 26.1% of patients were within the tacrolimus target predose concentration range, 67.4% had subtherapeutic tacrolimus concentrations and 6.5% supratherapeutic concentrations. After a median of 9.8 days (range 1.7 - 23.8 days) patients were within the tacrolimus target predose concentration range.

Genetic polymorphisms in *CYP3A5* partly explain the IIV in tacrolimus pharmacokinetics. In this study, 25.9% of the included children of whom the genotype was known, were CYP3A5 expressers. In agreement with previously published data, we report a significantly lower CL/F in children having the *CYP3A5*3/*3* genotype compared with children carrying at least one *CYP3A5*1* allele. Given the wide availability of TDM, genetic testing is most useful prior to initiation of tacrolimus

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

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

Contrary to what we expected, kidneys from a deceased donor had a higher tacrolimus CL than kidneys from a living donor. This finding was confirmed in the external validation of the model. A literature search was performed and showed no previous reports substantiating this observation. Dialysis prior to kidney transplantation or pre-emptive kidney transplantation was not significantly associated with the tacrolimus CL, and neither was the number of HLA mismatches. As tacrolimus undergoes hepatic metabolism, a higher tacrolimus CL in kidneys from a deceased donor seems highly unlikely. All patients received the same immunosuppressive protocol, regardless of the donor status. Only if there was a slow graft function, the start of tacrolimus was postponed and patients continued treatment with glucocorticoids. This occurred in four patients, but none of them experienced delayed graft function in sensu stricto (i.e. the need for dialysis in the first week after transplantation). However no correlation between slow graft function and tacrolimus CL was found in these patients. Furthermore glucocorticoid use was tested as a covariate and did not significantly influence the tacrolimus CL. The higher tacrolimus CL in kidneys from a deceased donor is probably caused by other and unknown parameters that could not be tested as covariates and therefore cannot be corrected for. One of these parameters could be the interaction between donor and age. Recipients of a deceased donor kidney tended to be younger than recipients of a living donor kidney transplant, although this was not statistically significant. Another explanation could be other non-investigated parameters like serum albumin, anemia and metabolic acidosis. Recipients of deceased donors are usually more catabolic and might therefore have a higher free fraction of tacrolimus. This is however, not supported by our data which show that the difference in CL between living and deceased donors does not decrease during the first six weeks post-transplantation.

Approximately 70-80% of tacrolimus is distributed in erythrocytes [43]. This indicates that low hematocrit concentrations will reduce the whole-blood concentrations of tacrolimus. In our cohort, children with a hematocrit level < 0.30 had an increased CL/F and thus required a higher tacrolimus dose. This is in line with previous findings in pediatric kidney recipients [10, 20].

More than 98% of tacrolimus in the plasma is bound to plasma proteins, and despite measuring the tacrolimus concentration in whole-blood, it is actually the unbound concentration that is pharmacologically active [44]. Hematocrit levels do not seem to influence the unbound fraction of tacrolimus in plasma [45]. Low albumin concentrations will increase the tacrolimus unbound fraction. Unfortunately, there were not enough albumin measurements available to determine if there was a relationship between albumin and tacrolimus CL/F. A recently published study reported the validation of an assay specifically developed for measurement of unbound tacrolimus concentrations [46].

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

There were no patients expressing *CYP3A4*22*, and therefore we could not confirm the reported relationship between *CYP3A4*22* and tacrolimus CL [22]. An association between the glucocorticoid dose and tacrolimus CL has been reported [53, 54]. We could not substantiate this finding, probably because all children were treated with a glucocorticoid-minimization regimen and prednisolone was only prescribed the first five days in a relatively low dose.

The main strength of this study was the extensive evaluation of the final model. Not only bootstraps, VPCs and an NPDE were performed, but the model was also externally validated. Another strength of the study is the large amount of blood samples per patient, including abbreviated tacrolimus pharmacokinetic curves. An extensive literature search was performed and all covariates known to influence the CL of tacrolimus, were tested. The population PK model was developed in patients treated according to the same immunosuppressive protocol. Other studies included children with different regimens, for example with azathioprine instead of mycophenolic acid or glucocorticoids in different doses, making it difficult to determine the effect of tacrolimus. The final strength of the study is that dosing guidelines for the starting dose of tacrolimus after renal transplantation were developed. These dosing guidelines will be prospectively tested.

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

CONCLUSIONS

The population PK of tacrolimus during the first 6 weeks after renal transplantation can be adequately described using the model presented in this paper. Higher bodyweight, lower eGFR and higher hematocrit levels resulted in lower tacrolimus clearance. CYP3A5 expressers and recipients who received a kidney from a deceased donor had a higher tacrolimus clearance. The tacrolimus weight-normalized starting dose should be higher in patients with lower bodyweight, CYP3A5 expressers and patients receiving a kidney from a deceased donor. By combining these parameters an individualized tacrolimus dosing regimen has been developed, which adequately predicts the target C_0 and hopefully will improve patient outcome.

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4

A POPULATION PHARMACOKINETIC MODEL DOES NOT PREDICT THE OPTIMAL STARTING DOSE OF TACROLIMUS IN PEDIATRIC RENAL TRANSPLANT RECIPIENTS IN A PROSPECTIVE STUDY; LESSONS LEARNED AND MODEL IMPROVEMENT

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ABSTRACT

Background and objective

Bodyweight based dosing of tacrolimus is considered standard care. Currently, at first steady state, a third of pediatric kidney transplant recipients has a tacrolimus predose concentration within the target range. We investigated whether adaptation of the starting dose according to a validated dosing algorithm could increase this proportion.

Methods

This was a multi-center, single arm, prospective trial with a planned interim analysis after 16 patients, in which the tacrolimus starting dose was based on bodyweight, *CYP3A5* genotype and donor status (living *vs*. deceased donor).

Results

At the interim analysis, 31% of children had a tacrolimus predose concentration within the target range. As the original dosing algorithm was poorly predictive of tacrolimus exposure, the clinical trial was terminated prematurely. Next, the original model was improved by including the data of the children included in this trial, thereby doubling the number of children in the model building cohort. Data were best described with a 2-compartment model with inter-individual variability, allometric scaling and inter-occasion variability on clearance. *CYP3A5* genotype, hematocrit and creatinine influenced the tacrolimus clearance. A new starting dose model was developed in which *CYP3A5* genotype was incorporated. Both models were successfully internally and externally validated.

Conclusion

The weight-normalized starting dose of tacrolimus should be higher in patients with a lower bodyweight and in those who are *CYP3A5* expresser.

Keypoints

- A validated dosing algorithm could poorly predict the individual starting dose of tacrolimus following renal transplantation in *CYP3A5* expressers receiving a kidney from a deceased donor.
- The dosing algorithm was improved and the weight-normalized starting dose of tacrolimus should be higher in patients with lower bodyweight and in those who are *CYP3A5* expressers.
- This study demonstrates that even though a model is validated on paper, it is not necessarily effective in clinical practice. Dosing algorithms should be tested in prospective studies first.

INTRODUCTION

Tacrolimus is the most commonly used immunosuppressant to prevent acute rejection following renal transplantation [1-4]. Due to its huge medical impact, tacrolimus was chosen by scientists as one of the five molecules to take to a remote island [5]. Nonetheless, prolonged use of tacrolimus leads to substantial toxicity, including increased rates of infection, post-transplant diabetes mellitus, nephrotoxicity, neurotoxicity, hypertension and gastrointestinal disturbances [6-9]. These adverse events contribute to the limited long-term patient and kidney allograft survival and patient non-adherence [10, 11]. Adverse events seem to be related to higher tacrolimus concentrations, whereas rejection rates seem to be related to lower concentrations [12, 13]. It is thus important to reach the tacrolimus target concentration as soon as possible to limit the risk of rejection and reduce toxicity [12, 14].

Tacrolimus is a critical dose drug with a narrow therapeutic index and large intra- and interpatient variability, for which therapeutic drug monitoring (TDM) is routinely performed [13]. Many factors, including age [15, 16], bodyweight [17-19], cytochrome P450 (CYP) 3A genotype [17, 19], drug-drug interactions [20, 21], ethnicity [22, 23] and hematocrit [17, 19, 24, 25] influence the pharmacokinetics of tacrolimus. Contrary to adults, most published pediatric population pharmacokinetic (PK) models have included either bodyweight or age as a significant covariate influencing clearance (CL) [21, 26]. To reach the target range, children younger than five years old require higher weight-normalized tacrolimus doses than older children [15]. Currently in clinical practice and at first steady state, only 30% of patients are within the target range. Two thirds of children have a concentration outside the target range and 63.5% have subtherapeutic concentrations and 6.5% supratherapeutic concentrations [17]. In daily practice, the starting dose is often based solely on bodyweight, subsequent doses are adjusted using TDM, which limits the time a patient is exposed to concentrations outside the target range, but it can still take up to three weeks before target concentrations are reached [17].

The use of a population PK model may help in predicting an individual's tacrolimus exposure and can be applied before the start of therapy. Recently, our group developed a dosing algorithm to predict the tacrolimus starting dose in pediatric renal transplant recipients [17]. In this model, the starting dose is based on bodyweight, *CYP3A5* genotype and donor status (living vs. deceased). The model was extensively validated, both internally (bootstrap analysis, visual predictive check (VPC) and normalized prediction distribution errors (NPDE)) and externally (VPC) in an independent cohort consisting of 23 pediatric renal transplant recipients.

Here we report the results of a prospective clinical trial in pediatric renal transplant recipients in which the tacrolimus starting dose was based on this dosing algorithm [17]. The aim of this trial was to determine if basing the starting dose of tacrolimus on the validated dosing algorithm leads to a higher proportion of patients reaching the tacrolimus target predose concentration (C_0) range (10-15 ng/mL) at day 3 after transplantation. The number of children we planned to include was 28 and an interim analysis was planned after the inclusion of 16 children. The interim analysis demonstrated that the algorithm did not adequately predict the tacrolimus exposure and therefore the trial was stopped. Subsequently, a new and improved dosing algorithm was developed in a cohort in which the total number of included children was doubled compared to the original cohort.

METHODS

Clinical trial

This was an investigator-initiated, prospective, open-label, multi-center clinical trial. Pediatric patients (2-18 years) who were scheduled to receive a single-organ, blood group AB0-compatible kidney at the Erasmus MC-Sophia Children's Hospital in Rotterdam or the Radboudumc-Amalia Children's Hospital in Nijmegen were eligible for participation. Patients who received immunosuppressive drug treatment in the 28 days prior to transplantation (with the exception of glucocorticoids) and/or used drugs known to interact with tacrolimus (Table S1) were not included in the study.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (Erasmus MC, Medical Ethical Review Board number 2017-393) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was registered in the Dutch national trial registry (https://www.trialregister.nl/trial/6694). Written informed consent was obtained from all patients and/or their parents depending on the age of the patient before inclusion in the study. The PK study was also approved by the institutional review board of the Erasmus MC (Medical Ethical Review Board number 2017-092).

Intervention

Patients were prescribed a tacrolimus starting dose based on a published dosing algorithm [17]. This original dosing algorithm is shown in equation 1:

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

The dose was adjusted based on bodyweight, *CYP3A5* status and donor type. The dosing guideline ranged from 0.27 to 1.33 mg/kg as shown in Table S2. Both twice-daily formulations Prograf® capsules and Modigraf® granules for suspension (Astellas Pharma, Leiden, The Netherlands) were used. All doses were divided into two equal daily doses administered every 12h. All patients were treated according to the TWIST protocol with basiliximab, tacrolimus, mycophenolic acid and a 5-day course of glucocorticoids [14]. Patients were followed for 10 days post-transplantation.

2.1.3 Endpoints

The primary endpoint was the proportion of patients within the tacrolimus C_0 target range (10-15 ng/mL) after five unaltered tacrolimus doses on day 3 (first steady state) after transplantation. Hereafter, the physician could change the tacrolimus dose based on the tacrolimus C_0 or the clinical status of the patient. Subsequently, C_0 were drawn frequently according to local hospital protocol. Clinicians were encouraged to use the following formula to calculate the new dose:

$$New\ tacrolimus\ dose = \left(\frac{desired\ tacrolimus\ C_0}{current\ tacrolimus\ C_0}\right) * current\ tacrolimus\ dose$$

Secondary endpoints included the proportion of patients within the target range on day 7 and 10 following transplantation, and the proportion of patients with markedly sub-therapeutic (<5.0 ng/mL) or supra-therapeutic (>20 ng/mL) tacrolimus C_0 on day 3. Clinical endpoints included the incidence of BPAR and serious adverse events during follow-up (10 days) and the 30 days after the follow-up period. DGF was defined as the need for dialysis in the first week after transplantation.

Laboratory analysis

Tacrolimus concentrations were determined using a validated LC-MS/MS method as described previously [17]. Genotyping for *CYP3A4* and *CYP3A5* was performed as described previously [17].

Statistical analysis

With standard tacrolimus dosing the percentage of patients with a tacrolimus C_0 within the therapeutic target range on day 3 after transplantation is 30% in our population [17]. With model-based dosing this was expected to increase to at least 55%. For the sample size of the study a minimax Simon 2-stage design was used [27]. With α = 0.1 and β = 0.10, the required number of patients was 25 with an interim analysis involving the first 16 included patients. If after the inclusion of 16 patients, 4 or less patients (or 25%) were on target, inclusion of further patients would be terminated. The conclusion of the trial would then be that model-based dosing does not result in a sufficiently high proportion of patients with a C_0 on target. If at least 5 patients (or 31%) had a tacrolimus C_0 within the target range, inclusion would be extended. The conclusion of the trial would then be that model-based dosing is effective and results in a sufficiently high proportion of patients with an adequate C_0 to warrant further research in this population. To account for 10% dropout, it was estimated that a total of 28 patients had to be included.

For the analysis an intention-to-treat approach was followed, which included all patients who received at least one dose of the assigned drug. All secondary endpoints are described for the study population, and also for the historic controls. These historic controls are the 46 children previously transplanted in the Erasmus MC [17]. Possible differences between data from both populations were not formally tested.

Improved dosing algorithm

The model building cohort consisted of a total of 95 children. Of these patients, 45 were included in the original model [17]. For the remaining patients, additional PK data were retrospectively retrieved from the medical records. 16 patients were included in the clinical trial described here. The remaining 34 patients were transplanted in the Erasmus MC (n =11) or in the Radboudumc (n= 23) and were transplanted between March 2012 and October 2017. The Ethics Review Board of the Erasmus MC provided a waiver for the Medical Research Involving Human Subjects Act for this study (Medical Ethical Review Board number 2017-092). No extra laboratory analyses were performed.

Base model and covariate model development

PK analysis was conducted by nonlinear mixed-effects modelling using NONMEM version 7.2 (FOCE+I, ICON Development Solutions, Ellicott City, MD, USA) and PsN version 4.6.0. Pirana software was used as an interface between NONMEM, R (version 3.2.2) and Xpose (version 4). Base model and covariate model development was conducted as described previously [17]. The following demographic, clinical and genetic characteristics were evaluated as potential covariates: weight, height, gender, age, ethnicity, co-medication (glucocorticoids and calcium channel blockers), glucocorticoid dose, *CYP3A4* and *CYP3A5* genotype, primary kidney disease, number of transplantations, renal replacement therapy prior to transplantation (pre-emptive, peritoneal dialysis or hemodialysis), donor status (living or deceased), human leucocyte antigen mismatches, time post-transplant, hematocrit, creatinine, estimated glomerular filtration rate (Schwartz formula [28]), aspartate aminotransferase, albumin, C-reactive protein and total protein.

2.2.2 Model evaluation

The final model was internally validated with a VPC with 500 simulated datasets and a NPDE analysis with 1000 simulations as described previously [17]. The VPC was stratified for the included covariates. To evaluate the effect of the significant covariates, simulations were performed using the final model with varying parameters for the covariate. All other parameters were fixed to the mean.

2.2.3 Starting dose model

To be able to predict the required tacrolimus starting dose, the final model was used to develop a starting dose model. Each significant covariate in the final model was evaluated if it was clinically relevant, feasible to use, and if it significantly influenced the starting dose of tacrolimus. The starting dose model was validated using the techniques mentioned above.

RESULTS

3.1 Clinical study

The study was conducted between 19 November 2017 (first patient, first visit) and 19 February 2019 (last patient, last visit). A total of 19 patients was screened for participation in the trial of which 17 were eligible (Figure 1). Sixteen patients gave written informed consent and were subsequently included (n = 13 in the Erasmus MC and n = 3 in Radboudumc). Patient characteristics are shown in Table 1. Of these patients, 15 completed the ten day follow up. One patient was accidently administered tacrolimus extended-release (Advagraf, Astellas Pharma, Leiden) on day 5 and discontinued the study.

CYP3A5 allele frequencies are shown in Table 1. The observed *CYP3A5* genotype distribution was in accordance with the Hardy-Weinberg equilibrium ($\chi^2 = 1.34$; $p = 0.25$).

Endpoints

At the pre-planned interim analysis (after the inclusion of n = 16 children), five children (31%) had a tacrolimus C_0 within the target range (10-15 ng/mL), 31% had a supratherapeutic C_0 and 38%

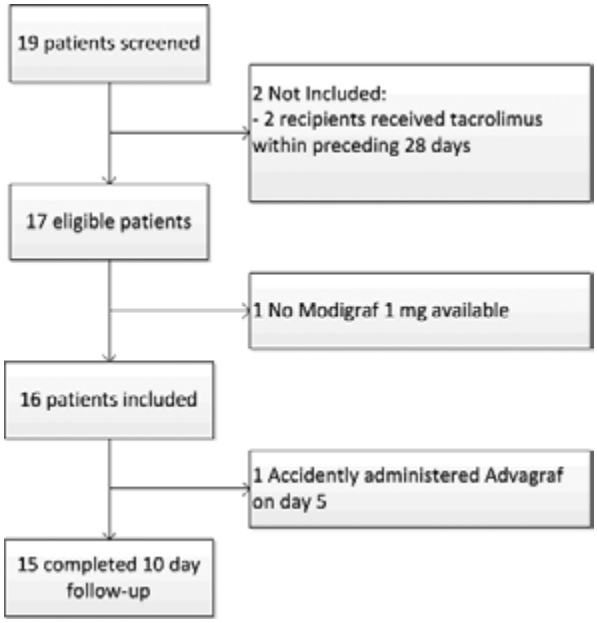

Figure 1. Trial flowchart. All patients who underwent a kidney transplantation and received at least one dose of tacrolimus according to the dosing algorithm were included in the intention-to-treat population.

a subtherapeutic C_0 on day 3 after transplantation (Table 2). This number was set before the study as a minimum to continue the study. Although the a priori criteria for a successful study were thus just met (31% of children on target at day 3 after transplantation), we decided to stop the study prematurely and to improve the model using new data. The reasons for this were twofold: First, at the interim analysis it was discovered that the model performed poorly in *CYP3A5* expressers receiving a kidney from a deceased donor which constituted 25% of all children included in this prospective study. Second, there was considerable concern among clinicians regarding overdosing in patients who were *CYP3A5* expresser and received a kidney from a deceased donor (see below).

The model predicted really high doses (*i.e.* 0.80 mg/kg/day) in patients who were *CYP3A5* expresser and received a kidney from a deceased donor (n = 3). Considering these high doses, a tacrolimus C_0 was measured already on day 1 or 2 following transplantation. These C_0 were too high, and the tacrolimus dose was subsequently reduced before day 3 (the primary endpoint). After TDM was performed, three children (20%) had a tacrolimus C_0 within the target range on day 7, compared with 5 (36%) on day 10. Two children (12.5%) had a markedly sub-therapeutic tacrolimus C_0 (<5 ng/mL) on day 3, and 3 (19%) had a markedly supra-therapeutic (> 20 ng/mL) tacrolimus C_0 on day 3 after transplantation. The individual tacrolimus C_0 during follow-up is shown in Figure 2. The tacrolimus C_0 on day 3, 7 and 10 is shown in Figure 3.

Safety

Overall patient and graft survival was 100%. The incidence of biopsy-proven acute rejection (BPAR) during the follow-up was 6.3% (n = 1). One child had antibody-mediated rejection on day 8 and

Table 1. Patient Characteristics.

	Clinical trial patients (n=16)
Sex	
Male	12 (75.0%)
Age (years)*	15.0 (4.6-16.8)
Ethnicity	
Caucasian	11 (68.8%)
Asian	0 (0%)
African descent	2 (12.5%)
Other	3 (18.9%)
Bodyweight (kg)*	50.3 (15.7-80.4)
Height (cm)*	161 (101-179)
Genotype	
CYP3A5	
*1/*1	1 (6.3%)
*1/*3	3 (18.9%)
*3/*3	12 (75.0%)
CYP3A4	
*1/*1	13 (81.3%)
*1/*1G	2 (12.5%)
*1G/*1G	1 (6.3%)
Primary diagnosis	
CAKUT	7 (43.8%)
Glomerular kidney disease	1 (6.3%)
Cystic kidney disease / nephronophthisis	3 (18.9%)
Other/unknown	5 (31.3%)
RRT prior to kidney transplantation	
Hemodialysis	5 (31.3%)
Peritoneal dialysis	3 (18.9%)
Pre-emptive	8 (50.0%)
Donor type	
Living	11 (68.8%)
Deceased	5 (31.3%)
Number of HLA mismatches	
0	2 (12.5%)
1	1 (6.3%)
2	3 (18.9%)
3	7 (43.8%)
4	3 (18.9%)

CAKUT congenital anomalies of the kidney and the urinary tract, CYP cytochrome P450, RRT renal replacement therapy
* Presented as median and range for continuous variables.

Table 2. Clinical trial results

			Day 3		Day 7		Day 10	
Bodyweight	**CYP3A5**	**Donor**	**Tac dose (mg/kg/day)**	**Tac C_0**	**Tac dose (mg/kg/day)**	**Tac C_0**	**Tac dose (mg/kg/day)**	**Tac C_0**
65	*3/*3	LD	0.31	11.3	0.31	17.8	0.25	14.9
80.4	*1/*3	DD	0.80	21.4*	0.22	35.0	0.22	15.7
48.2	*1/*1	DD	0.79	17.9*	0.29	15.3	0.37	4.6
51.3	*3/*3	DD	0.39	9.8	0.39	16.9	0.31	13.3
15.7	*3/*3	LD	0.38	7.4	0.51	12.0	0.51	9.7
70.1	*3/*3	LD	0.29	4.2	0.43	16.0	0.37	20.8
62.2	*1/*3	DD	0.64	11**	0.48	18.6	0.42	17.7
57.7	*3/*3	LD	0.29	11.9	0.33	22.2	0.26	13.7
29.9	*1/*3	LD	0.54	4.1	0.67	8.5	0.67	10.6
26.3	*3/*3	DD	0.46	7.2	0.61	14.6	0.53	14.4
39.3	*3/*3	LD	0.31	5.3	0.31	23.5	0.15	18.1
49.3	*3/*3	LD	0.28	24	0.20	17.4	0.12	16.7
41.5	*3/*3	LD	0.31	10.9	/***	/	/	/
63.6	*3/*3	LD	0.31	10.3	0.31	11.7	0.19	8.0
59.3	*3/*3	LD	0.30	10.8	0.24	21.3	0.17	22.3
19.3	*3/*3	LD	0.36	21.3	0.26	21.3	0.21	/

*Patients had a toxic tacrolimus C_0 on day 1-2 following transplantation. The tacrolimus dose was subsequently reduced.
**Patient had a concentration of 11 ng/mL after just one dose of tacrolimus. The tacrolimus dose was subsequently reduced.
***Patient discontinued the study after accidental administration of Advagraf.
C_0 Predose concentration, *CYP* cytochrome P450, *DD* Deceased donor, *LD* Living donor, *Tac* tacrolimus, / not available

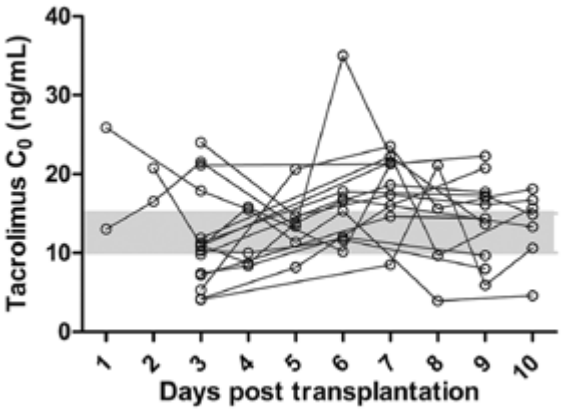

Figure 2. Individual tacrolimus predose concentrations (C_0) in the 10 days following kidney transplantation.

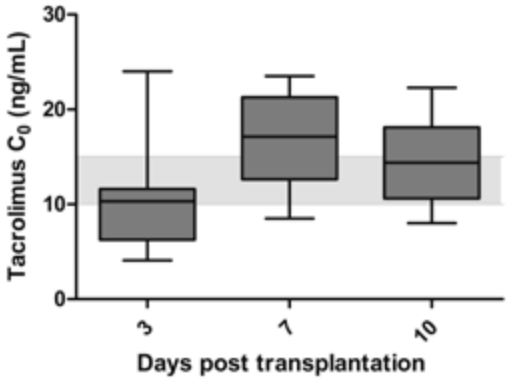

Figure 3. Boxplot depicting the tacrolimus predose concentrations (C_0) on day 3, 7 and 10 following transplantation. In this figure the 3 patients who had a dose reduction before day 3 are excluded.

was treated with methylprednisolone and immunoglobulins. At the time of biopsy the tacrolimus C_0 was 3.9 ng/mL. Just after the follow-up finished (day 13) one patient was diagnosed with acute cellular rejection Banff grade 2A and was treated with methylprednisolone. At the time of biopsy the tacrolimus C_0 was 18.7 ng/mL. Both children fully recovered. The incidence of delayed graft function (DGF) was 6.25% (n = 1). The tacrolimus C_0 on day 3 of this patient was 21.4 ng/mL. During the 30 days after the follow-up period had finished, one patient reported a serious adverse event. This child developed post-transplant diabetes mellitus, and was switched to ciclosporin-based immunosuppressive therapy, and fully recovered. At the time of diagnosis the tacrolimus C_0 was relatively high (18 ng/mL).

Improved dosing algorithm

As the trial was ended prematurely, a new dosing algorithm was developed in an extended cohort. The model building cohort was expanded to a total of 95 children. Patient characteristics are presented in Table 3. From these patients, a total of 1,138 blood samples were collected and analyzed for tacrolimus concentrations (range 1.8 – 109.0 ng/mL). No samples were below the lower limit of quantification. One sample was above the upper limit of quantification and was discarded. A fifth of the samples was drawn within the first week following transplantation. For 90 patients at least one PK profile at a median of 12.2 days (range 4.2 – 42.0 days) post-transplantation was available. The *CYP3A4* and *CYP3A5* allele frequencies are depicted in Table 3. There was no deviation from the Hardy-Weinberg equilibrium.

PK model development

The data were best described with a two-compartment model. Including inter-individual variability (IIV) on clearance (*CL/F*), volume of distribution of the central compartment (V_1/F), volume of distribution of the peripheral compartment (V_2/F) and the absorption rate constant (k_a) significantly improved the model fit. Allometric scaling with an estimated exponent on *CL/F* and fixed exponent on V_1/F (1) and V_2/F (1) significantly improved the model ($p < 0.001$).

Table 3. Patient characteristics PK model

	Model building cohort (n = 95)
Recipient sex	
Male	58 (61%)
Age of recipient (years)	11.4 (1.6-17.9)
Ethnicity	
Caucasian	71 (74%)
Asian	2 (2%)
African descent	9 (9%)
Other	13 (14%)
Bodyweight (kg)*	32.0 (10.4-87.5)
Height (cm)*	138 (73-188)
Laboratory measurements	
Hematocrit (L/L)	0.29 (0.16-0.52)
Creatinine (µmol/L)	84 (12-1454)
eGFR (mL/min) [28]	63 (2.9-274)
ASAT (U/L)	29 (7-217)
Albumin (g/L)	34 (11-52)
CRP (mg/L)	6.4 (0.3-268)
Total Protein (g/L)	61 (34-80)
CYP3A4	
*1/*1	34 (36%)
*1/*1G	8 (8%)
*1G/*1G	3 (3%)
*22	2 (2%)
Unknown	48 (51%)
CYP3A5	
*1/*1	3 (3%)
*1/*3	11 (12%)
*3/*3	52 (55%)
*3/*7	2 (2%)
Unknown	27 (28%)
Primary diagnosis	
CAKUT	46 (48%)
Glomerular kidney disease	22 (23%)
Cystic kidney disease / nephronophtisis	12 (13%)
Other/unknown	15 (16%)
Number of kidney transplantations	
First	90 (95%)
Second	5 (5%)
RRT prior to kidney transplantation	
Hemodialysis	28 (29%)
Peritoneal dialysis	23 (24%)
Pre-emptive	44 (46%)

Table 3. (continued)

	Model building cohort (n = 95)
Donor type	
Living	74 (78%)
Deceased	21 (22%)
Route of administration	
Suspension	24 (25%)
Capsule	89 (94%)
Co-medication	
Calcium channel blockers	
Amlodipine	51 (54%)
Nifedipine	23 (24%)
Antibiotics	
Erythromycin	1 (1%)
Antimycotics	
Fluconazole	2 (2%)
Voriconazole	1 (1%)
Distribution of tacrolimus samples	
Total samples	1338
0-7 days post-transplantation	286 (21%)
8-14 days post-transplantation	515 (38%)
15-21 days post-transplantation	218 (16%)
22-42 days post-transplantation	319 (24%)
Tacrolimus analysis	
Immunoassay	64 (4.8%)
LC-MS/MS	1274 (95.2%)

ASAT aspartate aminotransferase, C_0 predose concentration, *CAKUT* congenital anomalies of the kidney and the urinary tract, *CYP* cytochrome P450, *eGFR* estimated glomerular filtration rate, *RRT* renal replacement therapy
* Presented as median and range for continuous variables.

The OFV decreased further after adding inter-occasion variability (IOV) on *CL/F*. The residual error was described with a combined additive and proportional error model for the immunoassay measured concentrations, and a separate additive and proportional error model for the LC-MS/MS measured concentrations. Parameter estimates of the base model, final model and starting dose model are presented in Table 4.

After graphical analysis, the univariate analysis resulted in five significant covariates correlated with *CL/F*: hematocrit, *CYP3A5,* African descent, serum creatinine during follow-up and eGFR. After forward inclusion-backward elimination (stepwise covariate modelling method [29]), *CYP3A5*, hematocrit and serum creatinine remained in the final model. The final model for estimation of tacrolimus *CL/F* in the first six weeks after transplantation is shown in equation 2:

Table 4. Parameter estimates of the base model, final model and bootstrap analysis

Parameter	Base Model (RSE %) [shrinkage]	Final model (RSE %) [shrinkage]	Starting dose model (RSE %) [shrinkage]
t_{lag} (h) FIX	0.41	0.41	0.41
k_a (L/h)	2.1 (19)	1.7 (11)	1.85 (24)
CL/F (L/h/70 kg)	37.0 (6)	36.6 (12)	34.5 (6)
V_1/F (L/70 kg)	560 (12)	496 (22)	540 (12)
Q/F (L/h)	27.4 (16)	31.7 (19)	28.5 (12)
V_2/F (L/70 kg)	1600 (13)	1270 (13)	1660 (17)
Allometric scaling on CL	0.57 (10)	0.62 (20)	0.56 (9)
Covariate effect on CL			
*CYP3A5*1/*1* or **1/*3*	-	1.4	1.5
Hematocrit (L/L)	-	-0.60	-
Creatinine (μmol/L)	-	-0.1	-
IIV (%)			
CL/F	47.2 (8) [4]	42.1 (10) [5]	42.3 (11) [3]
V_1/F	89.0 (12) [11]	99.6 (12) [10]	93.0 (12) [8]
V_2/F	92.1 (15) [20]	85.2 (15) [22]	89.3 (15) [19]
k_a	172 (10) [23]	183 (11) [20]	178 (10) [22]
IOV (%)			
CL/F	20.7 (9)	20.1 (20)	20.1 (10)
Residual variability			
Additional			
Immunoassay	0.77	1.27	1.01
LC-MS/MS	0.94	0.87	0.96
Proportional			
Immunoassay	0.12	0.11	0.12
LC-MS/MS	0.23	0.23	0.24

CL clearance, *CYP* cytochrome P450, *F* bioavailability of oral tacrolimus, *IIV* inter-individual variability, *IOV* inter-occasion variability, K_a absorption rate constant, *LC-MS/MS* Liquid chromatography-mass spectrometry, *Q* intercompartmental clearance of tacrolimus, t_{lag} lag time, V_1 central compartment for tacrolimus, V_2 peripheral compartment for tacrolimus

$$CL/F = 36.6 * \left(\frac{weight}{70}\right)^{0.62} * [(1.0, if\ CYP3A5 * 3/* 3) or\ (1.4, if\ CYP3A5 * 1/* 3\ or\ CYP3A5 * 1/* 1)] * \left(\frac{Hematocrit}{0.29}\right)^{-0.6} * \left(\frac{Creatinine}{84}\right)^{-0.1}$$

The final model was used to develop a model to predict the starting dose of tacrolimus. As time after transplantation was not a significant covariate, the same database was used to create the starting dose model. The last measured hematocrit and serum creatinine before transplantation did not significantly influence the *CL/F*, and were not included in the starting dose model. Equation 3 describes the starting dose model:

$$CL/_F = 34.5 * \left(\frac{weight}{70}\right)^{0.56} * [(1.0, if\ CYP3A5 * 3/* 3) or\ (1.46, if\ CYP3A5 * 1/* 3\ \ or\ CYP3A5 * 1/* 1)]$$

To calculate the required dose the PK formula Dose = *CL/F* * AUC can be used. A C_0 of 10 ng/mL corresponded with an $AUC_{0\text{-}12h}$ of 185 ng h/mL, 12.5 ng/mL with 220 ng h/mL and 15 ng/mL with 254 ng h/mL. For a target of 12.5 ng/mL this leads to equation 4 for the starting dose based on a twice daily dose (improved dosing algorithm):

$$Dose\ (mg) = 220 * 34.5 * \left(\frac{weight}{70}\right)^{0.56} * [(1.0, if\ CYP3A5 * 3/* 3) or\ (1.46, if\ CYP3A5 * 1/* 3\ \ or\ CYP3A5 * 1/* 1)]/1000$$

An example of calculated doses according to equation 4 are given in Table S3.

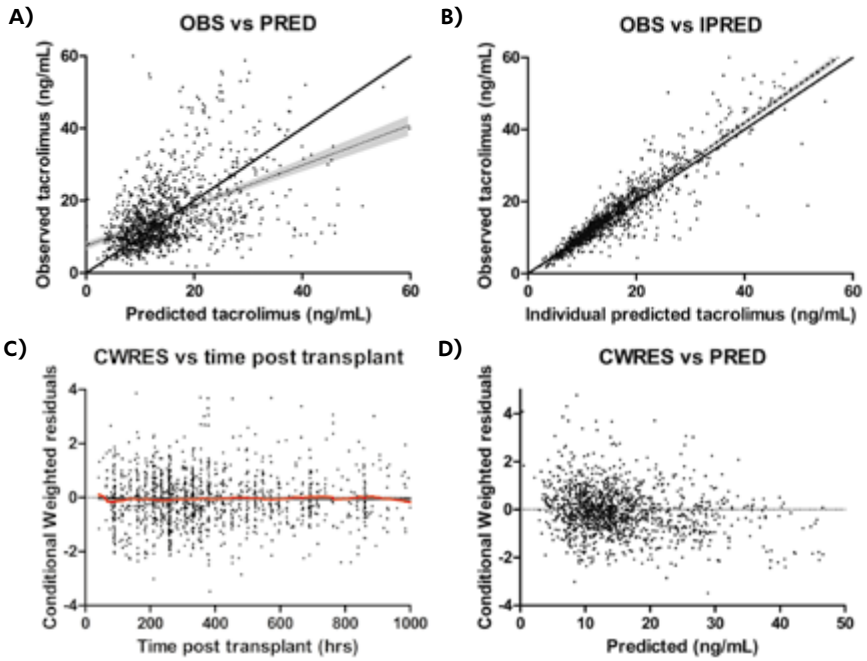

Figure 4. Goodness-of-fit plots of the final model. **A** DV plotted against PRED. **B** DV plotted against IPRED. **C** The correlation of CWRES with the time after the tacrolimus dose. **D** The correlation of CWRES with PRED. The line represents the line of identity. *CWRES* conditional weighted residuals, *DV* observed concentrations, *IPRED* individual predicted concentration, *OBS* observed concentration, *PRED* predicted concentration.

Evaluation of the Final Model and starting dose model

All estimates were within the limits, except for shrinkage on V_3 which was 22%. Goodness of fit plots of the final model and starting dose model showed both the individual and population predictions were evenly distributed around the line of unity (Figure 4). The median and variability of the C_0 fell within the corresponding simulations as shown in the prediction corrected VPCs (Figure S1). NPDEs were acceptably deviated from a normal distribution and values were mostly between -2 and 2, showing the adequate predictive ability of the model. A shark plot showed 68% of patients had a decrease in OFV with the final model compared with the base model.

Simulations

Based on the final model, *CYP3A5* expressers had a 1.4-fold higher CL/F than the *CYP3A5* non-expressers. A decrease in hematocrit levels from 0.35 to 0.25 L/L corresponded with a 18% higher tacrolimus *CL/F*. A decrease in serum creatinine concentration from 500 to 50 µmol/L was associated with a 21% increase in *CL/F*. The effect of *CYP3A5*, bodyweight, serum creatinine and hematocrit are shown in Figure S2. In total these covariates explained 39% of the variability in *CL/F*.

DISCUSSION

In this prospective trial, 31% (5 out of 16) of the children had a tacrolimus C_0 within the target range on day 3 following transplantation when prescribed a tacrolimus starting dose based on the original dosing algorithm. Two children (12.5%) had a markedly subtherapeutic tacrolimus C_0 (<5 ng/mL) on day 3, and 3 (19%) had a markedly supratherapeutic (>20 ng/mL) tacrolimus C_0. The original algorithm performed worse than anticipated and therefore the trial was ended prematurely.

Contrary to what we expected, only 31% of patients had a tacrolimus C_0 within the target range. When the tacrolimus starting dose is only based on bodyweight in children, 30% is on target on day 3 [17]. In order for the model to be a meaningful addition to standard bodyweight-based dosing, our estimate was that at least 55% would have to be on target. Based on our results it seems that basing the starting dose on the dosing algorithm we had developed earlier does not increase the percentage of patients on target and does not reduce the number of extreme high and low tacrolimus C_0 compared with standard bodyweight-based dosing.

There is compelling evidence that *CYP3A5* expressers require a 1.5 to 2-fold higher tacrolimus dose than non-expressers [15, 17-19, 30-36]. A randomized clinical trial of age and genotype-guided tacrolimus dosing in children concluded that *CYP3A5* genotype-guided dosing stratified by age resulted in earlier attainment of therapeutic tacrolimus concentrations and fewer out-of-range concentrations [37]. Clinical outcomes were not studied. Two large clinical trials in adults studied whether basing the tacrolimus dose on *CYP3A5* would lead to more patients within the target C_0 range. Both studies concluded that optimization of the initial tacrolimus dose using *CYP3A5* genetic testing, does not improve clinical outcomes when TDM is performed [38, 39]. As the variability in CL is not solely based on *CYP3A5*, basing the starting dose on a dosing algorithm including clinical, genetic and demographic factors seemed the sensible next step.

On day 3 following transplantation, five patients were on target. As they were all *CYP3A5* non-expressers who received a kidney from a living donor, they were all prescribed a dosage of approximately 0.3 mg/kg/day. This is the same dose as the standard bodyweight-based dose

according to the package leaflet [40]. These children would have been on target regardless if they participated in this trial.

Six children had a subtherapeutic tacrolimus C_0 on day 3. All of these children received a dose between 0.29-0.54 mg/kg/day, which is equal to or higher than the standard bodyweight-based dose of 0.3 mg/kg. This suggests that if they had not participated in the trial and received a standard, bodyweight-based dose, these children would also all have had a subtherapeutic tacrolimus exposure. Of these children one was a *CYP3A5* expresser and one received a kidney from a deceased donor. The doses were calculated correctly. It seems other factors not included in the original algorithm increased tacrolimus CL in these patients.

In three patients the tacrolimus dose was reduced on day 1-2 following transplantation due to a high tacrolimus C_0. If these doses had not been reduced, these patients would have likely had toxic tacrolimus C_0 on day 3. These patients all received a kidney from a deceased donor and were *CYP3A5* expressers. It seems that the original dosing algorithm overestimates the CL of tacrolimus in this group, and therefore overestimates the required tacrolimus dose. To our knowledge no other publication has found a relationship between donor type and tacrolimus CL. As tacrolimus undergoes hepatic metabolism, a higher tacrolimus CL in kidneys from a deceased donor seems highly unlikely. All patients received the same immunosuppressive protocol, no patients experienced delayed graft function. Dialysis prior to transplantation and the number of HLA mismatches was not significantly associated with the tacrolimus CL. The higher tacrolimus CL in kidneys from a deceased donor is probably caused by other and unknown parameters that could not be tested as covariates and therefore cannot be corrected for. The cohort in which the model was developed [17], consisted of 46 children of whom only 2 were a *CYP3A5* expresser and received a kidney from a deceased donor. It seems that there was insufficient power in the cohort in which the model was developed to determine adequate tacrolimus exposure predictions in this specific subgroup.

After improving the original dosing algorithm, *CYP3A5* expressers required a 1.4-fold higher tacrolimus dose than *CYP3A5* nonexpressers in the improved dosing algorithm. This is in line with previous research mentioned above. As approximately 70-80% of tacrolimus is distributed in erythrocytes, low hematocrit reduces the whole-blood concentration of tacrolimus [41]. In this study we concluded that patients with a higher hematocrit had a lower *CL/F*. Previous research substantiates these findings [17, 19, 31, 35, 36, 42-45]. Patients with higher serum creatinine levels had a decreased *CL/F*. Tacrolimus undergoes hepatic elimination and almost no renal elimination, thus the explanation for this observation remains unclear. Some studies have reported a correlation between creatinine and tacrolimus CL [31, 46, 47], whereas others found no such effect [48-50]. Four decades ago, Sheiner *et al*. concluded that forecasting a concentration based on covariates does not improve accuracy and precision as much as one previous concentration [51]. However, when predicting the starting dose no previous concentrations are available. In this first exploratory study we chose to focus on the starting dose with a historic cohort. For a new study it will be interesting to adjust the subsequent doses using the dosing algorithm in combination with the previous concentration rather than just TDM.

The main difference between the improved PK model and the original model which was used to determine the tacrolimus starting dose in this trial, is that donor status was no longer a significant

covariate on CL. It remains unclear why a recipient of a kidney from a deceased donor would have a higher tacrolimus CL. The other difference between the two models is that allometric scaling was coded with an estimated exponent on CL and *CYP3A5* expressers should receive a 1.46-fold higher dose in the improved algorithm compared with 1.82 in the original. Simulations of the improved model showed better description of the data compared with the previously published model, as shown in the VPC (Figure S3). It will be interesting to see if the improved model is able to adequately predict the tacrolimus exposure when used in clinical practice. We are currently planning a new prospective clinical trial using this new and improved algorithm.

The main strength of this study is that this is the first attempt at predicting the starting dose of tacrolimus in children in clinical practice with a dosing algorithm. Many PK models that have been published in literature were developed retrospectively but were not tested in prospective studies. This study has demonstrated that even though on paper the algorithm was validated extensively and performed well, in clinical practice it was simply inadequate. As the old proverb says, *the proof of the pudding is in the eating*. A second strong point is that due to the chosen methodology, a limited sample size was sufficient to answer the research question. The final strength of this study is that an improved starting dose model was developed designed for clinicians, making it easy to use the dosing algorithm in clinical practice.

The main limitation of this study is that in the PK model building cohort two different analytical techniques were used: immunoassay and LC-MS/MS. However, to solve this issue this difference was built into the residual error model. We chose not to exclude the immunoassay concentrations as they were included in the original model. Furthermore, the relatively large proportion of Caucasian patients in our center is a limitation as this may not reflect pediatric transplant populations worldwide.

CONCLUSIONS

In a prospective study, a validated tacrolimus dosing algorithm was poorly predictive of the individual tacrolimus starting dose following renal transplantation in children. On day 3, a total of only 31% of patients was within the target tacrolimus predose concentration range. The dosing algorithm was subsequently improved using data of children included in this trial and was able to adequately describe the tacrolimus PK the first six weeks following kidney transplantation. The weight-normalized starting dose of tacrolimus should be higher in patients with a lower bodyweight and in those who are *CYP3A5* expresser. However, as the negative result of this trial demonstrates, a prospective study is needed to demonstrate the accuracy of this improved model.

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SUPPLEMENTARY DATA

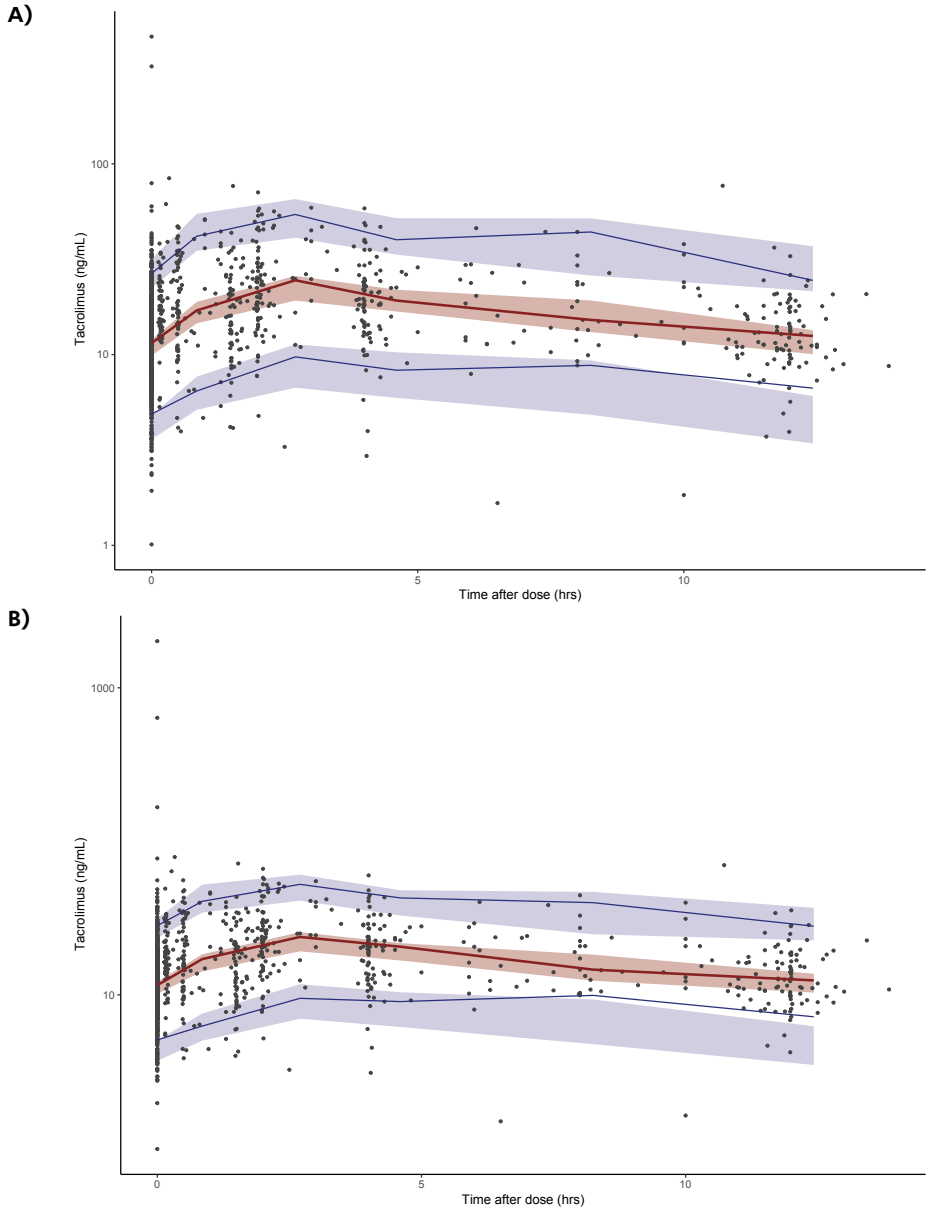

Figure S1. Visual predictive check showing how well the average trend of the observations (solid line) and how well the variability of the observed data (two dashed lines) fall within the model simulated average trend (red shaded area) and the model simulated variability (blue shaded areas) represented as 95% CI. The average and the variability of the observed data both fall within the corresponding simulations. **A** VPC of the final model. **B** VPC of the starting dose model.

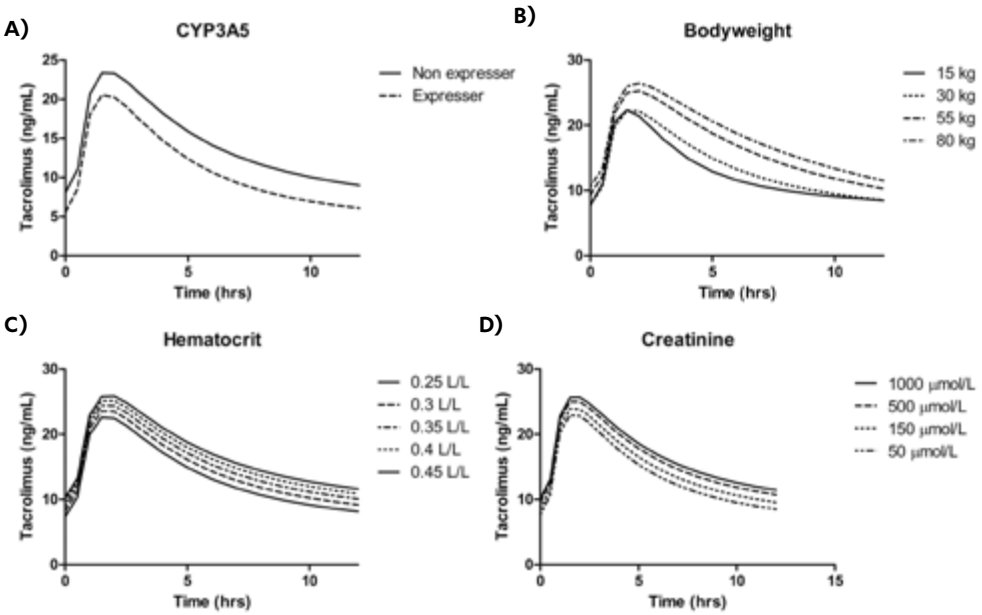

Figure S2. Simulated plasma profiles of tacrolimus at first steady state after transplantation. **A** Simulated plasma profiles of tacrolimus for CYP3A5 non-expressers (*CYP3A5*3/*3)* and CYP3A5 expressers (*CYP3A5*1/*1* or *CYP3A5*1/*3*). **B** Simulated plasma profiles of tacrolimus for patients with a bodyweight of 15, 30, 55 or 80 kg. **C** Simulated plasma profiles of tacrolimus for patients with plasma hematocrit levels of 0.2, 0.25, 0.3, 0.35, 0.4 and 0.45 L/L. **D** Simulated plasma profiles of tacrolimus for patients with plasma creatinine levels of 50, 150, 500 and 1000 µmol/L. C_0 predose concentration, *CYP* cytochrome P450.

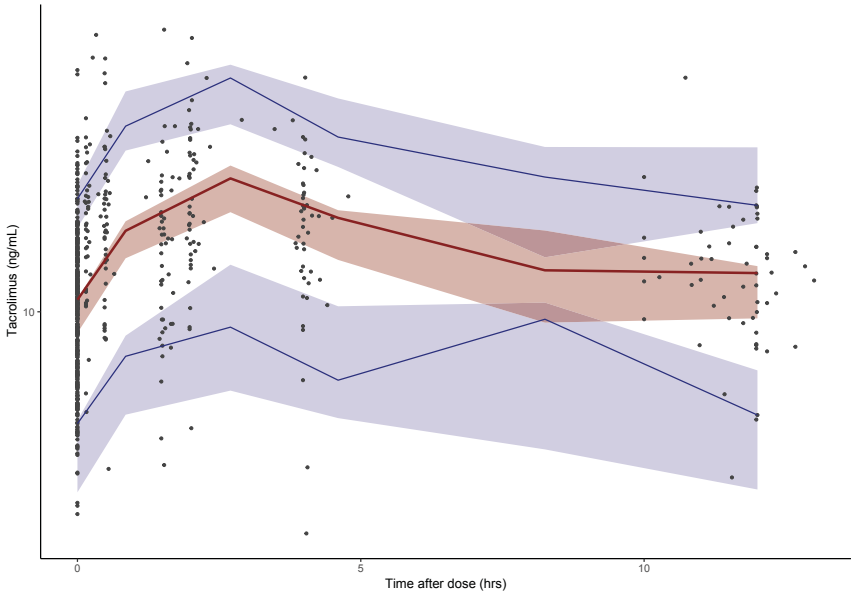

Figure S3. Visual predictive check of the previously published dosing algorithm.

Table S1. Drugs interacting with tacrolimus

Drugs
Drugs with a relevant pharmacokinetic interaction with tacrolimus:
Antibiotics
Clarithromycin
Doxycycline
Erythromycin
Rifampicin
Anti-epileptics
Carbamazepine
Phenobarbital
Phenytoin
Antihypertensive and antiarrhythmic agents
Amiodarone
Diltiazem
Verapamil
Antimycotics
Fluconazole
Itraconazole
Ketoconazole
Other
HIV protease inhibitors
Theophylline

Table S2. Tacrolimus dosing guidelines

Weight (kg)	Donor	CYP3A5 expresser Dose (mg/kg/day)	CYP3A5 non-expresser Dose (mg/kg/day)
10-15	Living	0.89	0.44
	Deceased	1.33	0.61
15-25	Living	0.76	0.37
	Deceased	1.06	0.53
25-35	Living	0.63	0.33
	Deceased	1.00	0.46
35-45	Living	0.61	0.31
	Deceased	0.91	0.44
45-55	Living	0.60	0.30
	Deceased	0.82	0.42
55-65	Living	0.55	0.30
	Deceased	0.80	0.40
65-75	Living	0.53	0.30
	Deceased	0.78	0.38
75-85	Living	0.52	0.27
	Deceased	0.78	0.38

Table S3. Tacrolimus example doses according to equation (4)

Weight (kg)	CYP3A5 expresser Dose (mg/kg/day)	CYP3A5 non-expresser Dose (mg/kg/day)
10	0.73	0.50
20	0.54	0.37
30	0.46	0.31
40	0.40	0.28
50	0.37	0.25
60	0.34	0.23
70	0.32	0.22
80	0.30	0.20

PREDICTING TACROLIMUS EXPOSURE IN ADULT RENAL TRANSPLANT RECIPIENTS

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5

PHARMACOGENETIC ASPECTS OF THE USE OF TACROLIMUS IN RENAL TRANSPLANTATION: RECENT DEVELOPMENTS AND ETHNIC CONSIDERATIONS

Expert Opin Drug Metab Toxicol. 2016 May;12(5):555-65.

ABSTRACT

Introduction

Tacrolimus (Tac) is effective in preventing acute rejection but has considerable toxicity and inter-individual variability in pharmacokinetics and pharmacodynamics. Part of this is explained by polymorphisms in genes encoding Tac metabolizing enzymes and transporters. A better understanding of Tac pharmacokinetics and pharmacodynamics may help to minimize different outcomes amongst transplant recipients by personalizing immunosuppression.

Areas covered

To examine the pharmacogenetic contribution of Tac metabolism, with focus on recent discoveries, new developments and ethnic considerations.

Expert opinion

The strongest and most consistent association in pharmacogenetics is between *CYP3A5* genotype and Tac dose requirement, with CYP3A5 expressers having a ~40-50% higher dose requirement compared to non-expressers. Two recent RCTs using *CYP3A5* genotype, however, did not show a decrease in acute rejections nor reduced toxicity. *CYP3A4*22, CYP3A4*26,* and *POR *28* are also associated with Tac dose requirements and may be included to provide the expected improvement of Tac therapy. Studies focusing on the intracellular drug concentrations and on CNI-induced nephrotoxicity seem also promising. For all studies, however, the ethnic prevalence of genotypes should be taken into account, as this may significantly impact the effect of pre-emptive genotyping.

INTRODUCTION

The calcineurin inhibitor (CNI) tacrolimus (Tac) is used to prevent acute rejection after solid organ transplantation (SOT).[1] Unfortunately, the clinical use of Tac is complicated by its considerable toxicity, narrow therapeutic window, and high inter-individual pharmacokinetic variability. [2] Therapeutic drug monitoring (TDM) is universally applied to individualize Tac therapy in SOT recipients. However, despite TDM many SOT recipients experience significant over- or underexposure to Tac. Part of the inter-individual variability in Tac pharmacokinetics is explained by genetic polymorphisms in genes encoding for Tac metabolizing enzymes and transporter proteins.[3-6] Genetic variation may also explain inter-individual differences in Tac's pharmacodynamics. In this article, the relevance of a pharmacogenetic approach to Tac therapy is discussed. The focus is on recent discoveries, new developments and ethnic considerations.

GENETIC VARIATION AND TAC PHARMACOKINETICS

Tac is a substrate of the drug-efflux pump ABCB1 (encoded by the *ABCB1* gene), which is expressed in the intestine and thought to limit the absorption of Tac. Inter-individual differences in the expression and/or function of ABCB1 determines the variability in the bioavailability of Tac.[7] Following absorption, Tac is metabolized in the intestine, liver, and to a limited degree in the kidney by cytochrome P450 (CYP) 3A4 and 3A5.[8] Inter-individual differences in CYP3A activity are the most important determinants of the variability in Tac clearance. Other enzymes/receptors including P450 oxidoreductase (POR), the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-α), and CYP2C8, play minor roles in the disposition of Tac. Nonetheless, polymorphisms in *PPARA*, *POR*, and *CYP2C8* may explain residual variability in the response to Tac.

CYP3A5

Polymorphisms in the *CYP3A5* gene explain 40-50% of the variability in Tac dose requirement. [9, 10] The best-studied single-nucleotide polymorphism (SNP) in *CYP3A5* is *CYP3A5*3*, which is an A to G transition at position 6986 within intron 3 (rs776746). The *CYP3A5**3 allele causes alternative splicing, which results in protein truncation and a severe decrease of functional CYP3A5 enzyme.[11] Other *CYP3A5* SNPs are *CYP3A5**6 (rs10264272) and *CYP3A5**7 (rs41303343): *CYP3A5**6 encodes a 14690G>A transition, causing a splice variant mRNA and deletion of exon 7, resulting in nonfunctional CYP3A5 protein. [11, 12] *CYP3A5**7 denotes a single base insertion at codon 346 causing a frameshift, resulting in a truncated mRNA and nonfunctional CYP3A5.[13]

Individuals homozygous for the *CYP3A5**3 allele are referred to as CYP3A5 non-expressers, whereas individuals carrying at least one *CYP3A5**1 allele are known as CYP3A5 expressers. The reduced enzymatic activity associated with the *CYP3A5**3 allele has been associated with a reduced Tac dose requirement (for a review see reference [14]). CYP3A5 expressers require a Tac dose that is about 50% higher than that of CYP3A5 non-expressers to reach the same exposure. This is a consistent finding and has been observed in both adults and children, and among recipients of either a kidney, liver, heart or lung transplant.[15-17]

Following standard, bodyweight-based dosing, CYP3A5 expressers are prone to have sub-therapeutic Tac concentrations in the early phase after surgery and may therefore be at an increased

risk for acute rejection. MacPhee *et al*.[18] demonstrated that CYP3A5 expressers did indeed have a delay in achieving the target Tac exposure, in spite of TDM. However, CYP3A5 expressers did not experience more biopsy-proven acute rejection, although rejection did occur earlier in CYP3A5 expressers compared with non-expressers with a median of 7 versus 13 days.[18] Other investigators have also reported that CYP3A5 expressers do not have a higher risk of developing acute rejection.[19-27].

Although numerous studies have reported the higher Tac dose requirement of CYP3A5 expressers compared to non-expressers, the clinical relevance of this association is unclear and has so far only been investigated in two randomized-controlled clinical trials (RCT). The Tactique study[28] was a multicenter RCT, including 280 renal transplant recipients. Patients were randomized 1:1 to receive either a standard starting dose of Tac (0.1 mg/kg twice daily) or a starting dose based on an individual patient's *CYP3A5* genotype (0.075 or 0.15 mg/kg twice daily for CYP3A5 non-expressers and expressers, respectively). The primary efficacy endpoint was the proportion of patients for whom the Tac predose concentration (C_0) was within the target range (10-15 ng/mL) after six unchanged doses of Tac. In the Tactique study this was day 10 because Tac was started on day 7 after transplantation. Throughout the first post-transplant week, all patients were Tac-free to allow for *CYP3A5* genotyping, and received high-dose mycophenolate mofetil (MMF; 3 g/day), glucocorticoids, and induction therapy with rabbit anti-thymocyte globulin (rATG; in 82.2% of patients) or IL-2 receptor antibodies (in 17.8 % of patients). In the TacTic study, *CYP3A5* genotype-based Tac (start)dosing led to significantly more patients reaching the target range 3 days after the start of Tac treatment as compared with standard, bodyweight-based Tac dosing: 43.2% versus 29.1%.[28] Also, the group that received a *CYP3A5* genotype-based Tac dose needed significantly less time and fewer dose adaptations to reach target. However, there were no differences between the two groups with regard to graft survival, acute rejection, delayed graft function or Tac toxicity.[28]

Recently, the long-term follow-up results of Tactique were published.[29] Pallet *et al*. reported that the incidence of biopsy-proven acute rejection and graft survival were similar between the control and the *CYP3A5* genotype-adapted Tac dose groups. There were also no differences between the two groups in terms of patient survival, the incidence of cancer, cardiovascular events, infections and kidney function. The authors concluded that optimization of initial Tac dosing using *CYP3A5* pharmacogenetic testing does not improve clinical outcomes.[29]

In a second RCT, 240 renal transplant recipients were randomized to receive a standard, bodyweight-based Tac starting dose (0.1 mg/kg twice daily) or a *CYP3A5* genotype-based starting dose (0.075 or 0.15 mg/kg twice daily for CYP3A5 non-expressers and expressers, respectively).[30] Unlike the Tactique study, this trial only included recipients of a living kidney donor (who were genotyped for *CYP3A5* during the work-up for transplantation) and Tac was started on the day of transplantation rather than at day 7 post-transplant. All patients received basiliximab induction therapy and a standard MMF starting dose of 2 g/day followed by TDM. The primary endpoint of this trial was again the proportion of patients within the Tac therapeutic range on day 3 after transplantation (*i.e.* at first steady state). Unlike in Tactique, there was no difference in the proportion of patients "on target" at day 3 after transplantation: 37.4% *versus* 35.6% for the standard-dose and the genotype-based groups, respectively. In addition, there was

no difference in the time-to-reach target concentration or the number of Tac dose modifications required to reach the target concentration. In line with the French trial, there were no differences in any of the clinical endpoints, including the incidence of acute rejection.

It is unknown why the *CYP3A5*-based Tac dosing approach was beneficial in terms of early Tac exposure in the Tactique study, whereas this was not the case in the second. The main difference between these studies was the day on which Tac was initiated (day 0 *versus* 7). Changes in glucocorticoid dosing or gastro-intestinal motility during the first post-operative week may have had a greater effect on Tac exposure than *CYP3A5* genotype. Of note, in both studies, the percentage of patients "on target" 3 days after initiation of Tac was low in spite of *CYP3A5* genotype adaptation. This observation demonstrates that there exists considerable residual variability in Tac pharmacokinetics that is not explained by *CYP3A5* genotype. The fact that neither study demonstrated a clinical benefit, does not support routinely genotyping kidney transplant recipients for *CYP3A5*. Possibly, donor genotype (including that of *CYP3A5*) may be more relevant for long-term clinical outcome than the genotype of the recipient.[31]

CYP3A4

The *CYP3A4* SNPs *CYP3A4**1B (rs2740574), and *CYP3A4**22 (rs35599367) have both been associated with altered Tac dose requirements. Individuals carrying the *CYP3A4**1B allele were reported to have a 35% lower Tac dose-adjusted C_0 concentration compared to individuals having the *CYP3A4* wild-type allele.[32-34] However, whether the *CYP3A4**1B allele is truly itself responsible for the altered Tac dose requirement remains a matter of debate as this SNP is in linkage disequilibrium with the *CYP3A5**1 allele.[35]

*CYP3A4**22 (rs35599367) is located in intron 6 of *CYP3A4* and is a C to T substitution at g.15389. Wang and Sadee [36] demonstrated that *CYP3A4*22* increases the formation of the nonfunctional CYP3A4 splice variant with partial intron 6 retention, thereby reducing the production of functional full-length CYP3A4 mRNA and reduced CYP3A4 enzymatic activity. Elens *et al*. [37] were the first to find that the *CYP3A4**22 variant is associated with lower Tac dose requirements after renal transplantation. When *CYP3A4* and *CYP3A5* genotypes of individual patients were combined, Elens *et al*. were able to predict Tac dose requirements better compared with the *CYP3A4* or *CYP3A5* genotype alone. Based on these observations, it has been proposed to prescribe different Tac doses for ultrarapid (CYP3A5 expressers and *CYP3A4* *1/*1), intermediate (CYP3A5 non-expressers and *CYP3A4**1/*1) and poor (CYP3A5 non-expressers and *CYP3A4**22 carriers) CYP3A metabolizers, respectively.[37-39] In pediatric heart transplantation, an association between *CYP3A4*22* and Tac dose requirement has also been observed.[40] *CYP3A4*22* carriers needed 30% less Tac to reach similar target concentrations compared with *CYP3A4**1/*1 carriers.

Recently a new and rare *CYP3A4* variant was described, which is now designated as *CYP3A4**26.[41] This variant is a c.802C>T transition and results in a premature stop codon at position 268 in exon 9 (R268*).[41] The resulting truncated CYP3A4 protein is non-functional. Werk *et al*. [42] first identified this mutation when they observed an unusually low Tac dose requirement in a kidney transplant recipient. This patient had very high Tac exposure following standard Tac dosing and only reached the therapeutic window once the Tac dose was reduced to 0.5 mg thrice

weekly. This patient was a *CYP3A5*3* homozygote and was also homozygous for *CYP3A4*26*, and therefore experienced complete failure of CYP3A enzyme activity.

POR

POR is a protein that functions as an electron donor for CYP enzymes (including CYP3A) and is essential for CYP-mediated drug oxidation.[43] More than 100 SNPs have been identified in the human *POR* gene and these may influence POR–CYP interaction and CYP activity.[43, 44] The *POR*28* SNP (rs1057868; C>T) induces an amino acid substitution (p.Ala503Val) at position 503 which influences the electron binding moiety of POR and likely modifies its interaction with CYP enzymes.[43-45] Individuals homozygous *for POR*28* have an increased *in vivo* CYP3A activity with regard to midazolam compared with wildtype *POR*.[46]

In a study in 71 healthy Chinese volunteers, Zhang *et al*. [47] demonstrated that CYP3A5 expressers carrying the *POR*28* variant allele had a Tac exposure that was about 40% lower than CYP3A5 expressers with wildtype *POR*. The increased Tac dose requirement of CYP3A5-expressing kidney transplant recipients carrying the *POR*28* (T) variant allele was recently confirmed by Elens and Lunde *et al*. [48, 49] Taken together, these studies suggest that the *POR*28* SNP leads to increased CYP3A5-mediated Tac metabolism, possibly resulting from a facilitated interaction between POR, CYP3A5 and Tac. In CYP3A5 non-expressers, Tac metabolism depends entirely on CYP3A4. and *POR*28* apparently does not influence CYP3A4 activity to a clinically relevant degree.

ABCB1

ABCB1 is thought to be responsible for the low oral bioavailability of Tac and is also considered important for the distribution of Tac throughout the body and its excretion into bile and urine.[50] The *ABCB1* gene contains more than 50 SNPs of which the 3435C>T (rs1045642), 1236C>T (rs1128503) and 2677G>T/A (rs2032582) SNPs, which are in linkage disequilibrium, have received the most attention. The functional significance of these SNPs on *ABCB1* expression and function remains unclear. It has been suggested that the synonymous *ABCB1* 3435C>T SNP affects the timing of co-translational folding and insertion of ABCB1 into the membrane, thereby altering the structure of substrate and inhibitor sites.[51]

Many studies have investigated the influence of *ABCB1* SNPs on Tac pharmacokinetics but results are conflicting and suggest no or at best a limited impact of *ABCB1* SNPs on Tac exposure. For an extensive review of these studies, the reader is referred to the literature.[52]

Because ABCB1 is also expressed in the membrane of lymphocytes, its activity may also impact the intracellular accumulation of Tac where the drug exerts its biologic effect. Vafadari *et al*. [53] found that patients with the *ABCB1* 3435CC genotype need more Tac for inhibition of IL-2 production in T-cells compared with 3435TT genotype patients. Capron *et al*. demonstrated that patients carrying the *ABCB1* 3435T or the 2677T/A allele had 1.3-fold higher Tac concentrations within circulating lymphocytes compared with wildtype homozygotes.[54] These studies provide evidence that *ABCB1* 3435C>T and 2677G>T/A affect Tac distribution into lymphocytes with the variant alleles being associated with an increased pharmacodynamic effect of Tac. In line with the above, *ABCB1* SNPs also may be relevant with regard to its nephrotoxicity, because tissue concentrations of Tac are believed to be related to its renal side effects (see paragraph 3.1).

PPAR-α and PXR

The nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-α) have recently been recognized as potential contributor to intra- and inter-individual variability in CYP3A expression and activity. Two sequence variants in the PPAR-α gene (*PPARA*) can affect PPAR-α expression. *In vitro*, *PPARA* c.209-1003G>A and c.208+3819A>G were associated with reduced expression of PPAR-α, and consistently related to lower CYP3A4 mRNA levels, protein expression, and enzymatic activity.[55] Recently, Lunde *et al*. found that expression of at least one *PPARA* variant allele was significantly associated with a higher Tac C_0/D ratio, when adjusting for *POR*28*, *CYP3A5*3*, and *CYP3A4*22* among 229 kidney transplant recipients.[49] A detailed analysis of the two *PPARA* sequence variants showed significantly increased Tac exposure in patients homozygous for *PPARA*-α c.209-1003G>A. These results are in concordance with the reduced CYP3A4 protein/activity levels previously observed *in vitro*.[55] At present, *PPARA* c.208+3819A>G appears to be the *PPARA* sequence variant with the strongest influence on Tac pharmacokinetics but this observation requires confirmation.

The human pregnane X receptor (PXR; encoded by *NR1I2*), is a nuclear transcription factor that regulates the expression of CYP3A and ABCB1. Several SNPs in *NR1I2* have been identified but conflicting results regarding their association with Tac dose requirement have been reported.[9, 56, 57]

GENETIC VARIATION AND CLINICAL OUTCOMES

Tac treatment is accompanied by adverse effects, including nephrotoxicity (both acute and chronic), post-transplant diabetes mellitus, neurotoxicity and hypertension. With TDM the majority of patients can be brought within the targeted window quite rapidly after transplantation. Nevertheless, some patients will experience acute rejection or Tac toxicity, despite being within the target range, reflecting differences in Tac pharmacodynamics. The relationship between genetic variation and Tac pharmacodynamics is the subject of the second part of this review.

Nephrotoxicity

Acute CNI-induced nephrotoxicity is caused by constriction of the afferent glomerular arteriole leading to a reduced renal blood flow and glomerular filtration rate (GFR). Chronic CNI-induced nephrotoxicity appears to be the result of structural changes in the kidney caused by chronic changes in renal hemodynamics. CNI-induced nephrotoxicity is likely to be related to intra-renal concentrations of CNIs which may not be properly reflected by whole-blood CNI concentrations.[58-61] CYP3A5 is the only CYP3A isozyme expressed in the kidney and may limit local exposure to CNIs by intra-renal metabolism.[62, 63] Zheng *et al*. [64] demonstrated that Tac concentrations in the renal epithelium of CYP3A5 expressers are 53% lower compared with CYP3A5 non-expressers.

Studies on the relationship between *CYP3A5* genotype and the risk of Tac-induced nephrotoxicity have reported contradictory results. Kuypers *et al*.[33] observed a higher incidence of biopsy-proven Tac-nephrotoxicity (defined as *de novo* arteriolar hyalinization) in CYP3A5-expressing kidney transplant recipients. In a follow-up study, which included more patients (n = 304), this group confirmed that CYP3A5 expressers have an increased risk for biopsy-proven Tac-induced nephrotoxicity.[65] These counter-intuitive findings may be explained by the fact that

it is not Tac itself but its metabolites, that are responsible for its nephrotoxicity. These metabolites might be formed at an increased rate in the renal parenchyma of CYP3A5 expressers. However, there is at present little evidence to support this hypothesis.

In contrast to the studies by the Leuven group, a Chinese study including 67 kidney recipients showed a higher incidence of nephrotoxicity in CYP3A5 non-expressers at one month post-transplant.[66] In patients with the *CYP3A5**3/*3 genotype, interstitial fibrosis and proximal tubular vacuolization were more severe than in patients with the *CYP3A5**1/*3 genotype. Similarly, in a study with 136 renal transplant recipients (121 Caucasians, 12 Africans and 3 Asians), those with the *CYP3A5**3/*3 genotype tended to have a higher incidence of biopsy-proven nephrotoxicity compared to *CYP3A5**1 allele carriers, although the difference was non-significant.[67] There are many reasons for these discrepancies, including differences in ethnicity, sample size and the definition of nephrotoxicity.

ABCB1 is expressed in the apical membrane of renal tubular epithelial cells, where it may facilitate excretion of CNIs (and their metabolites) in urine and thus protect the kidney against intra-renal CNI accumulation. Studies on the relationship between *ABCB1* genotype and the risk of Tac-induced nephrotoxicity are more consistent compared with those on *CYP3A5*. In a prospective cohort study of 252 renal transplant recipients Naesens *et al*.[58] observed a progressive increase in glomerulosclerosis, vascular intimal thickening and IF/TA over the first 3 years post-transplantation. A lower ABCB1 expression in kidney transplant biopsies was a risk factor for such chronic histologic damage in patients receiving Tac. In another study they reported that both donor and recipient homozygosity for *ABCB1* 3435TT was associated with a higher risk of Tac-associated kidney damage.[68] Combined donor-recipient *ABCB1* 3435TT homozygosity was also a risk factor for worse graft function after the first post-transplant year. The authors speculated that the relevance of the recipient genotype could possibly be explained by renal epithelial chimerism in the allograft. Recently, in a study including 368 African American and 314 European American deceased donors, Ma *et al*. found that the T allele at *ABCB1* 3435 of the kidney donor is associated with shorter renal allograft survival compared with 3435C for kidneys from European American donors. [69] A poorer renal function (*i.e*. a lower estimated GFR) was also observed among patients who received kidneys from donors with the *ABCB1* 3435TT genotype as compared with patients receiving *ABCB1* 3435CC kidneys.[70]

In contrast, Moore *et al*. reported contradictory results. In a very large cohort (n = 4471 white kidney transplant recipients) it was the 3435CC donor genotype (not 3435TT as in the study by Ma *et al*.) that was associated with a worse death-censored graft survival.[71]

The discrepancies between these studies are unexplained. Again differences in sample size, patient characteristics, and duration of follow-up may form an explanation. Perhaps more importantly, loss of renal function may have causes other than chronic CNI nephrotoxicity and in many of the larger genetic association studies, "pure" chronic CNI nephrotoxicity was not distinguished from, for example, recurrent primary kidney disease, chronic rejection or polyomavirus-associated nephropathy.[14] Finally, and perhaps most importantly, there is no "gold standard" to diagnose chronic CNI nephrotoxicity. Even renal histology has its shortcomings and is not specific enough for making a definitive diagnosis.[72] Possibly, in the future, we will see an increasing use in genetic association studies of surrogate markers for the chronic nephrotoxic

effects of CNIs. Preliminary data indicate that markers of epithelial-to-mesenchyme transition may serve as such.[73]

Some studies have investigated the association between CNI-nephrotoxicity and genetic variation in genes other than *ABCB1* and *CYP3A*. One such gene is *CYP2C8*, which is a member of the P450 superfamily and is expressed in the kidney where it is involved in the metabolism of arachidonic acid (AA) to biologically active epoxyeicosatrienoic acids (EETs). EETs help to maintain blood pressure, are involved in tubular reabsorption of water and sodium transport, protect against inflammation, and the maintenance of vascular smooth muscle tone.[74-76] Smith *et al*. [77] found that patients carrying one or more *CY2C8**3 variant alleles have a higher risk of developing CNI-induced nephrotoxicity after liver transplantation. Possibly, decreased production of EETs in patients with the variant *CYP2C8**3 allele may reduce the capacity of their kidneys to counter the vasoconstrictive effects of CNIs. Gervasini *et al*. observed that the rs1042032A>G SNP in *EPHX2*, the gene that encodes soluble epoxy hydrolase, the enzyme which metabolizes EETs to less active compounds, was associated with renal allograft function and the risk of acute rejection.[78]

Delayed graft function

Delayed graft function (DGF) is most commonly defined as the need for dialysis within the first week after transplantation.[79, 80] It is associated with reduced long-term allograft survival and is closely related to ischemia/reperfusion injury.[79, 81]

Hauser *et al*. [82] investigated the impact of *ABCB1, ABCC2,* and *PXR* polymorphisms of the donor and recipient on the development of DGF after renal transplantation. The *PXR* 8055TT genotype of the donor (but not the recipient) was significantly associated with an increased risk of DGF. Another study, including 304 kidney transplant recipients found that DGF was associated with higher initial Tac exposure which occurred more frequently in CYP3A5 non-expressers.[83] A recent study in renal transplant patients found that the *CYP3A4**22 allele was associated with a higher risk of DGF compared with *CYP3A4**1 homozygotes in cyclosporine (CsA)-treated patients.[84] There are no reports on the association between *CYP3A4**22 and DGF in patients treated with Tac. More recently, Gervasini *et al*.[85] investigated the association between DGF and the *CYP2C8**3 variant allele. They observed that subjects carrying one or two *CYP2C8**3 variant alleles had a higher risk of developing DGF and had a lower creatinine clearance one year after transplantation than *CYP2C8**1/*1 homozygotes.[85]

Acute rejection

The incidence of acute rejection may be related to genetic variation in the genes encoding the proteins involved in the absorption and elimination of immunosuppressive drugs (reviewed in references [4, 52]). However, at present, no consistent association between *CYP3A* and *ABCB1* SNPs and an individual's risk for rejection has been demonstrated and the additional risk posed by certain genetic variants, if any, appears to be small and is unlikely to be clinically relevant. [86, 87] That these studies did not find *CYP3A5* genotype to be associated with the risk of acute rejection may be perceived as a surprise, given the strong influence of *CYP3A5* genotype on Tac dose requirement. It thus seems that the very efficient process of TDM results in rapid correction

of Tac concentrations outside the target range. As a result the genotype induced under-exposure only lasts for a few days, which is not sufficient to cause a clinically important increased incidence of acute rejection episodes.[88]

Post-transplant diabetes mellitus

Post-transplant diabetes mellitus (PTDM) is a frequent complication of Tac therapy.[89] Tac is directly toxic to islets of Langerhans and impairs insulin secretion and insulin gene expression.[89] A patient's genetic background may contribute to the risk of PTDM. Among 101 renal transplant recipients receiving Tac-based immunosuppressive therapy, Elens *et al.* found that the *PPARA* rs4253728 A>G and *POR*28* variant alleles were both independently associated with an increased risk of developing PTDM with respective odds ratios of 8.6 (95%-CI 1.4 to 54.2) and 8.1 (95%-CI 1.1 to 58.3).[90] Several other investigators have reported associations between the risk of PTDM and polymorphisms in the vitamin D receptor gene, promoter region of the IL-6 gene, transcription factor 7-like 2 gene (rs7903146), and the zinc transporter-8 gene (*SLC30A8*; rs13266634) .[91-95]

The role of pharmacogenetics in PTDM is complex. CNIs, glucocorticoids and mTOR inhibitors are all diabetogenic but alternative immunosuppressive regimens (including anti-proliferative agents and the novel immunosuppressant belatacept) have been associated with higher rejection rates.[96] Possibly, genetic risk factors may be used together with non-genetic variables to estimate an individual's risk of developing PTDM. However, even if this becomes possible in the future, the current literature does not provide guidance on what the best immunosuppressive regimen would be for such patients.

Other Tac-related adverse events

Tac can cause hypertension and is neurotoxic. Tac causes hypertension by activating the renal sodium chloride co-transporter, which is under the control of the ''with-no-lysine''(WNK) kinase network. Ferrarresso *et al.* genotyped 92 Caucasian kidney transplant recipients receiving CsA or Tac and found that *CYP3A5**1 carriers had a higher blood pressure 1 week and 6 months after transplantation.[97] Torio *et al.* also found a trend towards higher blood pressure in *CYP3A5**1 carriers treated with a CNI, 6 and 24 months after kidney.[98] At present, it appears that *CYP3A5* genotype may relate to an individual's risk of developing hypertension but there is no convincing evidence that SNPs in *ABCB1*, *WNK4* or *SPAK* do the same (see reference [99] for an extensive review on the genetic basis of hypertension).

Using pharmacogenetics to guide antihypertensive therapy in Tac-treated patients appears to be more readily clinically applicable. Diltiazem is a calcium channel antagonist that interacts with Tac by inhibiting CYP3A-mediated Tac metabolism. Kidney transplant patients expressing CYP3A5 were much more susceptible to the inhibitory effects of diltiazem than non-expressers. [100]

Neurotoxic effects of Tac include tremor, headache, insomnia, and peripheral neuropathy. [101] Although the exact pathophysiology of Tac-induced neurotoxicity is unclear, penetration of Tac into the central nervous system (CNS) is considered important. ABCB1 is an important component of the blood brain barrier and loss of its function leads to accumulation of Tac in the CNS, at least in mice.[102, 103] However, no clinically meaningful associations between *ABCB1* genotype and the risk of developing Tac-induced neurotoxicity have been identified. Yamauchi *et al.*[104] found

that transplant recipients carrying the *ABCB1* 2677T/A allele had an increased risk of neurotoxicity, whereas carriers of an *ABCB1* 3435T allele had a decreased risk. Yanagimachi *et al*. [105] reported that among 30 pediatric patients who received CsA for the prevention of graft-*versus*-host disease the *ABCB1* 1236CC genotype tended to be associated with neurotoxicity after adjustment for age, hypertension and renal dysfunction (*P*=0.07). In the same study, the *CYP3A5**1 allele was found to be associated with an increased risk for neurotoxicity. Yanagimachi *et al*. suggested that it may not be Tac itself but its metabolites that cause neurotoxicity. Such metabolites might be formed locally at an increased rate in CYP3A5 expressers. An increased risk for neurotoxicity in association with the *ABCB1* 1236C or 2677G alleles was also observed by a Spanish research group.[25] Prospective studies that measure Tac concentrations in the cerebrospinal fluid of affected patients may shed more light on the pathophysiology of Tac-induced neurotoxicity and the role of genetic variation therein.

ETHNIC CONSIDERATIONS

Ethnicity may play an important role in inter-individual variability of drug metabolism and response. Genetic variations of drug-metabolizing enzymes show pronounced differences between populations. Allelic frequencies of the most common SNPs in *CYP3A5*, *CYP3A4*, *ABCB1* and *POR*28* in various ethnic groups are presented in Table 1. The most striking difference is the marked variation in the allelic frequency of the *CYP3A5*3* allele which is common among Caucasian patients but less frequently seen in patients of Asian or African descent. Patients of African descent require higher doses of Tac to reach the target concentration range. Vadivel *et al*. found that for patients of African descent a Tac starting dose of 0.3 mg/kg per day is probably more effective than the currently recommended starting dose of 0.2 mg/kg per day.[106] This higher dose requirement appears to result in part from the high number of CYP3A5 expressers among patients of African descent.[107]

The lower exposure to Tac following standard dosing may be responsible for the higher acute rejection risk after kidney transplantation in recipients of African descent.[108] By contrast, *CYP3A5* genotype appears not to be a risk factor for the poorer long-term kidney allograft survival observed in patients of African descent, despite its well-characterized influence on Tac dose requirement. [109]

Table 1. Allele frequencies (by ethnic group) of relevant Tac metabolizing enzymes and transporters.

	Caucasians	Africans	Indians	Asians	Reference
*CYP3A5**3	90-93%	32%	66, 68%	60-73%	[129, 130]
*CYP3A5**6	0-4.3%	8.6-15%	ND	0%	[129]
*CYP3A5**7	0%	5-12%	ND	0%	[129]
*CYP3A4**1B	2-9.6%	35-67%	3.5%	0%	[117]
*CYP3A4**22	8.3%	4.3%	ND	4.3%	[38, 131]
ABCB1 3435C	48-62%	68-83%	38%	51-62%	[118]
ABCB1 1236C	55-59%	85%	ND	35-41%	[118]
POR*28	26%	19%	30%	37%	[44]

ABCB1:ATP-binding cassette subfamily B member 1; CYP:Cytochrome P450; POR:Cytochrome P450 oxidoreductase

In addition to *CYP3A5**3, the *CYP3A5**6 and *CYP3A5**7 variant alleles can also lead to the absence of functional CYP3A5 protein. These SNPs are rare or absent in Asian or Caucasian populations, but are found commonly in African populations. The presence of *CYP3A5**6 and *CYP3A5**7 in African populations may compensate for the relatively low frequency of the *CYP3A5**3 allele, resulting in a metabolic phenotype similar to those of Caucasians. The guidelines recommend increasing the starting dose by 1.5–2 times in extensive metabolizers (*CYP3A5**1/*1) and intermediate metabolizers (*CYP3A5**1/*3, *1/*6, or *1/*7) and to prescribe a standard dose in poor metabolizers (*CYP3A5**3/*3, *6/*6, *7/*7, *3/*6, *3/*7 or *6/*7).[110] This recommendation is supported by the findings of a study in African-American kidney transplant recipients (n=354). In this study, it was observed that *6 and *7 allele carriers required lower Tac doses. In this African-American population, one or more nonfunctional *CYP3A5* alleles (*3, *6 or *7) were identified in 74.5%.[111] This study demonstrated that there are considerably more CYP3A5 non-expressers in African populations than was previously presumed. In 197 adult African kidney transplant recipients, Oetting *et al*.[112] also found that the variants *CYP3A5**3, *CYP3A5**6, and *CYP3A5**7 explained a great proportion of the observed Tac C_0 variability in African recipients. Taken together, these studies illustrate the importance ethnicity-specific genotypes (*CYP3A5**6 and *CYP3A5**7) for Tac clearance. Using dosing models that account for these genotypes may lead to a more precise dosing of Tac.

Given the size and ethnic diversity of the Chinese population, it is very important to investigate the inter-ethnic variability in this particular group. In a study of six different Chinese ethnic groups, Lai *et al*.[113] found that significantly higher frequencies of *CYP3A5**3 variant alleles were observed in Uygur Chinese (88.1%), Kazakh Chinese (84.5%) and Tibetan Chinese (80.3%) than in Han (67.3%) and Bai Chinese (70.2%). The lowest frequency of the *CYP3A5**3 variant alleles was observed in Wa Chinese (56.3%). This result was consistent with what was reported previously by Li *et al*. [114] (Uygur Chinese 84.8%, Kazakh Chinese 86.6%, and Han Chinese 72.7%). The frequency of the *CYP3A5**3 variant allele in Uygur, Kazakh and Tibetan Chinese appears to be more similar to Caucasians as compared with Han Chinese. Other studies, however, did not report significant differences in the *CYP3A5**3 allelic frequency among Uygur and Kazakh Chinese and Caucasians.[11, 115, 116]

The frequency of *CYP3A4*1B* in African Americans (35-67%) is the highest amongst all ethnic groups.[117] The frequencies of the *ABCB1* 3435C and the 1236C alleles are also much higher in individuals of African descent than in populations of other ethnicity.[118] Recently, a novel *CYP3A4* loss-of-function allele (*CYP3A4*20*) was identified and was shown to be present in 1.2% of the Spanish population. This polymorphism has, however, not been investigated in relation to Tac dose requirement nor toxicity.[119, 120]

In conclusion, ethnic variation in the prevalence of *CYP3A5, CYP3A4* and *ABCB1* genotypes is high and clinically relevant. Given the fact that *CYP3A5* genotype has the strongest and most consistent association with Tac dose requirement, a *CYP3A5* genotype-based Tac dosing approach may be especially relevant for patients of African descent who are more often CYP3A5 expressers than Caucasians. How this genetic variability affects the metabolizing phenotype in the non-Caucasian and non-African population is incomplete and should be the subject for future studies.

DOSING ALGORITHMS

Dosing algorithms have only fairly recently been proposed to better individualize the Tac starting dose.[121] In 2011 Passey *et al*. created the first dosing algorithm using a combination of genetic information and clinical factors in adult kidney transplant recipients. The algorithm included *CYP3A5* genotype, days post-transplant, age, steroid and calcium channel blocker use. Interestingly, other factors such as sex, ethnicity and bodyweight did not have a statistically significant influence on Tac clearance.[122] The dosing algorithm was later successfully validated in an independent cohort of 795 kidney transplant recipients.[123] In 2013, the developed dosing algorithm was prospectively tested by an independent research group in the UK. Unfortunately the dosing algorithm was not able to predict estimated Tac clearance accurately.[124] As mentioned before, not all pharmacokinetic variability is explained by the *CYP3A5* genotype. It was recently shown that the algorithm designed by Passey was improved by incorporating the *CYP3A4*22* allele.[125]

More recently, Størset *et al*. [126] used the dosing software BestDose, including fat-free mass, hematocrit, time after transplantation, Tac dosing history and the patient's previously measured Tac concentrations, but not *CYP3A5* genotype, to determine the Tac starting dose in renal transplant recipients. They found that computerized dose individualization improved target achievement of Tac compared with conventional dosing early after renal transplantation and that the computer software may also potentially improve long-term outcome.[126] One advantage of not basing the dose predictions on genotype in this study is that it is useful also for centers without the opportunity to perform pre-transplant genotyping. To our knowledge this dosing algorithm has not been further tested or improved. This Norwegian study mainly included Caucasian patients. If algorithms such as these are to gain widespread (clinical) acceptance their performance should not only be validated but this should also be done in populations of different ethnicity.[126]

CONCLUSIONS

In conclusion the *CYP3A5* and *CYP3A4* genotype of the transplant recipient has an impact on Tac dose requirement in SOT recipients. Other variants, such as *CYP3A4*22, CYP3A4*26,* and *POR*28* are also associated with Tac dose requirement. Besides these pharmacokinetic considerations, ethnicity plays an important role in inter-individual variability in Tac metabolism. Unfortunately the evidence that implementing genotype-based Tac dosing will improve clinical outcome is missing. Recent studies have shown that dosing algorithms which incorporate genetics with demographic and clinical factors may allow for more precise Tac dosing. However, further research is necessary to elucidate the role of pharmacogenetics in the pharmacodynamic effects of Tac.

EXPERT OPINION

Immunosuppressive drug therapy is necessary to prevent acute rejection after SOT. Tac is the preferred CNI and it is to be expected that in the next 10 years many SOT recipients will continue to receive Tac as part of their immunosuppressive maintenance regimen. There is convincing evidence that the *CYP3A5* and the *CYP3A4* genotype of the recipient have a significant impact on Tac dose requirement. However, despite the strong genetic effect on Tac dose requirement, the evidence that implementing genotype-based dosing will improve clinical outcome is missing.

Two RCTs using *CYP3A5* genetic information to guide Tac dosing have been performed. The first of these showing a small increase in the proportion of patients reaching the target Tac concentration, but without reduction in the incidence of acute rejection, and a second study, which even failed to show an improvement of the achievement of the pharmacokinetic outcome parameters.

CYP3A5 is currently the strongest known genetic predictor of Tac dose requirement, but it does not explain all variability. Other variants, including *CYP3A4*22, CYP3A4*26,* and *POR*28* are also associated with Tac dose requirements. These other variants may need to be taken into consideration. Given its pro-active nature, pharmacogenetics may still be a potential complimentary tool to TDM for optimizing immunosuppressive therapy.

A more precise and rational strategy to optimize early Tac exposure is to use a dosing algorithm that incorporates more than just the bodyweight and *CYP3A5* genotype. Such dosing algorithms may include genetics and demographic and clinical factors. Although the implementation of dosing algorithms is appealing, they do have some limitations. The developed dosing algorithms are all very different and many have not been validated in independent data sets. Most algorithms are published in pharmacokinetic journals, which make them less accessible to clinicians. Refining of Tac dose prediction is possible using dosing algorithms. We propose that newly developed dosing algorithms for the starting dose need to be validated and subsequently tested in an independent cohort of patients. If successful, a clinical trial should be conducted with the amount of patients on target on day 3 after initiation as primary endpoint. Clearly, the rapid adjustment of Tac dose based on TDM can correct for any variability in Tac exposure resulting from genetic differences within a matter of days. Therefore, it is questionable if the transplant community will adopt the strategy of genotyping recipients prior to transplantation for these metabolizing enzymes and transporters.

To get the most out of the efficacy and safety of Tac, more effort has to be put into a better understanding of pharmacogenetics of the pharmacodynamic effects of the drug. It has been demonstrated that ABCB1 and CYP3A5 expression within the kidney transplant is associated with CNI-induced nephrotoxicity. However, with regard to the other toxic effects of Tac, like hypertension, neurotoxicity and PTDM, the results about the relationship between these side effects and pharmacogenetics are conflicting and seem to be of little value for the clinician. Prospective studies using novel techniques such as mass spectrometry to detect Tac metabolites and tissue drug concentrations, should be developed to elucidate the role of pharmacogenetics in Tac nephrotoxicity. Possibly, genetic information may predict the occurrence of drug toxicity and provide guidance to clinicians to choose for Tac-free or reduced-dose Tac immunosuppressive protocols in those at high risk.[127, 128]

Ethnicity plays an important role in inter-individual variability of drug metabolism and response. Where Caucasian patients are mostly CYP3A5 non-expressers, patients from Asian descent are CYP3A5 expressers in about one-third of cases, and those from African descent in two-thirds of cases. Pre-emptive genotyping for *CYP3A5* or other relevant Tac metabolizing enzymes may be more promising in ethnic populations containing higher proportions of expressers. The two prospective randomized trials were both performed in populations containing largely Caucasian patients.

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OVERWEIGHT KIDNEY TRANSPLANT RECIPIENTS ARE AT RISK OF BEING OVERDOSED FOLLOWING STANDARD BODYWEIGHT-BASED TACROLIMUS STARTING DOSE

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ABSTRACT

Background

Bodyweight-based dosing of tacrolimus is considered standard care, even though the available evidence is thin. An increasing proportion of transplant recipients is overweight, prompting the question if the starting dose should always be based on bodyweight.

Methods

For this analysis, data were used from a randomized-controlled trial in which patients received either a standard tacrolimus starting dose or a dose that was based on *CYP3A5* genotype. The hypothesis was that overweight patients would have tacrolimus overexposure following standard bodyweight-based dosing.

Results

Data were available for 203 kidney transplant recipients, with a median BMI of 25.6 (range 17.2 - 42.2). More than 50% of the overweight or obese patients had a tacrolimus predose concentration above the target range. The CYP3A5 non-expressers tended to be above target when they weighed more than 67.5 kg or had a BMI of 24.5 or higher. Dosing guidelines were proposed with a decrease up to 40% in tacrolimus starting doses for different BMI groups. The dosing guideline for patients with an unknown genotype was validated using the FDCC dataset.

Conclusion

This study demonstrates that dosing tacrolimus solely on bodyweight results in overexposure in more than half of overweight or obese patients.

INTRODUCTION

The oral starting dose of tacrolimus (Tac) in the first trial in humans was 0.15 mg/kg. This starting dose was based on animal studies[1, 2]. A few years later, in most transplant centers, oral Tac therapy was initiated with doses ranging from 0.10 to 0.20 mg/kg per day administered in two equally divided doses[3]. This recommended starting dose has not changed since [4].

Tac has a narrow therapeutic window and displays large pharmacokinetic variability[5]. Even with therapeutic drug monitoring (TDM) it can take up to 14 days to reach target concentrations[6]. In theory, the sooner target Tac concentrations are attained post transplantation, the more effective it is likely to be in preventing acute rejection[1]. Previous studies have suggested that underexposure of Tac could be related to low bodyweight and overexposure to higher bodyweight in kidney transplant recipients[7-10]. Available data suggests that being overweight or obese at time of kidney transplantation has a negative effect on both patient and allograft outcomes[11]. Overweight kidney transplant recipients have an increased mortality, more delayed graft functions, more acute rejection episodes, more wound infections, and a longer hospital stay compared to patients with a BMI of less than 30[12]. The KDIGO Transplant Workgroup states that dosing of Tac is important and that it is relatively under-investigated[1]. Although the Tac starting dose is based on bodyweight in many centers, dosing algorithms have demonstrated that bodyweight does not have a statistically significant influence on Tac clearance[13, 14]. The evidence that Tac elimination is linearly related to total bodyweight remains thin.

In 2003 the majority (60%) of kidney transplant recipients were overweight (BMI 25 – 30) or obese (BMI ≥ 30) at the time of transplantation[11]. With global obesity on the rise, this number is likely to increase even further and prompts the question if it is justified to continue to base the Tac starting dose on bodyweight. In this study, we investigated whether Tac dosing based on bodyweight leads to the achievement of Tac target whole-blood exposure in overweight patients.

PATIENTS AND METHODS

Aims

The aim of this study was to investigate whether a Tac starting dose based on bodyweight leads to the achievement of Tac target whole-blood predose concentrations (C_0) in overweight patients on day 3 after transplantation. This was defined as the first steady state concentration attained after five unaltered Tac doses. The subsequent dose was determined using TDM. The pre-specified Tac C_0 target range was 10-15 ng/mL. The hypothesis was that overweight patients are overdosed if the starting dose is strictly based on bodyweight alone. Furthermore, the influence of *CYP3A5* genotype on the relationship between bodyweight (or BMI) and Tac exposure was investigated.

Patients

The patients in this study were kidney transplant recipients who participated in a randomized-controlled trial investigating whether adaptation of the Tac starting dose according to *CYP3A5* genotype increases the proportion of kidney transplant recipients reaching the target Tac predose concentration range (10-15 ng/mL) at first steady-state. After day 3, the physicians were allowed to adapt the Tac dose based on whole-blood concentration measurements. The primary outcomes of this trial were presented in a separate publication[15].

In this trial, patients were randomized to receive Tac in either the standard, bodyweight-based dose of 0.20 mg/kg/day according to the package insert[4], or to receive a dose based on their *CYP3A5* genotype. CYP3A5 expressers (*i.e.* carriers of one or two *CYP3A5*1* alleles) received 0.30 mg/kg/day, whereas the non-expressers (*CYP3A5*3* homozygotes) received 0.15 mg/kg/day. The additional immunosuppressive therapy was identical for all patients and consisted of basiliximab, mycophenolic acid and prednisolone[15]. The only inclusion criteria for the *post-hoc* analysis presented here, was that there had to be a Tac C_0 available on day 3 after transplantation.

Tac concentration measurement

As described previously[15], the Tac C_0 was measured in the laboratory of the Erasmus MC using two different immunoassays: antibody-conjugated magnetic immunoassay (ACMIA) and enzyme multiplied immunoassay technique (EMIT). The measurements obtained with EMIT were systematically ~15% higher than those obtained with ACMIA. The weight of patients measured with the two different assays was not significantly different. The mean weight of the patients measured with ACMIA was 79.6 kg (95% CI 77-82.2 kg) and for EMIT 78.4 kg (95% CI 74-82.9 kg). The lower limits of detection were 1.5 ng/mL (ACMIA) and 2.0 ng/mL (EMIT). In this study, the lowest Tac concentration was 2.6 ng/mL. The upper limit of detection was 30.0 ng/mL. For calculation purposes, Tac C_0 above the detection limit were set at 30.0 ng/mL. For further details on the tacrolimus concentration measurement, the reader is referred to our previous publication[15].

Dosing guidelines

Dosing guidelines were developed and validated retrospectively in an independent cohort of patients that participated in the FDCC study[16]. All Tac concentrations were scaled to the theoretical starting dose of 0.2 mg/kg. The percentage of patients on target, and the median Tac concentration on day 3 after transplantation were compared before and after utilization of the dosing guideline.

Statistical analysis

For the analysis, the data were divided into three groups: the standard-dose group (SDG), the genotype-based group (GBG), and all patients (SDG plus GBG) scaled to the standard bodyweight dose. The latter group was created to be able to compare the C_0 of all patients, irrespective of whether the starting dose was adjusted according to genotype. The scaled C_0 was calculated by dividing the C_0 by the actual dose per kg bodyweight, and multiplying this by 0.2. Categorical variables are reported using frequency tables and percentages, and continuous variables are expressed as medians with ranges. Tac overexposure was defined as a Tac concentration above 15 ng/mL, and underexposure as below 10 ng/mL. The correlation between Tac C_0 and bodyweight (or BMI) was investigated by calculating the goodness of fit. Dosing guidelines were calculated using linear regression lines. Descriptive statistics were generated and all tests were two-tailed and statistical significance was defined as a p-value <0.05. Data were analyzed using IBM SPSS version 21 (SPSS Inc., Chicago, IL).

RESULTS

For this *post-hoc* analysis, data were available for 203 kidney transplant recipients. A total of 34 patients were not included in this analysis because no data on C_0 was available on day 3 after transplantation due to logistic issues (usually missed sample collection on Sunday)[15]. The patient characteristics are described in Table 1. The median bodyweight was 78.9 kg with a range of 37.6 kg – 123.1 kg, whereas the median BMI was 25.6 kg/m^2 with a range of 17.2 – 42.2 kg/m^2. Of all patients, 102 (50.2%) were overweight (BMI > 25), 40 (19.7%) were obese (BMI > 30) and 1 patient was morbidly obese (BMI > 40).

Bodyweight

In the SDG, the overweight or obese patients had significant higher median Tac C_0 (15.9 ng/mL) after five unaltered Tac doses than patients with a BMI smaller than 25 (12.0 ng/mL), with a p-value of 0.011. Of the overweight or obese patients, 57.8% were overexposed. The CYP3A5 expressers on average had lower Tac C_0 than the CYP3A5 non-expressers (10.7 ng/mL *vs*. 16.2 ng/mL, p=0.001). On day 3 after transplantation, a considerable proportion (47%) of non-expressers was overexposed (Figure 1A). In CYP3A5 expressers, only 13% was overexposed (p=0.003).

Figure 1B shows the relationship between bodyweight and Tac C_0 in the GBG. In this group, all non-expressers received 0.15 mg/kg and the expressers 0.30 mg/kg. Of all patients dosed

Table 1. Patient characteristics

	Standard-dose group	Genotype-based group	p-value
Recipient gender			0.864
Male	64 (64.6%)	65 (62.5%)	
Female	35 (35.4%)	39 (37.5%)	
Age of recipient (years)*	55.5 (19-77)	55.0 (19-79)	0.945
Ethnicity			0.914
White	77 (77.8%)	83 (79.8%)	
Asian	10 (10.1%)	8 (7.7%)	
African descent	10 (10.1%)	10 (9.6%)	
Other	2 (2%)	3 (2.9%)	
Bodyweight (kg)*	75.4 (37.6-122.8)	81.4 (43.6-123.1)	0.247
Height (cm)*	174.0 (148.0-203.0)	173.5 (151.0-193.0)	0.800
BMI (kg/m^2)*	24.7 (17.2-37.8)	26.5 (18.1-42.2)	0.093
CYP3A5 genotype			0.658
**3/*3*	76 (77%)	76 (73%)	
**1/*3*	19 (19%)	25 (24%)	
**1/*1*	4 (4%)	3 (3%)	
Tac starting dose (mg/kg)*			
Non-expresser	0.20 (0.19-0.21)	0.15 (0.14-0.16)	**<0.05**
Expresser	0.20 (0.19-0.21)	0.30 (0.29-0.31)	**<0.05**

*Presented as median and range

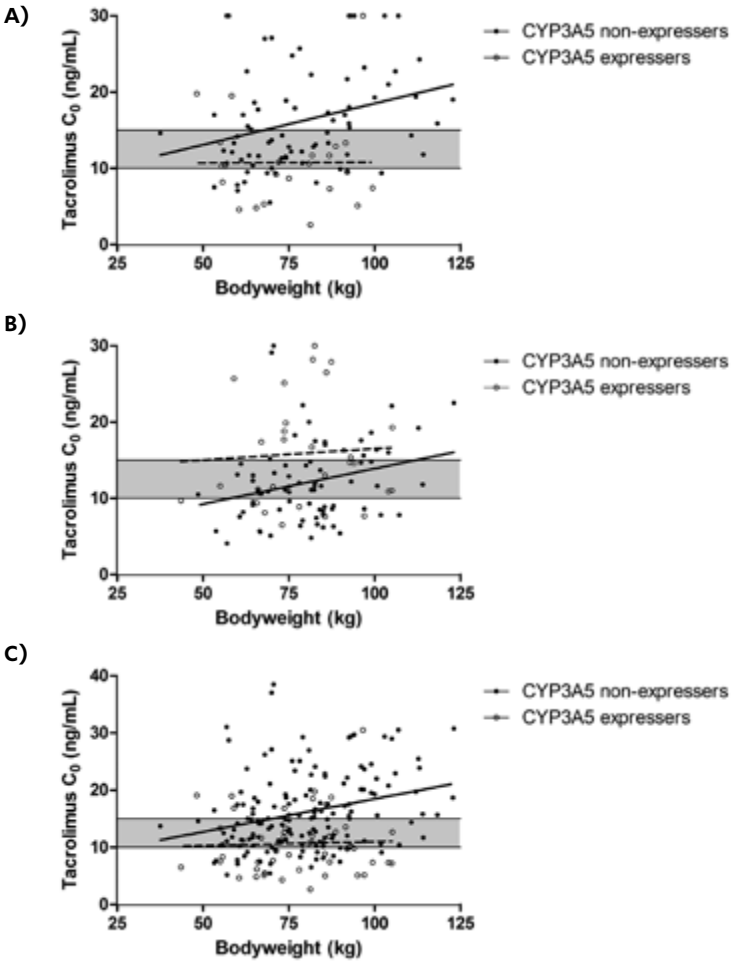

Figure 1. The association between bodyweight, *CYP3A5* genotype and Tac C_0 on day 3 after transplantation, with the target concentration of 10-15 ng/mL shown (shaded area). 1A: Patients who received 0.20 mg/kg tacrolimus. 1B: Patients who received tacrolimus in a dose of 0.15 mg/kg (CYP3A5 non-expressers) or 0.30 mg/kg (CYP3A5 expressers). 1C: All patients scaled to 0.2 mg/kg dose.

according to genotype, 35.4% of all overweight or obese patients were overexposed. Of all patients in this group, 30% was overexposed, whereas 35% was underexposed.

Figure 1C depicts all patients in the study scaled to the theoretical dose of 0.2 mg/kg/day. Due to scaling of the dose, the maximum Tac concentration depicted in the figure increased to 38.5 ng/mL, rather than the 30 ng/mL cut-off. Of all overweight or obese patients, 53.6% were overexposed. In line with the SDG, a substantial proportion of non-expressers was above the target range (36%) and over a quarter (27%) was underexposed. The correlation line crosses the upper limit of the target range at 67.5 kg (calculated with the regression line $y = 0.12x + 6.9$, $r^2 = 0.08$). Of all patients weighing more than 67.5 kg, 40% of non-expressers was above the target range and 25% was below the target range.

BMI

In the SDG, the non-expressers were overexposed if the BMI increased, whereas paradoxically, the expressers had fairly stable exposure regardless of BMI (see Figure 2A). In the GBG, a considerable amount of expressers was overexposed (Figure 2B), whereas the non-expressers were below target if the BMI was lower than 22.3 kg/m^2 and above target if it was higher than 33.2 kg/m^2 ($y = 0.46\,x - 0.26$, $r^2 = 0.15$).

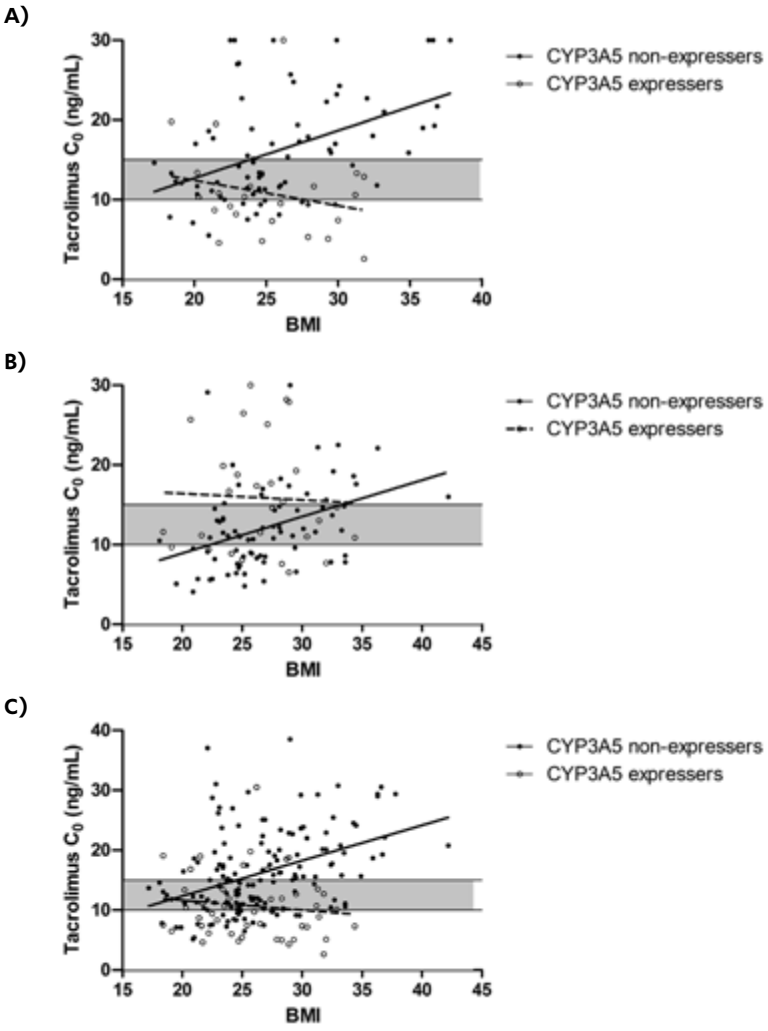

Figure 2. The association between BMI, *CYP3A5* genotype and Tac C_0 on day 3 after transplantation, with the target level of 10-15 ng/mL shown (shaded area). 2A: Patients who received 0.20 mg/kg tacrolimus. 2B: Patients who received tacrolimus in a dose of 0.15 mg/kg (CYP3A5 non-expressers) or 0.30 mg/kg (CYP3A5 expressers). 2C: All patients scaled to 0.2 mg/kg dose.

When scaled to the theoretical standard dose, a considerable amount (36%) of non-expressers was overexposed (Figure 2C). The correlation line crosses the upper limit of the target range at 24.5 kg/m^2 (calculated with the regression line y = 0.59 x + 0.53, r^2 = 0.17). Of all patients with a BMI greater than 24.5 kg/m^2, 44% of non-expressers were above the target range and 24% were below target.

Overweight patients with a BMI > 25 kg/m^2 took a median of 6 days to reach the target concentration, compared to 4.5 days in patients with a normal bodyweight (p = 0.083).

Dosing guidelines

Dosing guidelines for overweight patients based on BMI are shown in Table 2. The recommendations were calculated with a target C_0 of 10 – 15 ng/mL on day 3. A distinction is made between patients in whom the *CYP3A5* genotype status is known and in whom it is unknown. In accordance with the CPIC guideline[17] it is not common practice in most centers to genotype patients before transplantation, therefore the guideline for unknown genotype was developed. Unfortunately, there wasn't a sufficient number of *CYP3A5* expressers in our population to be able to calculate specific dosing guidelines based on BMI for this group. No guidelines for patients with a BMI > 35 could be developed due to a lack of patients in this category. For a typical patient with a BMI of 28 kg/m^2 and an unknown *CYP3A5* genotype, the dose should be 0.17 mg/kg (85%) if the standard starting dose is 0.20 mg/kg based on the developed dosing guideline (Table 2). If this same patient is a known CYP3A5 non-expresser, the dose should be 0.16 mg/kg (80%).

The recommended dosing guidelines were validated retrospectively in an independent cohort of patients that participated in the FDCC study[16] by comparing the percentage on

Table 2. Dosing guidelines (mg/kg) based on a patient's BMI and *CYP3A5* genotype.

	***CYP3A5* genotype unknown**	***CYP3A5* Non-expresser**
BMI < 25	0.20 (100%)	0.20 (100%)
BMI 25-30	0.17 (85%)	0.16 (80%)
BMI 30-35	0.15 (75%)	0.14 (70%)

Table 3. Validation of the dosing guidelines: median Tac C_0 (ng/mL)

	CYP3A5 unknown				**CYP3A5 non-expressers**			
	n	**before**	**after**	**p-value**	**n**	**before**	**after**	**p-value**
All patients	137	16.8	15.1	<0.05	111	18.5	16.4	**<0.05**
BMI < 25[a]	82	15.3	n/a	n/a	65	15.4	n/a	n/a
BMI 25-30	42	17.8	15.1	<0.05	36	18.5	14.8	**<0.05**
BMI 30-35	11	15.2	11.4	<0.05	8	20.0	14.0	**<0.05**

The median Tac C_0 is shown on day 3 post transplantation before and after correction with the developed dosing guidelines. All concentrations were scaled to a dose of 0.2 mg/kg per day.
[a] Not assessed because the developed dosing guidelines recommend a dose of 100%.

target before and after utilization of the guideline, and the median Tac concentration on day 3 after transplantation. The results are shown in Table 3. If *CYP3A5* genotype is unknown, 21.8% of overweight patients were on target before utilization of the guideline, and 32.7% afterwards. The percentage of patients overexposed dropped from 70.9% to 47.2%. Especially overweight patients (BMI 25-30) benefit of the dosing guideline with 16.7% of patients on target before, and 31% after applying the dosing guideline. The percentage of patients on target before and after utilization of the dosing guideline are shown in figure 3. When underweight patients (BMI smaller than 20 kg/m^2) were excluded from the analysis, the proportion of patients overexposed and on target remained the same (data not shown).

DISCUSSION

This study demonstrates that dosing Tac solely on bodyweight results in overexposure in a considerable proportion of patients. This is especially the case for overweight and obese patients. Based on the goodness of fit, the association between BMI and Tac exposure seems stronger than the one between bodyweight and Tac. To our knowledge only two studies have investigated the relationship between bodyweight or BMI and Tac exposure at first steady state. Rodrigo *et al*. concluded that overweight renal transplant recipients are more prone to develop initial high exposure (*i.e*. a C_0 >15 ng/mL) compared to non-overweight recipients[9]. Rodrigo *et al*. demonstrated that the first Tac C_0 tended to be higher than 15 ng/mL in overweight, older kidney transplant recipients[9]. Sawamoto *et al*. demonstrated that the average Tac maintenance dose in patients with a BMI greater than 25 kg/m^2 is significantly lower compared with that in patients with a BMI ≤ 25 kg/m^2 [18]. Our study substantiates these findings.

Dosing guidelines for overweight and obese patients were developed and validated for patients with an unknown genotype and CYP3A5 non-expressers. As a validation cohort we used patients who participated in the FDCC trial. In this multi-center study tacrolimus was dosed according to

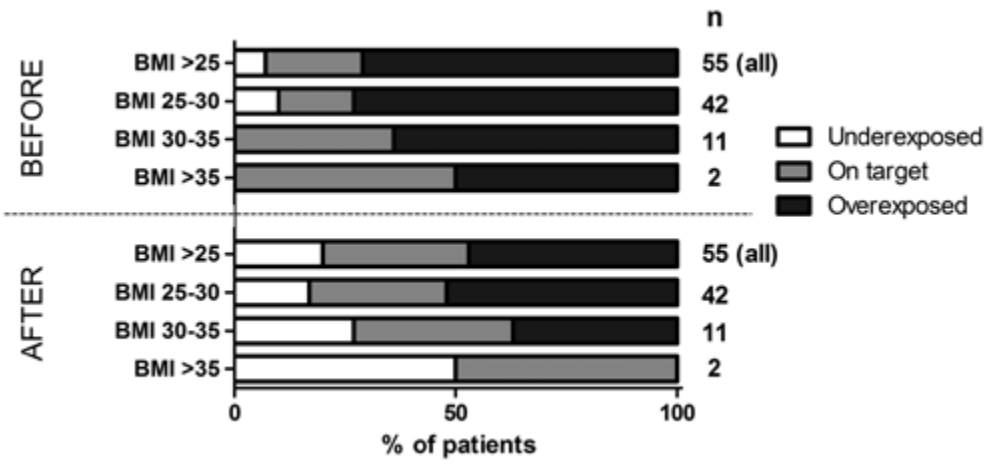

Figure 3. The percentage of patients underexposed, on target and overexposed before and after utilization of the developed dosing guideline in a validation cohort of patients with an unknown genotype.

local clinical practice, and as a consequence this is a very heterogeneous group. Despite this, we managed to validate our guidelines.

Unfortunately no dosing guideline could be developed for CYP3A5 expressers because there were too few CYP3A5 expressers in our population. Also insufficient obese patients with a BMI > 35 were available to validate the dosing guideline in that group. Establishing separate dosing guidelines for overweight patients is not unusual. Other drugs such as aminoglycosides are dosed using ideal body weight in overweight patients[19]. However, aminoglycosides are hydrophilic drugs, whereas Tac is lipophilic. Overweight patients have a larger fat compartment, and therefore adjusting the dose of hydrophilic drugs based on total bodyweight would lead to overexposure. However, for lipophilic drugs, a larger volume of distribution would be expected in overweight patients, and therefore choosing the dose based on total bodyweight seems logical. Miyamoto *et al*. demonstrated that the tacrolimus concentration in fat tissue was lower than what one would expect based on the lipophilicity of the drug[20]. This substantiates the present finding that the tacrolimus dose should not be entirely based on bodyweight in obese patients. An explanation for this counter-intuitive finding could be that tacrolimus is extensively distributed into erythrocytes which have a high content of FK-binding proteins to the receptor of tacrolimus[4]. Although with an increasing bodyweight, the blood plasma volume also increases, this occurs to a lesser extent. Theoretically the tacrolimus initial dose should be based on this increase in blood plasma volume rather than the increase in bodyweight.

To our knowledge this is the first study to give dosing guidelines for overweight and obese transplant recipients. A subject for future research could be the influence of other genetic polymorphisms in for example *CYP3A4* which has also been associated with altered tacrolimus clearance. Especially *CYP3A4*22* is interesting as it is associated with a lower tacrolimus dosage after renal transplantation[21].

The biggest limitation of this study is that it is a *post-hoc* analysis. It was unplanned and therefore the apparent differences and associations could be coincidental. The second limitation is that more than three quarters of the studied population was Caucasian, and only 10% was from African descent. Research has shown that African-American kidney transplant recipients require a higher Tac dose regardless of their *CYP3A5* genotype[22, 23]. The third limitation is that Tac concentrations were measured with immunoassays, instead of using mass spectrometry which is nowadays considered the gold standard. In our center immunoassays were used for the routine determination of Tac at the start of the trial. Many transplant centers worldwide still rely on immunoassays for TDM of Tac. The final limitation is that the target range of 10-15 ng/mL we aimed for, may now be regarded as rather high although the precise therapeutic range for tacrolimus is still unclear[24-27].

In conclusion, basing the Tac starting dose solely on bodyweight leads to overexposure in more than one-third of patients, and over half of overweight or obese patients. Basing the Tac starting dose on BMI in overweight patients, whether or not in combination with *CYP3A5* genotype, could reduce overexposure of Tac early after transplantation.

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7

A POPULATION PHARMACOKINETIC MODEL TO PREDICT THE INDIVIDUAL STARTING DOSE OF TACROLIMUS IN ADULT RENAL TRANSPLANT RECIPIENTS

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ABSTRACT

Aims

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

Methods

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

Results

A total of 4,527 tacrolimus blood samples collected from 337 kidney transplant recipients were available. Data were best described using a two-compartment model. The mean absorption rate was 3.6 h^{-1}, clearance was 23.0 L/h (39% IIV), central volume of distribution (Vd) was 692 L (49% IIV) and the peripheral Vd 5340 L (53% IIV). Inter-occasion variability was added to CL (14%). Higher body surface area (BSA), lower serum creatinine, younger age, higher albumin and lower hematocrit levels were identified as covariates enhancing tacrolimus clearance. Cytochrome P450 (CYP) 3A5 expressers had a significantly higher tacrolimus clearance (160%), whereas *CYP3A4*22* carriers had a significantly lower clearance (80%). From these significant covariates, age, BSA, *CYP3A4* and *CYP3A5* genotype were incorporated in a second model to individualize the tacrolimus starting dose:

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

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

Conclusions

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

INTRODUCTION

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

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

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

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

the model was extensively validated, both internally and externally. A separate starting dose model was developed.

METHODS

Study Design

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

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

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

Immunosuppression

All patients were treated with oral twice-daily tacrolimus (Prograft®, Astellas Pharma, Leiden, The Netherlands) in combination with mycophenolic acid. Tacrolimus doses were tailored using TDM. The tacrolimus predose concentration (C_0) was measured for the first time on day 3 following transplantation in the Rotterdam cohort [33]. In the Leiden cohort, blood samples were drawn before tacrolimus ingestion, and 1, 2, 3, 4, 5 and 6 hours post ingestion. This is routine clinical care in Leiden. In the Leiden cohort, tacrolimus concentrations for the pharmacokinetic curve were obtained at steady state, with a median of 2 weeks after transplantation. The validation cohort consisted of 304 patients; of which 7 patients participated in the Symphony-Elite study. In this study, blood samples were drawn before tacrolimus ingestion, and 0.3, 0.7, 1.3, 2, 3, 4, 6, 8, 10 and 12 hours post ingestion [35]. For the remaining 297 patients only C_0 were available [12].

In the Rotterdam cohort, the target tacrolimus C_0 range was 10.0-15.0 ng/mL in week 1-2, 8.0-12.0 ng/mL in weeks 3-4, and 5.0-10.0 ng/mL after week 4 post transplantation. In the Leiden cohort, the target AUC was 210 ng*hr/mL with a corresponding C_0 range of 10.0-15.0 ng/mL the first six weeks following transplantation. After week 6 post transplantation, the target AUC was 125 ng*hr/mL with a corresponding C_0 range of 4.0-9.0 ng/mL.

Laboratory Analysis

Genotyping for *CYP3A5*3* and *CYP3A4*22* was performed as described previously [33, 34]. Deviations from Hardy-Weinberg equilibrium were tested using the Chi-squared goodness-of-fit (GOF) test. Tacrolimus concentrations in the Rotterdam cohort were analyzed in whole-blood samples using two different immunoassays: the antibody-conjugated magnetic immunoassay (ACMIA) and the enzyme multiplied technique (EMIT), as described previously [33]. The lower

limits of quantification (LLOQ) were 1.5 ng/mL (ACMIA) and 2.0 ng/mL (EMIT). The upper limit of quantification (ULOQ) was 30.0 ng/mL. Tacrolimus concentrations in the Leiden cohort were measured using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method [34]. The LLOQ was 1.0 ng/mL and the ULOQ 50.0 ng/mL. In the validation cohort, samples were measured using a validated LC-MS/MS [12].

Population Pharmacokinetic Modelling

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

Base Model Development

One and two compartment models were considered based on visual inspection of the data and a review of the literature. Typical values for lag-time (t_{lag}), absorption rate constant (k_a), central volume of distribution (V_1), peripheral volume of distribution (V_2), CL and inter-compartmental clearance (Q) were estimated. As bioavailability (F) could not be estimated, F was fixed to 1 and certain values were estimated as ratios: *CL/F*, *Q/F*, V_1/F and V_2/F. Inter-individual variability (IIV) and inter-occasion variability (IOV) were modeled for each pharmacokinetic parameter using an exponential model. An occasion was defined as the measurement of a C_0. Residual variability was incorporated as an additive and proportional error for immunoassay, and as a proportional error for LC-MS/MS. For all model parameters for which IIV was estimated, shrinkage was calculated. A shrinkage value below 25% was considered acceptable [36]. Minimum objective function values (OFVs, $p < 0.01$), parameter precision, error estimates, shrinkage value and visual inspection of the GOF plots were considered for model selection.

Covariate Model Development

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

First, the relationship between IIV and covariates was investigated graphically. Covariates with a visually apparent relationship and a clinically plausible relationship with the pharmacokinetic

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

Internal Model Evaluation

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

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

External Model Evaluation

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

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

Starting dose model

The final model was used to develop a model for the starting dose of tacrolimus after kidney transplantation. Each significant covariate in the final model was evaluated if it was clinically relevant, feasible to use, and if it significantly influenced the starting dose of tacrolimus. The starting dose model was then validated using the techniques mentioned in paragraphs 2.4.3 and 2.4.4.

Simulation trial

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

RESULTS

A total of 337 patients were included in the model building group. Patient characteristics are presented in Table 1. From these patients, 4,527 blood samples were collected and analyzed for

tacrolimus concentrations (range 1.6 – 96.0 ng/mL). A quarter of the blood samples in the model building groups was drawn the first week following transplantation. In total, 3 samples (0.07%) in the Rotterdam cohort were below the lower limit of quantification of the immunoassay and were discarded. A total of 40 samples (0.88%) in the Rotterdam cohort were above the upper limit of quantification. These samples were estimated by NONMEM and after every critical model building step checked if the estimate was plausible. The allele frequencies of the tested single-nucleotide polymorphisms (SNPs) are depicted in Table 1. There was no deviation from the Hardy-Weinberg equilibrium.

Base Model

The data were best described by a two-compartment model with first order absorption. Including IIV on *CL/F*, V_1/F, V_2/F and *Q/F* significantly improved the model fit. The OFV decreased further, and parameter precision, error estimates and GOF plots improved after introduction of IOV on *CL/F*. Building the different analytical techniques for tacrolimus into the residual error model improved the base model. The residual error was described with a combined additive and proportional error model for the immunoassay measured concentrations, and with a separate proportional error model for the LC-MS/MS measured concentrations. Parameter estimates of the base model, final model and simulation model are presented in Table 2.

3.2 Covariate Analysis

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

Equation 1:

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

The NONMEM control stream for the analysis has been included in the supporting information.

Table 1. Patient Characteristics

	Model building group 1 (n = 237)	Model building group 2 (n = 100)	Model validation group (n = 304)
Recipient gender			
Male	148 (62.4%)	56 (56.0%)	200 (65.8%)
Age of recipient (years)	58.5 (19.4-79.4)	54.0 (15.0-77.0)	52.0 (17.0-83.0)
Ethnicity			
Caucasian	186 (78.4%)	78 (78.0%)	304 (100%)
Asian	23 (9.7%)	8 (8.0%)	0 (0%)
African descent	23 (9.7%)	1 (1.0%)	0 (0%)
Other	5 (2.1%)	13 (13.0%)	0 (0%)
Bodyweight (kg)*	79.4 (37.6-132.0)	74.0 (40.0-114.0)	68.0 (40.0-106.0)
Height (cm)*	183 (145-203)	172 (141-195)	166 (145-190)
BMI (kg/m^2)	25.8 (17.2-42.2)	24.8 (15.6-38.2)	24.7 (16.3-44.1)
Body Surface Area (m^2)	2.03 (1.24-2.66)	1.90 (1.33-2.48)	1.78 (1.18-2.36)
Ideal Bodyweight (kg)	68.3 (46.8-89.8)	65.3 (41.7-83.9)	60.9 (45.7-80.2)
Lean Bodyweight (kg)	64.0 (33.1-85.3)	55.9 (33.6-81.7)	51.4 (34.9-76.3)
Fat mass (kg)	21.7 (12.0-44.0)	26.0 (14.1-49.5)	25.5 (11.3-50.2)
Laboratory measurements*			
Haematocrit (L/L)	0.34 (0.15-0.80)	0.34 (0.24-0.45)	0.33 (0.18-0.59)
Creatinine (µmol/L)	137 (38-1885)	124 (62-920)	139 (47-1284)
ASAT (U/L)	21 (<5-662)	Unknown	Unknown
Albumin (g/L)	42 (12-57)	Unknown	Unknown
Bilirubin (µmol/L)	6 (<2-305)	Unknown	Unknown
Total Protein (g/L)	64 (23-86)	Unknown	Unknown
CRP (mg/L)	11 (<0.3-320)	Unknown	Unknown
Genotype			
CYP3A4			
**1*	205 (86.5%)	91 (91.0%)	275 (90.5%)
**22*	22 (9.3%)	9 (9.0%)	29 (9.5%)
Unknown	10 (4.2%)	0 (0%)	0 (0%)
CYP3A5			
**1/*1*	9 (3.8%)	4 (4.0%)	0 (0%)
**1/*3*	56 (23.6%)	17 (17.0%)	49 (16.1%)
**3/*3*	172 (72.6%)	76 (76.0%)	255 (83.9%)
**3/*6*	0 (0%)	3 (3.0%)	0 (0%)
ABCB1 3435C>T			
CC	55 (24.3%)	Unknown	Unknown
CT	111 (49.1%)	Unknown	Unknown
TT	60 (26.5%)	Unknown	Unknown
*POR*28*			
CC	128 (56.4%)	Unknown	Unknown
CT	78 (34.4%)	Unknown	Unknown
TT	21 (9.3%)	Unknown	Unknown

Table 1. (continued)

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

Model building group 1 consists of patients transplanted in the Erasmus MC (Rotterdam cohort). Model building group 2 consists of patients transplanted in the LUMC (Leiden cohort).
ASAT aspartate aminotransferase, *CRP* C-reactive protein, *CYP* cytochrome P450, *POR*28* P-450 oxidoreductase*28, *RRT* renal replacement therapy
* Presented as median and range over a three-month period for continuous variables.

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

Parameter	Base Model (RSE %) [shrinkage]	Final Model (RSE %) [shrinkage]	Starting Dose Model (RSE %) [shrinkage]
t_{lag} (h)	0.29 (17)	0.38 (49)	0.39 (12)
k_a (L/h)	3.26 (19)	3.58 (40)	3.70 (13)
CL/F (L/h)	25.9 (3)	23.0 (3)	22.5 (3)
V_1/F (L)	655 (7)	692 (8)	685 (5)
Q/F (L/h)	10.5 (7)	11.6 (10)	10.6 (6)
V_2/F (L)	6320 (14)	5340 (22)	6590 (14)
Covariate effect on CL			
*CYP3A5*1*	-	1.63 (15)	1.62 (14)
*CYP3A4*22*	-	0.80 (32)	0.81 (36)
Haematocrit (L/L)	-	-0.76 (11)	-
Creatinine (µmol/L)	-	-0.14 (26)	-
Albumin (g/L)	-	0.43 (30)	-
Age (years)	-	-0.43 (19)	-0.50 (15)
BSA (m^2)	-	0.88 (24)	0.72 (29)
Covariate effect on V_1			
Lean bodyweight (kg)	-	1.52 (20)	-
IIV (%)			
CL/F	46.3 (5) [10]	38.6 (6) [8]	39.4 (6) [10]
V_1/F	50.2 (11) [19]	49.2 (7) [25]	54.0 (11) [19]
V_2/F	52.3 (14) [62]	53.0 (16) [58]	53.7 (13) [61]
Q/F	79.6 (12) [29]	78.7 (11) [28]	79.6 (11) [29]
IOV (%)			
CL/F	15.1 (9)	13.6 (10)	14.6 (9)
Residual variability			
Proportional (%)			
Immunoassay	16.6 (6) [26]	17.7 (7) [22]	16.9 (6) [25]
LC-MS/MS	24.7 (5) [12]	24.5 (5) [12]	24.4 (5) [12]
Additive Immunoassay (µg/L)	1.02 (9) [26]	0.88 (13) [22]	1.02 (10) [25]

CL clearance, *CYP* cytochrome P450, *F* bioavailability of oral tacrolimus, *IIV* inter-individual variability, *IOV* inter-occasion variability, K_a absorption rate constant, *OFV* objective function value, *Q* intercompartmental clearance of tacrolimus, *RSE* residual standard error, t_{lag} lag time, V_1 central compartment for tacrolimus, V_2 peripheral compartment for tacrolimus

Evaluation of the Final Model

All estimates were within the limits, given the criteria as defined in the Methods section, with the exception of shrinkage for V_2 and Q. The population and individual predictions were evenly distributed around the line of unity. The conditional weighted residuals were normally distributed. (Fig. 1). The median and variability of the C_0 fell mostly within the corresponding simulations as shown in the VPCs, with concentrations in simulations slightly lower than the measured concentrations approximately 2.5-4 hours after dose (Fig. 2A). NPDE analysis showed adequate predictive ability with distribution of the NPDEs within an acceptable deviation from a normal distribution (Supporting information). Evaluation of the individual's influence on a change in OFV

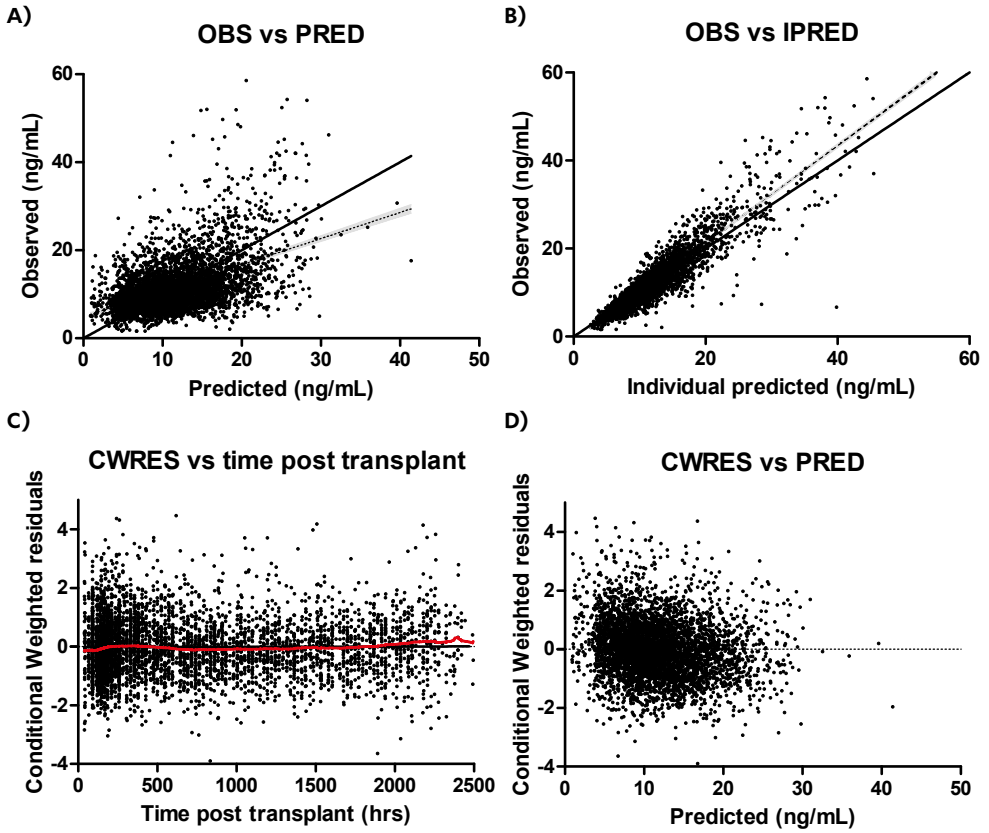

Figure 1. Goodness-of-fit plots of the final model. **A** DV plotted against PRED. **B** DV plotted against IPRED. **C** The correlation of CWRES with the time after the tacrolimus dose. **D** The correlation of CWRES with PRED. The line represents the line of identity. *CWRES* conditional weighted residuals, *DV* observed concentrations, *IPRED* individual predicted concentration, *OBS* observed concentration, *PRED* predicted concentration.

by shark plot showed that 73% of patients had a decrease in OFV with the final model compared with the base model. In the external validation, the median of the observed data was close to the lower bound of the simulated data in the second half of the curve. However, for an external validation in clinical data, the median was acceptably described (Fig. 2B). Unfortunately, we had no albumin levels at our disposal in the external validation cohort and therefore we fixed the albumin concentration to the population albumin median in the external validation.

Simulations

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

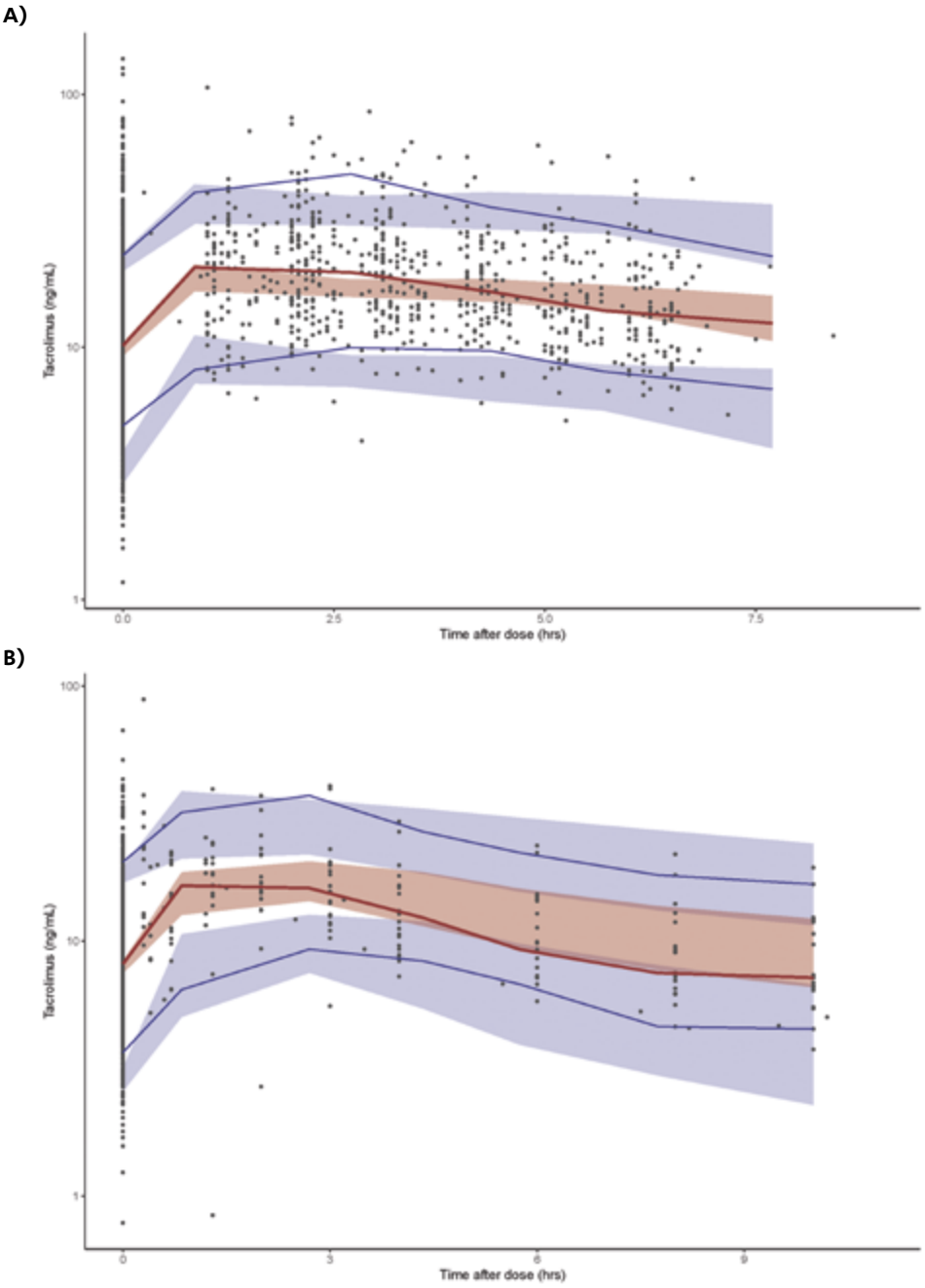

Figure 2. Prediction corrected visual predictive check showing how well the average trend of the observations (red line) and how well the variability of the observed data (blue lines) fall within the model simulated (n = 500) average trend (red shaded area) and the model simulated variability (blue shaded areas) represented as 95% CI. The average and the variability of the observed data both fall within the corresponding simulations. **A** prediction corrected VPC of the final model (internal dataset). **B** prediction corrected VPC of the final model (external dataset). *VPC* Visual Predictive Check.

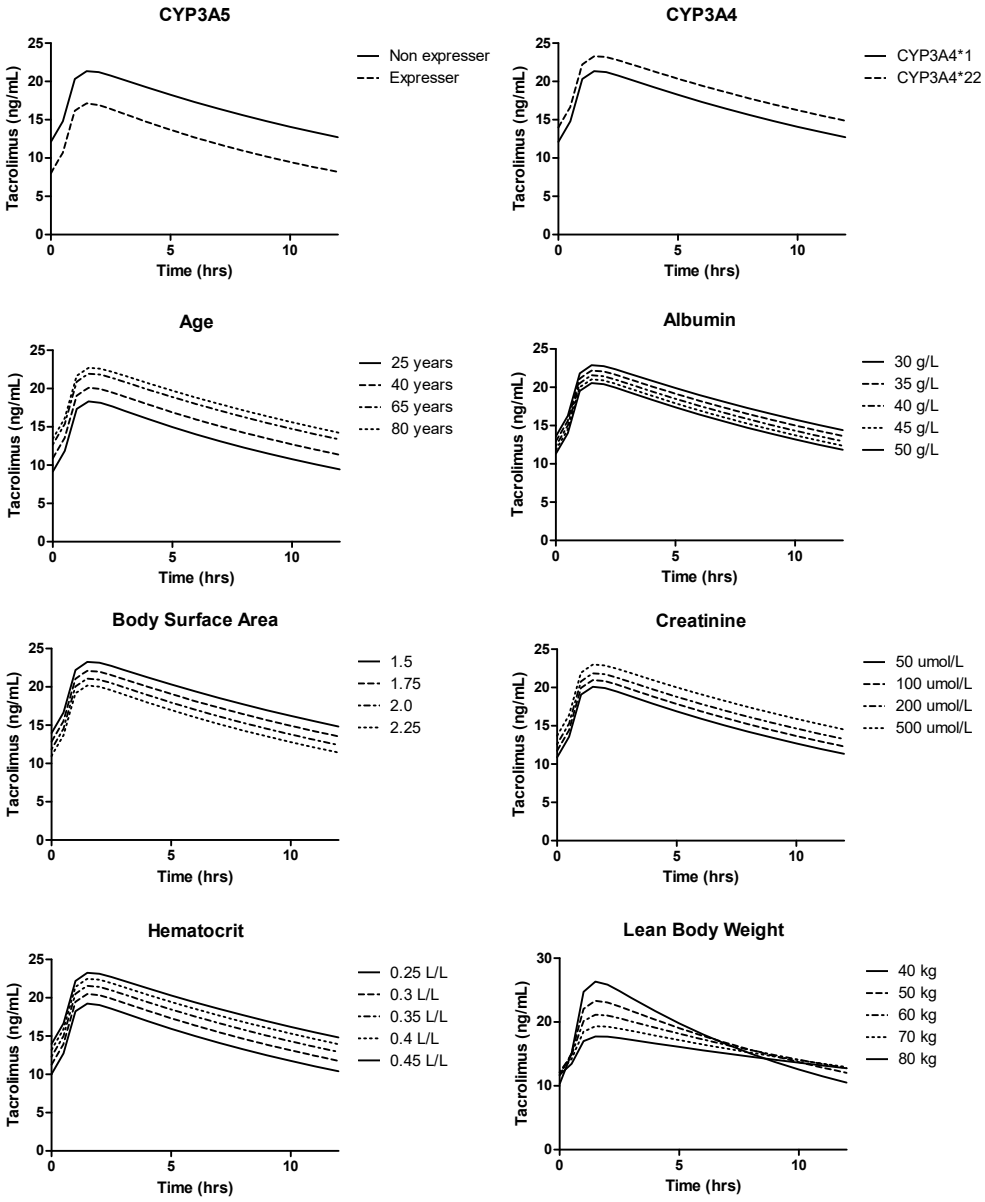

Figure 3. Simulated plasma profiles of tacrolimus at first steady state after transplantation. **A** Simulated plasma profiles of tacrolimus for CYP3A5 non-expressers (*CYP3A5*3/*3)* and CYP3A5 expressers (*CYP3A5*1/*1* or *CYP3A5*1/*3*). **B** Simulated plasma profiles of tacrolimus for patients carrying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

Starting dose model

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

Equation 2:

$$CL/_F = 22.5 * [(1.0, if\ CYP3A5*3/*3)\ or\ (1.62, if\ CYP3A5*1/*3\ or\ CYP3A5*1/*1)] * [(1.0, if\ CYP3A4*1\ or\ unknown)\ or\ (0.814, if\ CYP3A4*22)] * \left(\frac{Age}{56}\right)^{-0.50} * \left(\frac{BSA}{1.93}\right)^{0.72}$$

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

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

Equation 3:

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

The NONMEM control stream for the analysis is shown in the supporting information.

Simulation trial

The results are shown in Fig. 5. In the standard bodyweight-based group, 26.1% were on target (10-15 ng/mL) [38] *versus* 33.0% in the model-based group. In the bodyweight-based group, 44.5%

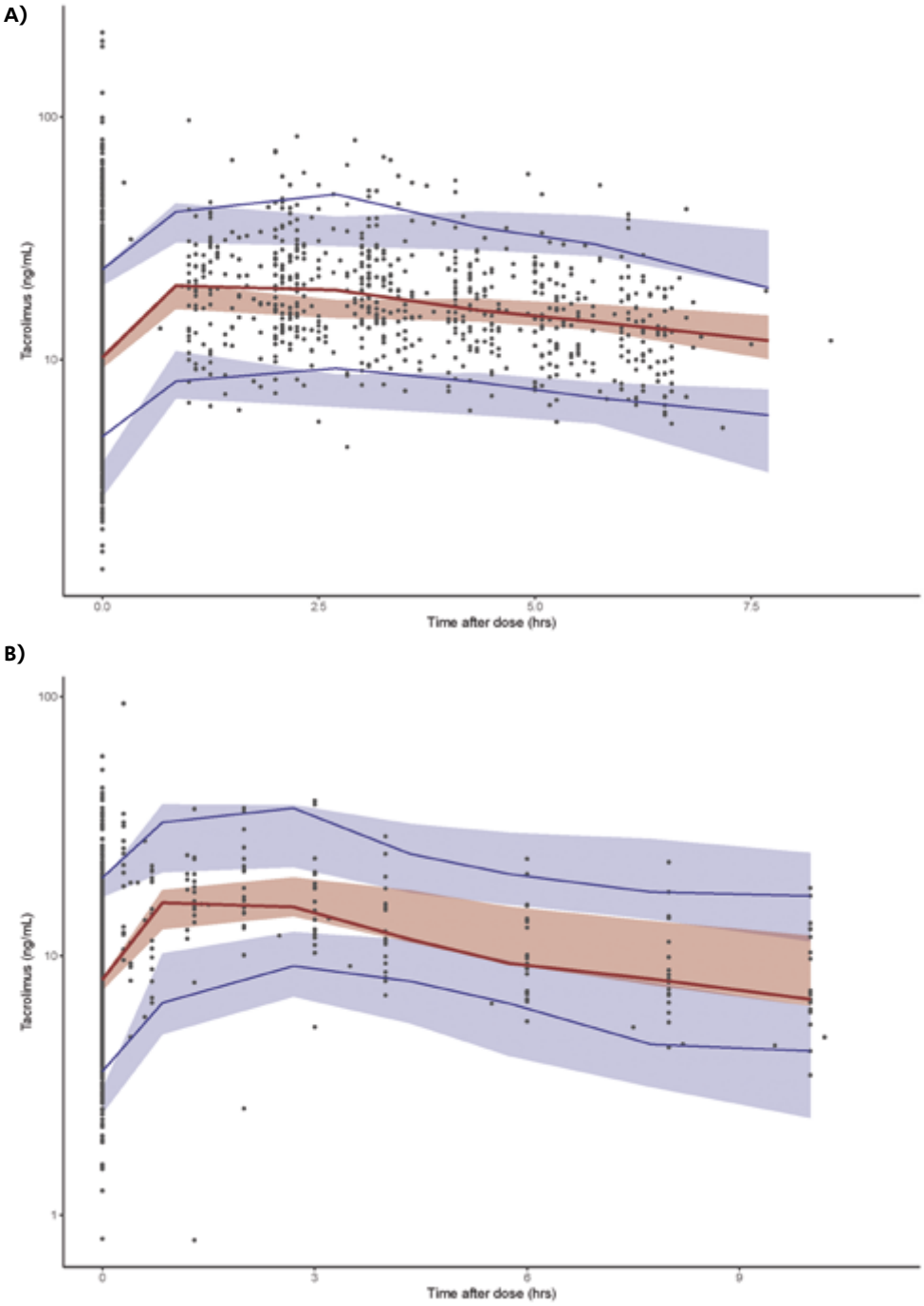

Figure 4. Prediction corrected visual predictive check of the starting dose model. **A** prediction corrected VPC of the starting dose model (internal dataset). **B** prediction corrected VPC of the starting dose model (external dataset). *VPC* Visual Predictive Check.

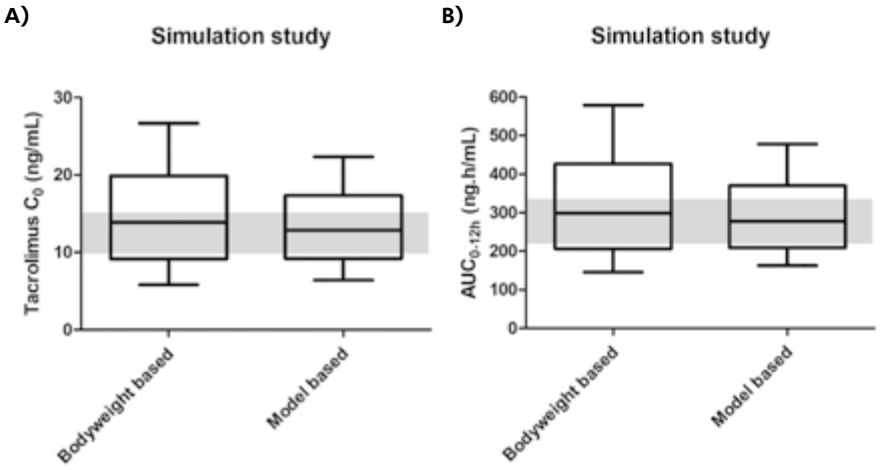

Figure 5. Boxplot with 10-90 percentile whiskers comparing simulations of the standard bodyweight-based dose and a dose based on the starting dose model. **A** Simulated predose concentrations. The median tacrolimus C_0 in the bodyweight-based dose group was 13.9 ng/mL, and in the model-based dose group 12.9 ng/mL. **B** Simulated AUCs. The median tacrolimus AUC in the bodyweight-based dose group was 298.5 ng.h/mL, and in the model-based dose group 277.9 ng.h/mL. *AUC* Area Under the Curve.

were above target compared with 36.8% in the model-based group. The median tacrolimus C_0 in the bodyweight-based dose group was 13.9 ng/mL, and in the model-based dose group 12.9 ng/mL. There were fewer extreme concentrations in the model-based dose group, with 5.2% markedly sub-therapeutic (<5 ng/mL) compared to 7.2% in the bodyweight-based group. In the model-based dose group, 15.6% were markedly supra-therapeutic (>20 ng/mL) compared to 24.6% in the bodyweight-based group.

DISCUSSION

This study demonstrates that multiple clinical (albumin, creatinine, haematocrit), demographic (age, BSA, LBW), and genetic (*CYP3A4* and *CYP3A5* genotype) factors significantly influence the pharmacokinetics of tacrolimus in the first 3 months following renal transplantation. Together these covariates explained 30% of the total variability in tacrolimus *CL/F*. A model for the starting dose was developed incorporating *CYP3A5* genotype, *CYP3A4* genotype, age and BSA. A simulation showed that more patients were on target when the starting dose proposed by the model was used compared with the standard bodyweight-based dose group (33.0% *versus* 26.1%).

In this study, *CYP3A5* expressers required a 1.6-fold higher tacrolimus dose than *CYP3A5* non-expressers. This is in line with previous research [39-43]. Patients carrying the *CYP3A4*22* allele required 20% less tacrolimus than the *CYP3A4*1* carriers independent of *CYP3A5* genotype status, confirming findings from previous research [7, 12, 44-46]. Given the wide availability of TDM, genotyping patients for *CYP3A* is most useful prior to initiation of tacrolimus therapy. Two RCTs demonstrated that optimization of the initial tacrolimus dose using *CYP3A5* genetic testing does not improve clinical outcomes when TDM is performed [24, 33]. However, this model is more sophisticated than basing the dose solely on bodyweight and *CYP3A5* genotype. For example, it

has been suggested that the *CYP3A4*22* allele should be included in the Clinical Pharmacogenetics Consortium guidelines when considering a Caucasian population [39, 47].

As approximately 70-80% of tacrolimus is distributed in erythrocytes, low haematocrit concentrations will reduce the whole-blood concentrations of tacrolimus [48]. We found in our study that patients with a lower haematocrit had higher *CL/F*. This underlines previous findings [12, 14, 23, 26, 27, 49, 50]. The unbound concentration of tacrolimus is pharmacologically active. Haematocrit levels do not influence the unbound fraction. However, low albumin concentrations will increase the unbound fraction [49]. In contrast to what we expected, patients with hypoalbuminemia had a lower tacrolimus *CL/F*. We did not find a similar effect on V_1 which one would expect if the correlation would be due to protein binding. A possible explanation could be that the reduced CL/F is caused by an underlying inflammatory response, as described previously [51]. Hypoalbuminemia can be an expression of inflammation, and inflammation can result in reduced CYP3A activity [52-54]. Unfortunately, no C-reactive protein levels were available to test this hypothesis. Patients with lower serum creatinine concentrations, had an increased CL/*F*. Tacrolimus undergoes almost no renal elimination and therefore the explanation for the observed association remains unclear. Some studies have reported a significant correlation between serum creatinine and tacrolimus CL [55, 56], whereas others found no such effect [57-59]. Research has shown that *CYP3A5* expresser genotype is associated with a greater extent of renal tacrolimus metabolism and a lower apparent urinary tacrolimus CL compared with subjects having the *CYP3A5*3/*3* genotype. This is indicative of substantial intra-renal CYP3A5-dependent tacrolimus metabolism. Patients with poor renal function, and especially patients with delayed graft function, may therefore have a lower tacrolimus CL [60]. It is unclear whether this is caused by decreased intrinsic metabolic capacity of the kidney, or whether this is an indirect effect of uremic toxins on hepatic metabolism [61].

Younger patients had an increased tacrolimus CL compared with older patients. A few years ago, Jacobson *et al*. examined age-related changes in the metabolism of tacrolimus and nicely demonstrated that older patients (>65 years) had significantly higher weight-normalized tacrolimus C_0 than younger patients (<34 years) [62]. Other developed pharmacokinetic models have found a similar effect [10, 12, 63]. Research has shown that basing the tacrolimus starting dose solely on bodyweight, will result in overexposure in a considerable proportion of patients [19]. BSA is a better indicator of metabolic mass than bodyweight because it is less affected by abnormal adipose mass. In both cohorts of the model building group this correlation between CL and BSA was seen. To the best of our knowledge this is the first pharmacokinetic model to incorporate BSA as a covariate.

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

The main strength of this study is the extensive validation of both models. The models were validated both internally and externally with clinical data using VPCs, and an NPDE was performed. Another strength of the study is the large number of patients included, and the high proportion of

patients for whom an AUC was available. Furthermore, the Rotterdam data were of high quality as they were collected in a large RCT, rather than routinely collected clinical data. Another strength is the usage of data collected in four different centers. The final strength of the study is that a separate model was developed to predict the starting dose of tacrolimus.

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

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

CONCLUSION

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

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SUPPLEMENTARY DATA

Supporting information 1. Example NONMEM control stream

Example control stream FINAL model

```
$PROBLEM Tacrolimus in adult renal transplant recipients
$INPUT ID TIME DV MDV EVID AMT II ADDL SS TAD FLAG CREAT HCT ALB AGE LBW CYP3A5 CYP3A4 BSA
$DATA tacrolimus.csv IGNORE=#
$SUBROUTINE ADVAN4 TRANS4

$PK
; --- COVARIATE ANALYSIS V2
V2LBW = ((LBW/58.94)**THETA(15))
V2COV=V2LBW

; --- COVARIATE ANALYSIS CL
IF(CYP3A4.EQ.0) CYP3A4 = 1  ; Most common
IF(CYP3A4.EQ.1) CYP3A4 = ( 1 + THETA(14))
IF(CYP3A4.EQ.-99) CYP3A4 = 1  ; Missing data

IF(CYP3A5.EQ.0) CLCYP3A5 = 1  ; Most common
IF(CYP3A5.EQ.1) CLCYP3A5 = ( 1 + THETA(13))

CLHCT = ((HCT/0.34)**THETA(12))
CLCREAT = ((CREAT/134.98)**THETA(11))
CLALB = ((ALB/42)**THETA(10))
CLAGE = ((AGE/55.72)**THETA(9))
CLBSA = ((BSA/1.93)**THETA(16))

CLCOV=CLAGE*CLALB*CLCREAT*CLHCT*CLCYP3A5*CLCYP3A4*CLBSA

; --- INTEROCCASION VARIABILITY (IOV) ; extended to 41 occasions
FLAG1=0
FLAG2=0
FLAG3=0
IF(FLAG.EQ.1) FLAG1=1
IF(FLAG.EQ.2) FLAG2=1
IF(FLAG.EQ.3) FLAG3=1

; --- PK PARAMETERS OF TACROLIMUS
ALAG1 = THETA(8)
KA = THETA(3)

TV2 = THETA(4)
TVV2 = V2COV*TV2
V2 = TVV2 * EXP(ETA(2))

TVV3 = THETA(6)
V3 = TVV3 * EXP(ETA(3))

TVQ = THETA(7)
Q = TVQ * EXP(ETA(4))
```

```
CL = THETA(5)
TVCL = CLCOV*CL
ECLA =FLAG1*ETA(5)+FLAG2*ETA(6)+FLAG3*ETA(7)
CL = TVCL * EXP(ETA(1)+ECLA)

K = CL / V2
K23 = Q / V2
K32 = Q / V3
S2 = V2 / 1000

$ERROR
IPRED=F
IF (DVID.EQ.2) Y=F + F*EPS(1)*THETA(1)
IF (DVID.EQ.1) Y=F + F*EPS(2)*THETA(2) +EPS(3)*THETA(17)
IF(DV.EQ.0) IPRED=0
IF (DVID.EQ.2) W=SQRT(F*F*THETA(1)**2)
IF (DVID.EQ.1) W=SQRT(THETA(17)**2+F*F*THETA(2)**2)
IRES=DV-IPRED
IWRES=IRES/W

$THETA
(0,0.2) ; 1 PROP LCMS
(0,0.2) ; 2 PROP Immunoassay
(0,2.7) ; 3 KA
(0,645) ; 4 V2
(0,22.5) ; 5 CL
(0,5395) ; 6 V3
(0,13) ; 7 Q
(0,0.37) ; 8 Lagtime

$THETA  ( -2.00,-0.38,  2.00) ; CLAGE
$THETA  ( -2.00,0.53,  2.00) ; CLALB
$THETA  ( -2.00,-0.14,  2.00) ; CLCREA
$THETA  ( -2.00,-0.79,  2.00) ; CLHCT
$THETA  ( -5.00,0.60,  5.00) ; CLCYP3A5
$THETA  (-1,-0.19,5) ; CLCYP3A4
$THETA  ( -5.00,1.4,  5.00) ; V2LBW
$THETA  (-5.00,1,  5.00) ; CLBSA
$THETA  (0,0.2) ; ADD immunoassay

$OMEGA
0.15  ;      1 CL
0.38  ;      2 V2
0.29  ;      3 V3
0.60  ;      4 Q

$OMEGA  BLOCK(1) 0.016  ;       CL
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
```

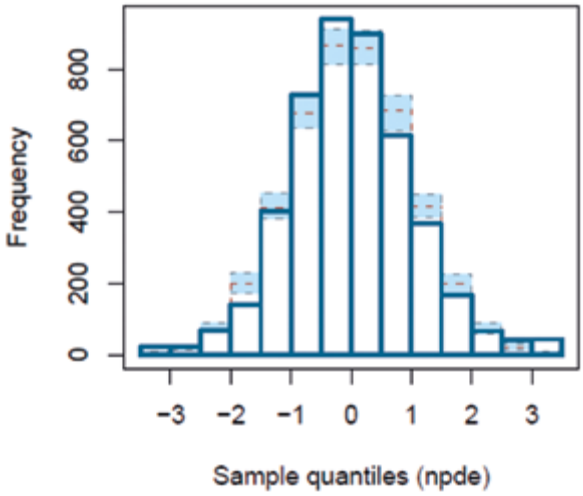
```
$SIGMA
1  FIX  ; residual variability
1  FIX  ; residual variability
1  FIX  ; residual variability

$ESTIMATION SIG=3 MAXEVAL=9999 NOABORT POSTHOC PRINT=5 METHOD=1 INTERACTION
$COVARIANCE PRINT=E UNCONDITIONAL
```

Example control stream starting dose model

```
$PROBLEM Tacrolimus in adult renal transplant recipients
$INPUT ID TIME DV MDV EVID AMT II ADDL SS TAD FLAG AGE CYP3A5 CYP3A4 BSA
$DATA tacrolimus.csv IGNORE=#
$SUBROUTINE ADVAN4 TRANS4

$PK

; --- COVARIATE ANALYSIS CL
IF(CYP3A4.EQ.0) CLCYP3A4 = 1  ; Most common
IF(CYP3A4.EQ.1) CLCYP3A4 = ( 1 + THETA(11))
IF(CYP3A4.EQ.-99) CLCYP3A4 = 1  ; Missing data

IF(CYP3A5.EQ.0) CLCYP3A5 = 1  ; Most common
IF(CYP3A5.EQ.1) CLCYP3A5 = ( 1 + THETA(10))

CLAGE = ((AGE/55.72)**THETA(9))
CLBSA = ((BSA/1.93)**THETA(12))

CLCOV=CLAGE*CLCYP3A5*CLCYP3A4*CLBSA

; --- INTEROCCASION VARIABILITY (IOV) ; extended to 41 occasions
FLAG1=0
FLAG2=0
FLAG3=0
IF(FLAG.EQ.1) FLAG1=1
IF(FLAG.EQ.2) FLAG2=1
IF(FLAG.EQ.3) FLAG3=1

; --- PK PARAMETERS OF TACROLIMUS
ALAG1 = THETA(8)
KA = THETA(3)

TV2 = THETA(4)
V2 = TV2 * EXP(ETA(2))

TVV3 = THETA(6)
V3 = TVV3 * EXP(ETA(3))

TVQ = THETA(7)
Q = TVQ * EXP(ETA(4))

CL = THETA(5)
TVCL = CLCOV*CL
ECLA = FLAG1*ETA(5)+FLAG2*ETA(6)+FLAG3*ETA(7)
CL = TVCL * EXP(ETA(1)+ECLA)

K = CL / V2
K23 = Q / V2
K32 = Q / V3
```

```
S2 = V2 / 1000

$ERROR
IPRED=F
IF (DVID.EQ.2) Y=F + F*EPS(1)*THETA(1)
IF (DVID.EQ.1) Y=F + F*EPS(2)*THETA(2) +EPS(3)*THETA(13)
IF(DV.EQ.0) IPRED=0
IF (DVID.EQ.2) W=SQRT(F*F*THETA(1)**2)
IF (DVID.EQ.1) W=SQRT(THETA(13)**2+F*F*THETA(2)**2)
IRES=DV-IPRED
IWRES=IRES/W

$THETA
(0, 0.24) ; 1 PROP LCMS
(0, 0.16) ; 2 PROP Immunoassay
(0, 3.6) ; 3 KA
(0, 692) ; 4 V2
(0, 23) ; 5 CL
(0, 6500) ; 6 V3
(0, 11) ; 7 Q
(0, 0.38) ; 8 Lagtime
(-2, -0.43,2) ; CLAGE
(-5, 0.63,5) ; CLCYP3A5
(-1, -0.2,5) ; CLCYP3A4
(-5, 0.75,5) ; CLBSA
(0, 1) ; ADD immunoassay

$OMEGA
 0.14 ;      1 CL
 0.28 ;      2 V2
 0.28 ;      3 V3
 0.62 ;      4 Q
$OMEGA BLOCK(1) 0.018 ;        CL
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME

$SIGMA
 1 FIX  ; residual variability
 1 FIX  ; residual variability
 1 FIX  ; residual variability

$ESTIMATION SIG=3 MAXEVAL=9999 NOABORT POSTHOC PRINT=5 METHOD=1 INTERACTION
$COVARIANCE PRINT=E UNCONDITIONAL
```

Supporting information 2. Normalized prediction distribution error (NPDE) plot for the starting dose model showing NPDE quantiles.



IV

SUMMARY, GENERAL DISCUSSION AND APPENDICES

8

GENERAL DISCUSSION AND SUMMARY

Treatment with low-dose tacrolimus combined with the antimetabolite mycophenolate and glucocorticoids seems to offer the best outcomes after kidney transplantation in terms of renal function, allograft survival, and acute rejection rates, as compared with ciclosporin based-regimens [1]. 2019 marks the thirtieth anniversary of tacrolimus [2]. More than a decade ago, tacrolimus largely replaced ciclosporin as the calcineurin inhibitor (CNI) of choice and has remained so ever since [3]. Tacrolimus exerts its immunosuppressive properties by inhibiting the phosphatase activity of calcineurin (CN) after binding to the intracellular FK-binding protein 12 (FKBP12) [4]. This inhibition subsequently leads to decreased de-phosphorylation and activation of the nuclear factor of activated T cells (NFAT), which activates the transcription of genes important for T cell activation including interleukin (IL)-2 and interferon (IFN)-γ. This eventually results in a diminished inflammatory alloreactive response [5, 6].

Belatacept is a novel, non-nephrotoxic immunosuppressive agent which blocks the CD80/86 – CD28 co-stimulatory signal necessary for T-cell activation [7]. Belatacept-based immunosuppression may result in improved long-term patient and graft survival but it is less effective than tacrolimus in preventing acute rejection [8, 9]. It thus remains to be seen whether belatacept will replace tacrolimus as the first line immunosuppressive drug anytime soon [10-12]. In the foreseeable future no other novel immunosuppressants are likely to emerge that can replace tacrolimus.

Short-term kidney allograft survival has greatly improved with the use of immunosuppressive drug combination therapy [13, 14]. However, prolonged use of immunosuppressive drugs leads to substantial toxicity, including increased rates of infections, hypertension, post-transplant diabetes mellitus, neurotoxicity and nephrotoxicity [15-18]. Long-term allograft failure remains an important problem with 3 to 5% of kidney allografts being lost annually after the first transplant year [19, 20]. These adverse events augment the limited long-term renal allograft survival and the high cardiovascular morbidity and mortality of transplant recipients [20, 21]. Rejection rates and most of the adverse events seem to be concentration related, with higher tacrolimus concentrations being related to toxicity and lower concentrations to an increased risk of acute rejection [22, 23]. Although the causes of long-term kidney allograft failure are multifactorial, chronic CNI-associated nephrotoxicity [24, 25] and antibody-mediated acute rejection [26] are considered important causes.

Due to a narrow therapeutic index and its large interpatient and intrapatient pharmacokinetic variability, therapeutic drug monitoring (TDM) is routinely performed for individualization of the tacrolimus dose to maintain drug efficacy and minimize the consequences of overexposure [27]. As allografts are nowadays rarely lost as a consequence of acute rejection, adverse events associated with long-term immunosuppression have become increasingly evident [28]. Reducing the toxic effects of immunosuppression has become a major goal in the treatment of transplant recipients [29]. The most frequently used means of tacrolimus monitoring is the measurement of the predose concentration (C_0) in whole blood.

The oral starting dose of tacrolimus in the first trial in humans was 0.15 mg/kg. This starting dose was based on animal studies [2, 3]. A few years later, in most transplant centres, oral tacrolimus therapy in adults was initiated with doses ranging from 0.10 to 0.20 mg/kg per day administered in two equally divided doses [30]. This recommended starting dose has not changed since [31]. The KDIGO Transplant Workgroup states that dosing of tacrolimus is important and that it is

relatively under-investigated [3]. Subsequent doses are adjusted by means of TDM, which limits the time a patient is exposed to concentrations outside the target range, although it may take up to 14 days in adults and 21 days in children to reach the target exposure [32, 33]. Therefore, patients are at an increased risk of sub- or supra-therapeutic tacrolimus exposure during these first weeks after transplantation, and may have an increased risk of developing adverse events [34].

The tacrolimus target range and its association with toxicity and rejection is discussed in detail in a review we published [35]. The area under the concentration *versus* time curve (AUC) is the best marker of tacrolimus exposure. However, in many centers, it is not feasible to perform TDM by means of a full-dosing interval AUC because of logistic and financial constraints. In addition, it poses a considerable burden on patients. Another limitation of TDM by means of tacrolimus AUC is the absence of hard evidence to support targeting a specific AUC. Nonetheless, some centers prefer to monitor tacrolimus by means AUC [36]. Calculation of the AUC based on a limited number of blood samples strategy (LSS) using Bayesian estimation has been proposed as a solution [37].

It is unclear which tacrolimus AUC should be targeted in both the early and late phase after kidney transplantation. Undre *et al.* and Squifflet *et al.* both suggested an AUC >200 ng/h/mL in the early phase after transplantation to be highly discriminatory for the risk of acute rejection [38, 39]. The study by Scholten *et al.*, performed an AUC-guided dosing study in 15 renal transplant recipients. Targets for AUC were as follows: 210 ng/h/mL for weeks 2–6 (corresponding with a C_0 of 12.5 ng/mL) and 125 ng/h/mL for weeks 6–52 (corresponding with a C_0 of 7.5 ng/mL). The authors suggest an AUC target of 150-200 ng/h/mL [40]. However, since the publication of the Symphony-Elite study [1], which demonstrated lower rates of acute rejection and improved graft function associated with low-exposure tacrolimus (target C_0 3–7 ng/mL) in combination with MMF and glucocorticoids, the corresponding AUC may be targeted to a lower range.

Before any new monitoring strategy can be recommended, further studies are required to clarify the relationship between (abbreviated) AUC monitoring and clinical outcome. For population pharmacokinetic models and Bayesian forecasting to be useful clinically, this next step must be taken to evaluate how closely dosage predictions with these models actually achieve AUC targets and improve clinical outcomes.

When the starting dose is based solely on bodyweight, approximately 37% of the adult and 26% of paediatric renal transplant recipients have a tacrolimus C_0 within the target concentration range at first steady state after five unaltered tacrolimus doses [33, 41]. Pharmacokinetic studies in adults have provided conflicting results, demonstrating that bodyweight is [32, 42-45] or is not [22, 46-48] statistically significantly associated with the clearance (CL) of tacrolimus. Contrary to adults, most paediatric PK studies do demonstrate that either bodyweight or age significantly associates with the tacrolimus CL [49]. To improve the percentage of patients on target in both children and adults, a different dosing strategy should be applied.

In 2003 the majority (60%) of kidney transplant recipients in the United States were overweight (BMI 25 – 30) or obese (BMI ≥ 30) at the time of transplantation [50]. With global obesity on the rise, this number is likely to increase even further and prompts the question if it is justified to continue to base the tacrolimus starting dose on bodyweight. We investigated the pharmacokinetics of tacrolimus in overweight patients in chapter 6. We concluded that basing the tacrolimus starting dose solely on bodyweight leads to overexposure in more than one-third of patients, and in 58% of overweight or obese patients. Basing the tacrolimus starting dose on BMI in overweight patients,

whether or not in combination with *CYP3A5* genotype, could reduce overexposure of tacrolimus early after transplantation [51].

PHARMACOGENETIC ASPECTS OF TACROLIMUS EXPOSURE

The inter-individual variability in tacrolimus pharmacokinetics is partly explained by genetic polymorphisms in genes encoding for tacrolimus metabolizing enzymes. CYP3A5, and to a lesser extent CYP3A4, are considered the fundamental enzymes involved in the metabolism of tacrolimus [52]. Individuals carrying at least one *CYP3A5*1* allele are referred to as CYP3A5 expressers, whereas individuals homozygous for the *CYP3A5*3* allele are known as CYP3A5 non-expressers. The *CYP3A5*3* allele causes alternative splicing which results in protein truncation and a severe decrease of functional CYP3A5 enzyme [53]. It has been consistently demonstrated that CYP3A5 expressers require at least a 1.5-fold higher tacrolimus dose compared to CYP3A5 non-expressers to reach the same exposure [54]. Following standard, bodyweight-based dosing, CYP3A5 expressers also appear to have a delay in achieving target tacrolimus exposure, in spite of TDM [34]. In one study, CYP3A5 expressers developed acute rejection earlier compared to non-expressers [34].

Two randomized-controlled trials in adults and one in paediatric transplant recipients in which patients either received the standard, bodyweight-based tacrolimus dosage or a *CYP3A5* genotype-based tacrolimus dosage (with expressers getting a higher than standard dose and non-expressers receiving a lower dose) have been conducted. In the first trial, significantly more genotype-dosed patients reached the target range at first steady state compared with bodyweight-based tacrolimus dosing, 43.2% *versus* 29.1% ($p = 0.03$) [32]. The second trial, however, found no such advantage of *CYP3A5*-guided tacrolimus dosing [41]. The trial in children concluded that *CYP3A5* genotype-guided dosing stratified by age resulted in earlier attainment of therapeutic tacrolimus concentrations and fewer out-of-range concentrations [55]. However, no study demonstrated a decreased risk of acute rejection or any other clinical benefit, and the conclusions were that optimization of the initial tacrolimus dose using *CYP3A5* pharmacogenetic testing does not improve clinical outcomes when TDM is performed [32, 41]. These findings do not support routinely genotyping kidney transplant recipients for *CYP3A5*. The CPIC guidelines state that if the *CYP3A5* genotype is available, it should be used to determine the starting dose [56].

CYP3A4 genotype has also been associated with altered tacrolimus CL [57]. The recently discovered *CYP3A4*22* single-nucleotide polymorphism (SNP) has been associated with lower tacrolimus dose requirements after renal transplantation. After combining both *CYP3A4* and *CYP3A5* genotype, Elens *et al*. were able to better predict the tacrolimus-metabolizing phenotype of kidney transplant patients [58]. Fairly recently, the *CYP3A4*26* SNP was described, which results in exceptionally low tacrolimus dose requirement [59].

DOSING ALGORITHMS

Besides pharmacogenetic factors, multiple other factors influence the CL of tacrolimus, including haematocrit [60], age [22, 61], ethnicity [62, 63] and drug-drug interactions [64]. As the variability in CL is not solely based on *CYP3A5*, basing the starting dose on a population PK model including

clinical, genetic and demographic factors seems the sensible next step. The use of a dosing algorithm may help in predicting an individual's response to tacrolimus and can be applied before the start of therapy.

In this thesis two different population PK models are described. The first model (chapter 3) was developed using retrospective data of 46 children [33]. The model was extensively validated both internally [bootstrap analysis, visual predictive check (VPC) and normalized prediction distribution errors (NPDE)] and externally (VPC) in an independent cohort consisting of 23 paediatric renal transplant recipients. In the final model, higher bodyweight, lower estimated glomerular filtration rate (eGFR), and higher haematocrit levels resulted in lower tacrolimus CL. *CYP3A5* expressers and recipients who received a kidney from a deceased donor had a higher CL. This final model was subsequently used to develop a model for the starting dose of tacrolimus (described in chapter 3). As time after transplantation was not a significant covariate, the same database consisting of data the first 6 weeks following transplantation was used to create the starting dose model. We chose for a more rich database rather than only using data of the first days after transplantation. The last measured haematocrit and eGFR before transplantation did not significantly influence the CL/F, and because they change substantially after transplantation, hematocrit and serum creatinine were not included in the starting dose model. The tacrolimus weight-normalized starting dose should be higher in children with a lower bodyweight, *CYP3A5* expressers and those who receive a kidney from a deceased donor. The equation for the starting dose is:

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

The second model described in this thesis (chapter 7) was developed using retrospective data of 337 adult renal transplant recipients. The model was extensively validated both internally (VPC and NPDE) and externally (VPC) in an independent cohort consisting of 304 patients. Higher body surface area (BSA), lower serum creatinine, younger age, higher albumin and lower haematocrit levels were identified as covariates enhancing tacrolimus CL. *CYP3A5* expressers had a significantly higher tacrolimus CL (1.6), whereas *CYP3A4*22* carriers had a significantly lower CL (0.8). From these significant covariates, age, BSA, *CYP3A4* and *CYP3A5* genotype were incorporated in a second model to individualize the tacrolimus starting dose:

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

When comparing both models a few things stand out. First of all, the similarities: both models found *CYP3A5* expressers require a more than 1.5-fold higher tacrolimus dose than *CYP3A5* nonexpressers. Bodyweight was also a significant covariate in both models, and in adults it was included indirectly as BSA. Both models also concluded that lower haematocrit levels enhance tacrolimus CL. Contrary to what we expected as tacrolimus is metabolized in the liver, both models found renal function influenced the tacrolimus CL: the serum creatinine concentration was a significant covariate in the adult model and eGFR in the paediatric model.

There are also a few differences between both models. First of all, in the paediatric model, a recipient of a deceased donor had an increased tacrolimus CL. It remains unclear why a recipient of a kidney from a deceased donor would have a higher tacrolimus CL, and no other study has found this relationship. As it was also seen in the validation cohort, it is probably a surrogate for other untested variables. The adult model found *CYP3A4*22*, albumin and age to significantly influence the tacrolimus CL, whereas this was not the case in the paediatric cohort.

The paediatric model was developed using a much smaller cohort of patients than the adult model: 46 *versus* 337 patients. A sample size of 46 children is reasonable given the nature and size of the patient population studied. However, as the age of these 46 children ranged from 2.4 to 17.9 years and their bodyweight form 11.6 to 83.7 kg, this is a very heterogeneous group. We tried to correct for this by incorporating allometric scaling in the model and thus scaling all the children to a bodyweight of 70 kg. This is perhaps also the reason why age was not a significant covariate. If the sample size of the paediatric model were to be increased, the model would likely be different. Perhaps donor status would no longer be significant or at least have a smaller effect on CL. Covariates such as *CYP3A4*22* and albumin could be tested in a larger sample size as in the current model none of the children were *CYP3A4*22* carriers and insufficient number of albumin levels were available.

PROSPECTIVE CLINICAL TRIALS

After retrospectively developing and validating the dosing algorithms, they should be subsequently tested in newly transplanted patients. In a first exploratory study the PK endpoints should prevail, investigating if the use of the algorithm reduces the time to reach the target concentration, and reduces the number of patients with a sub or supra-therapeutic concentrations. As it will be the first time the dosing algorithm is used to determine the starting dose, we feel that first a hypothesis-generating single arm study with a historic control arm should be considered. We believe that preferably a design with a limited sample size and a planned interim analysis with sufficient power to answer the research question should be chosen. After this single-arm hypothesis generating trial, we think enough information will be gathered to design a randomized controlled trial with PK and clinical endpoints.

What the primary endpoint for such trials should be is open for discussion. Incidence of acute rejection or tacrolimus-related toxicity are obvious possibilities. For the incidence of acute rejection, we would expect a 25% reduction (from 20% to 15% in the first 6 months after kidney transplantation). An adequately powered study to detect this clinically important difference in rejection incidence would require almost one thousand patients in each arm of the study. A downside of acute rejection as the endpoint, is that even when biopsy-proven acute rejection is

chosen requirement for the endpoint, it is still highly subjective. There is substantial disagreement between pathologists in evaluating biopsies drawn from transplanted kidneys. Another option is tacrolimus-related toxicity as the primary endpoint. This is however, extremely difficult to quantify. Nephrotoxicity and neurotoxicity may also be due to other factors, and in the transplant literature there are no broadly accepted methods to diagnose either of these toxicities. Perhaps better clinical primary endpoints would be graft loss, quality of life, post-transplant diabetes mellitus or (deterioration of) renal function.

We currently live in the age of 3D printing, next-generation sequencing and virtual reality. With all these innovations it makes sense to also implement novel trial techniques rather than the classic prospective studies. A few years ago, a study was conducted in which patients either received a tacrolimus dose based on computerized dose individualization using population PK models, or the conventional tacrolimus dose. The authors concluded that computerized dose individualization improves target concentration achievement compared with conventional dosing, with 90% *versus* 78% ($p < 0.001$) on target in standard risk patients. They also found lower 2-hour plasma glucose levels ($p = 0.008$) in the computer group, which may indicate that in the long-term perhaps less patients will develop diabetes mellitus [65]. Using a new modelling approach which recognizes the repeated measures in a same patient, Daher Abdi *et al*. demonstrated an association between mycophenolate exposure and acute rejection in a retrospective cohort [66]. The conduction of a large clinical trial is expensive, patients are subjected to novel treatment approaches that might not benefit them, and usually trials take years to finish. An approach that in our opinion should be used more often is to simulate a randomized controlled trial. Van Hest *et al*. investigated the usefulness of TDM of mycophenolate with a computer simulation model [67]. By simulating the trial, more insight can be gained into the issue. Trial simulations give an impression of the outcomes of the study and can be used to select the design of the clinical trial.

PRACTISE WHAT YOU PREACH

This thesis describes the results of the single arm hypothesis-generating study in children (chapter 4). The aim of this trial was to determine if basing the starting dose of tacrolimus on the validated dosing algorithm leads to a higher proportion of patients reaching the tacrolimus target concentration range on day 3 after transplantation (*i.e.* at first steady-state). In total 16 children were prescribed a tacrolimus starting dose based on the developed and validated dosing algorithm. The interim analysis demonstrated that the algorithm did not adequately predict the tacrolimus exposure and therefore the trial was stopped prematurely. The cohort in which the model was developed [33], consisted of 46 children of whom only 2 were a *CYP3A5* expresser and received a kidney from a deceased donor. It seems that there was insufficient power in the cohort in which the model was developed to determine adequate tacrolimus exposure predictions in this specific subgroup. The PK model demonstrated that *CYP3A5* expresser genotype and the type of kidney donor (deceased *versus* living) had an additive effect, but in clinical practice this does not seem to be the case. In hindsight the interaction between *CYP3A5* and donor type should perhaps have been investigated more thoroughly.

At the interim analysis, precisely enough patients were on target to continue the study. However, due to the poor model performance in *CYP3A5* expressers receiving a kidney from a deceased donor

and due to concerns of the clinicians regarding overdosing in patients who were *CYP3A5* expresser and received a kidney from a deceased donor (in some cases the model required a Tac starting dose as high as 0.8 mg/kg daily to be prescribed), we decided to stop the study prematurely and improve the dosing algorithm using new data. The model building cohort was increased from 46 to 95 children. A VPC demonstrated that the improved model better described the data. The weight-normalized starting dose should be higher in patients with lower bodyweight and in those who are *CYP3A5* expressers. Type of kidney donor was no longer a significant covariate. Since no plausible explanation for this association was found, this probably was a spurious association which may have been caused by limited power. We are currently planning a new prospective clinical trial using this new and improved algorithm.

The most important lesson learnt from this trial, is that a dosing algorithm needs to be tested in newly transplanted patients. This algorithm was validated extensively. In an external cohort it could adequately predict tacrolimus exposure retrospectively. Yet in clinical practice it did not deliver what we expected. It proves it was the smart decision to start with a hypothesis generating study, rather than embark on a large randomized controlled trial which would have taken years and would have been much more expensive to conduct.

A prospective clinical trial in adult renal transplant recipients in which the starting dose is based on the second dosing algorithm presented in this thesis (chapter 7) is still ongoing. The design is similar to the clinical trial presented in chapter 4. The results of this study are expected at the beginning of 2020.

INTRACELLULAR TACROLIMUS CONCENTRATIONS

As the site of action of tacrolimus is within the lymphocyte, it seems logical to assume that the tacrolimus concentration at its target site is more relevant than the concentration in whole blood to predict the efficacy of treatment [68]. Over the last few years several assays have been published that were able to measure tacrolimus in peripheral blood mononuclear cells (PBMC), obtained following gradient density centrifugation. In 2007, the first published assay was an immunoassay [69], but since then several (UP)LC-MS/MS assays have been published [70-72]. Capron *et al*. studied the intracellular tacrolimus concentration in 96 renal transplant recipients. They concluded that the intracellular concentrations seemed to be strongly dependent on *ABCB1* polymorphisms [73]. Based on histological findings, there tended to be an association between acute rejection episodes in renal transplant recipients and significantly lower tacrolimus intracellular concentrations [73]. The same research group conducted a study in liver transplant recipients and observed that patients experiencing clinical rejection one week after transplantation had significantly lower tacrolimus PBMC concentrations on day 7 after transplantation than patients who did not suffer from a rejection episode. In contrast to the intracellular concentration, the whole blood tacrolimus concentration was not associated with clinical rejection. The authors concluded that the tacrolimus concentration in PBMCs could be a better matrix for the measurement and TDM of tacrolimus [74]. Lemaitre *et al*. failed to demonstrate a relationship between tacrolimus whole blood concentrations, tacrolimus PBMC concentrations, and intracellular CN activity. This was probably caused by the small cohort of patients (n = 10) [75]. That same year Pensi *et al*. were able to characterize the PBMC compartment as a significant tacrolimus reservoir in 37 pediatric

liver transplant recipients, with intracellular concentrations being approximately 12.7 times higher than whole blood concentrations. For the first time, a correlation between intracellular and whole blood tacrolimus concentrations was demonstrated [72]. Fairly recently the relationship between tacrolimus concentrations in PBMCs, the whole blood tacrolimus concentration, the factors affecting this relationship and the risk of rejection was studied in 213 renal transplant recipients [76]. The correlation between whole blood and intracellular tacrolimus concentration was linear. This relationship was affected by sex, hematocrit, and time after transplantation. The tacrolimus ratio (intracellular concentration divided by whole-blood concentration) was not significantly associated with acute rejection [76].

The intracellular tacrolimus concentration could be a better matrix to ensure adequate tacrolimus exposure in addition to whole blood tacrolimus levels. The major drawback of implementing intracellular tacrolimus concentration measurement in clinical practice is the complex analytical technique [77]. Furthermore, there is only limited evidence that the intracellular concentration correlates better with clinical outcomes than whole blood exposure. Given the fact that one of the determinants of the ratio between intracellular and whole blood tacrolimus concentration is the activity of efflux pumps in the cell membrane of PBMCs, and as polymorphisms in the genes encoding for these pumps (such as ABCB1) will result in different ratios between individuals, it is expected that intracellular concentration will offer a better reflection of biological action than whole blood tacrolimus concentrations.

The next step is to develop a PK model of tacrolimus with an extra compartment for the intracellular concentration. This would probably be a three compartment model. It will be interesting to see if a dosing algorithm can be developed using these intracellular and whole blood tacrolimus concentrations. How much of the variability in CL will this model describe? Is intracellular tacrolimus the holy grail that we have been looking for? Only time will tell.

FUTURE PERSPECTIVES

It is likely that even if we could demonstrate that more than 55% of patients are on target with model-based dosing, physicians would still prefer standard bodyweight-based dosing in adults. By performing TDM we are quite effective in reaching target concentrations. We have previously shown that 10 days after transplantation the actual tacrolimus concentrations in renal transplant patients expressing *CYP3A5*, and those not expressing *CYP3A5*, were not different [78]. Tacrolimus dosages were substantially different, as a result of repetitive dose adjustments within these first 10 days. It is questionable if a few days' delay in reaching target concentrations will translate into impaired clinical outcome, such as more acute rejection episodes, or more delayed graft function, nephrotoxicity or neurotoxicity. We should not forget that patients are typically treated with three or four immunosuppressive drugs, in the first weeks after transplant. Especially adults are usually prescribed high doses of glucocorticoids.

Incorporating the use of a dosing algorithm in clinical practice, entails a couple of obstacles. Firstly, *CYP3A5* genetic testing needs to be performed prior to transplantation. Secondly it requires more effort from the treating physician to calculate the correct dose, especially if the physician works in a hospital where fixed starting doses are commonly used. Rationally, it seems likely that a dosing algorithm will only be incorporated worldwide in clinical practice if a prospective trial

with clinical endpoints demonstrates this genotype-based dosing strategy to be superior over traditional bodyweight-based dosing. However, one could dispute that with the current techniques genotyping for *CYP3A5* is a relatively easy, and there is evidence that computerized dosing of tacrolimus improves target concentration achievement and may even decrease the incidence of complications such as post-transplantation diabetes mellitus [65]. The actual modelling is challenging, however if the model is then translated into a formula for the dose calculation, this is easy to use. Especially if the model is uploaded on a website, application for a smartphone or even an excel spreadsheet.

In paediatric transplantation a dosing algorithm could probably be introduced after a successful trial with only PK endpoints. Paediatric nephrologists are used to precision dosing and calculating drug dosages based on bodyweight, BSA and other parameters. It is highly unlikely that after a successful PK study, a large RCT will be performed in children. In paediatric transplant recipients it is common to use steroid-sparing protocols. For example, in the TWIST protocol [79] steroids are given for only 5 days. After this the patient only receives tacrolimus and mycophenolate. One could therefore argue that, in contrast to adult patients, in children, a few days' delay in reaching the tacrolimus target concentration would translate into impaired clinical outcome.

In the future there should be more focus on pharmacokinetic-pharmacodynamic models of tacrolimus. Preferably they should not only predict tacrolimus exposure, but also the chance of rejection, adverse events and the response to other clinical outcomes such as the response to anti-viral treatment. Biomarkers should be incorporated in these models. Perhaps also biopsy findings can be implemented. Not just the starting dose, but all tacrolimus doses should be predicted with this model. Perhaps to acquire a large enough sample size, this should be a joint effort between several modelling groups. As mentioned before the models need to be published in a manner that clinicians can use the models in clinical practice. We feel that preferably applications for smartphones should be developed to facilitate the use in clinical practice. In local hospitals the model could be built in the clinical decision support system. When prescribing tacrolimus, the clinician would immediately receive a tacrolimus dose advice based on the built in model, including individual patient characteristics and clinical response.

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9

NEDERLANDSE SAMENVATTING

Momenteel is niertransplantatie de aangewezen behandeling voor patiënten met eindstadium nierfalen. Getransplanteerde patiënten leven in het algemeen langer, hun kwaliteit van leven verbetert en een transplantatie is goedkoper dan de zeer kostbare dialyse behandeling. Na de transplantatie wordt de patiënt ingesteld op een geneesmiddelen combinatie om afstoting van de nier te voorkomen. Deze combinatie bestaat in de meeste gevallen uit tacrolimus, mycofenolzuur en corticosteroïden. Tacrolimus werkt goed in het voorkomen van een afstoting. Helaas heeft dit geneesmiddel wel vervelende bijwerkingen. Bij meer dan 10% van de patiënten ontstaat misselijkheid, diarree, hoge bloeddruk, nierfunctiestoornissen, diabetes mellitus, hoge kalium concentratie in het bloed, hoofdpijn en slapeloosheid. Bij minder dan 1% van de patiënten komen hartritmestoornissen en leverfunctiestoornissen voor.

Afstoting en de eerdergenoemde bijwerkingen van tacrolimus, zijn gerelateerd aan de tacrolimus concentratie (spiegel) in het bloed. Een hogere bloedspiegel is gerelateerd aan bijwerkingen en een lagere bloedspiegel aan een verhoogde kans op afstoting. Tacrolimus is een lastig geneesmiddel om te doseren, omdat het een smalle therapeutische breedte heeft. Dit betekent dat het verschil tussen de werkzame en de toxische concentratie in bloed relatief klein is. Daarnaast is het een probleem dat de farmacokinetiek van tacrolimus tussen patiënten onderling sterk varieert. Farmacokinetiek beschrijft wat het lichaam met het medicijn doet. Het is onderverdeeld in absorptie (de opname van het geneesmiddel in het bloed), distributie (de verdeling van het geneesmiddel over het lichaam), metabolisme (de afbraak van het geneesmiddel) en eliminatie (de uitscheiding van het geneesmiddel). Bij tacrolimus is dit proces verschillend tussen patiënten. De hoeveelheid tacrolimus die na het passeren van de lever in het bloed terecht komt, is gemiddeld 30%, maar kan ook 5% of 90% zijn. Daarnaast is meer dan 90% van het tacrolimus in bloed gebonden aan hematocriet. Tacrolimus wordt in de lever afgebroken door de enzymen CYP3A5 en CYP3A4. Al deze factoren betekenen dat het lastig is om de juiste balans te vinden tussen werkzaamheid en bijwerkingen voor de patiënt. Het geneesmiddel moet voldoende in het bloed aanwezig zijn om te kunnen werken, maar niet in een zo'n hoge concentratie dat een patiënt bijwerkingen krijgt.

Momenteel worden bovengenoemde factoren niet mee genomen in de bepaling van de startdosering van tacrolimus. De startdosering wordt nu berekend op basis van het gewicht van de patiënt. Dit is opmerkelijk aangezien het niet duidelijk is of er een relatie is tussen het lichaamsgewicht en de blootstelling van tacrolimus in het lichaam. Vervolgens wordt er na enkele dagen een tacrolimus bloedspiegel afgenomen en aan de hand hiervan een vervolg dosering bepaald. Dit wordt Therapeutic Drug Monitoring (TDM) genoemd. TDM zorgt ervoor dat de patiënt zo kort mogelijk wordt blootgesteld aan te lage of te hoge concentraties. Het blijft echter 'trial-and-error'. Bij volwassenen kan het 2 weken duren en bij kinderen zelfs 3 weken, eer de streef concentratie tacrolimus bereikt wordt.

Door demografische (o.a. leeftijd, gewicht), klinische (labwaarden) en genetische factoren (*CYP3A4* en *CYP3A5* genotype) te combineren, zou een doseringsalgoritme voor de startdosering gemaakt kunnen worden. Hierdoor wordt de dosering berekend op basis van al deze parameters i.p.v. alleen het lichaamsgewicht.

Het doel van het onderzoek beschreven in dit proefschrift was om de startdosering van tacrolimus te optimaliseren bij kinderen en volwassenen na niertransplantatie. Hiermee kan

de tacrolimus streef concentratie sneller bereikt worden en kunnen geneesmiddel-gerelateerde complicaties zoveel mogelijk worden voorkomen.

In **hoofdstuk 1** van dit proefschrift wordt een overzicht gegeven van alle reeds gepubliceerde doseringsalgoritmen waarmee de startdosering van tacrolimus kan worden aangepast. Deze doseringsalgoritmen nemen verschillende factoren mee om de startdosering te bepalen, zoals hematocriet, co-medicatie, *CYP3A5* genotype, leeftijd en gewicht. Het baseren van de startdosering op een algoritme waar genetische, demografische en klinische factoren in worden meegenomen, is rationeler dan een startdosering gebaseerd op slechts het lichaamsgewicht. In **hoofdstuk 2** worden de onderzoeksvragen uiteengezet.

Het tweede deel van dit proefschrift gaat over het voorspellen van de tacrolimus blootstelling in kinderen na een niertransplantatie. **Hoofdstuk 3** beschrijft de ontwikkeling en validatie van een doseringsalgoritme voor de startdosering tacrolimus bij kinderen. Er is hiervoor retrospectief onderzocht welke factoren de klaring van tacrolimus uit het lichaam beïnvloeden. In totaal zijn er 722 tacrolimus concentraties gemeten bij 46 kinderen in de eerste zes weken na transplantatie. Een hoger lichaamsgewicht, een slechtere nierfunctie (eGFR) en een hogere hematocriet resulteerden in een lagere klaring van tacrolimus. Mensen met het *CYP3A5*1* allel en ontvangers van een nier van een overleden donor, hadden een hogere tacrolimus klaring. Deze gegevens zijn vervolgens gebruikt om een doseringsalgoritme te ontwikkelen voor de startdosering van tacrolimus. De relatieve startdosering van tacrolimus dient hoger te zijn bij kinderen met een lager lichaamsgewicht, *CYP3A5*1* allel dragers en ontvangers van een nier van een overleden donor. Het algoritme is gevalideerd met een externe database bestaande uit 23 kinderen.

Hoofdstuk 4 beschrijft de resultaten van de studie waarin het doseringsalgoritme dat in hoofdstuk 3 beschreven wordt in de praktijk getest is. Het plan was om 28 kinderen een tacrolimus startdosering te geven op basis van het algoritme. Na 16 patiënten is een geplande tussentijdse analyse uitgevoerd waarbij bleek dat slechts 31% van de kinderen de streefconcentratie tacrolimus had bereikt op dag 3 na transplantatie. Wanneer de standaarddosering op basis van het lichaamsgewicht wordt gegeven, was dit 30%. Het bleek dat door de toepassing van het doseringsalgoritme, sommige kinderen (*CYP3A5* expressers die een nier van een overleden donor kregen) een zeer hoge dosering kregen. Bij deze kinderen is er extra vroeg een tacrolimus concentratie gemeten en bleek deze te hoog. De dosering werd hierdoor direct verlaagd. Na de analyse van 16 patiënten is er vanwege de tegenvallende resultaten besloten de studie voortijdig te staken. Daarnaast hebben we het doseringsalgoritme verbeterd, o.a. met patiënten geïncludeerd in deze studie. Hieruit bleek dat de relatieve startdosering hoger dient te zijn bij kinderen met een lager lichaamsgewicht en dragers van het *CYP3A5*1* genotype.

Het derde deel van dit proefschrift gaat over het voorspellen van de tacrolimus blootstelling in volwassenen na een niertransplantatie. Het begint met **hoofdstuk 5** waarin een overzicht wordt gegeven van de genetische factoren die de farmacokinetiek van tacrolimus beïnvloeden. Het meeste is bekend over het *CYP3A5* genotype, waarbij bekend is dat dragers van het *CYP3A5*1* allel een 1.5 keer hogere tacrolimus dosering nodig hebben om de streef concentratie te bereiken. Er is steeds meer bewijs dat ook de *CYP3A4*22, CYP3A4*26* en *POR*28* allelen geassocieerd zijn met een afwijkende tacrolimus dosering. Het tweede deel van dit hoofdstuk gaat over de relatie tussen

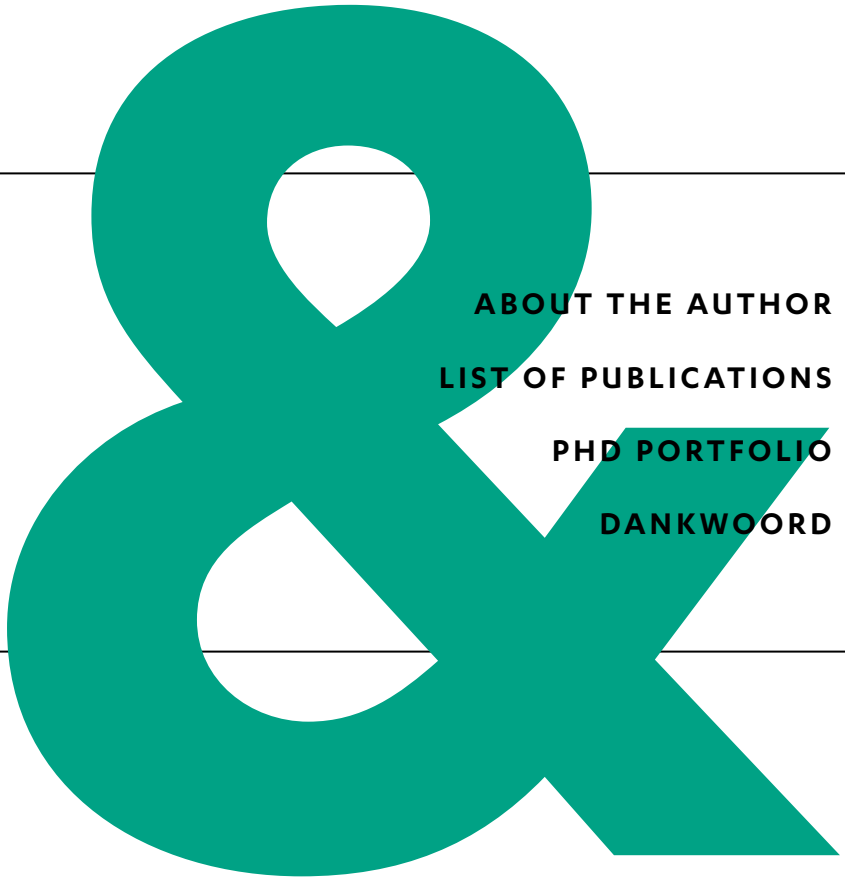
genetica en klinische effecten zoals afstoting en toxiciteit. Tot slot wordt er een overzicht gegeven van de rol van etniciteit op de tacrolimus blootstelling.

Hoofdstuk 6 beschrijft een onderzoek naar de tacrolimus dosering bij mensen met (extreem) overgewicht. De startdosering van tacrolimus wordt gebaseerd op lichaamsgewicht ondanks dat het bewijs hiervoor vrij mager is. Aangezien overgewicht wereldwijd een steeds groter probleem vormt, kun je je afvragen of de dosering altijd op lichaamsgewicht moet worden gebaseerd. Bij 203 niertransplantatie patiënten werd retrospectief onderzocht wat de tacrolimus blootstelling was op dag 3 na transplantatie. Daaruit bleek dat bij meer dan de helft van de patiënten met overgewicht (een body mass index, BMI > 25 kg/m^2) de tacrolimus concentratie direct na transplantatie (veel) te hoog was. In deze studie is een richtlijn opgesteld voor de startdosering van tacrolimus bij patiënten met overgewicht of obesitas. Bij patiënten met een BMI van 25-30, zou 85% van de dosering gegeven moeten worden, en bij patiënten met een BMI van 30-35 zou dit 75% moeten zijn.

In **hoofdstuk 7** wordt de ontwikkeling en validatie van een doseringsalgoritme voor de startdosering van tacrolimus bij volwassenen na niertransplantatie beschreven. Er is hiervoor retrospectief onderzocht welke factoren de farmacokinetiek van tacrolimus beïnvloeden. In totaal zijn 4527 tacrolimus concentraties gemeten bij 337 patiënten in de eerste drie maanden na transplantatie. Een groter lichaamsoppervlak, een lagere kreatinine concentratie in het bloed, een jongere leeftijd, een hoger serum albumine en een lagere hematocriet werden geïdentificeerd als factoren die de klaring van tacrolimus verhogen. Patiënten met het *CYP3A5*1* allel hadden een hogere klaring terwijl dragers van *CYP3A4*22* juist een lagere tacrolimus klaring bleken te hebben. Deze gegevens zijn gebruikt om een doseringsalgoritme te ontwikkelen voor de startdosering van tacrolimus. De tacrolimus startdosering dient hoger te zijn bij patiënten die het *CYP3A5*1* allel dragen, jongere patiënten en mensen met een groter lichaamsoppervlak. De startdosering dient lager te zijn bij dragers van het *CYP3A4*22* allel. Het algoritme werd gevalideerd met een externe database met 304 patiënten. Er werd binnen dit onderzoek ook een simulatiestudie uitgevoerd waaruit bleek dat er meer mensen de streef concentratie behalen indien de startdosering wordt gebaseerd op het doseringsalgoritme vergeleken met de standaard lichaamsgewicht dosering.

Momenteel is een klinische studie gaande waarbij 60 volwassen patiënten een dosering krijgen gebaseerd op het doseringsalgoritme beschreven in hoofdstuk 7. De resultaten van deze studie worden begin 2020 verwacht.

In **hoofdstuk 8** wordt de algemene discussie van dit proefschrift beschreven en hierbij worden ook gedachten ten aanzien van toekomstig onderzoek gepresenteerd. De verwachting is dat tacrolimus de komende jaren de eerste keus behandeling zal blijven na niertransplantatie. Voordat de ontwikkelde algoritmen kunnen worden toegepast in de klinische praktijk, dient er eerst een prospectieve studie uitgevoerd te worden om de toegevoegde waarde van het algoritme te controleren. In de discussie wordt beargumenteerd waarom eerst een kleine studie met farmacokinetische eindpunten dient te worden uitgevoerd en daarna pas een grote gerandomiseerde trial. In de toekomst zou de aandacht naar de ontwikkeling van complete modellen moeten gaan, dus niet alleen tacrolimus dosering, maar ook het risico op afstoting en bijwerkingen van de medicamenteuze behandeling zouden meegenomen moeten worden. Het ontwikkelde algoritme zou vervolgens in een app voor de smartphone gebouwd kunnen worden, zodat dokters en apothekers het makkelijker in de praktijk kunnen toepassen.



ABOUT THE AUTHOR

LIST OF PUBLICATIONS

PHD PORTFOLIO

DANKWOORD

ABOUT THE AUTHOR

Louise Marijke Andrews was born on the 8th of November 1985 in Bradford, England. She moved to the Netherlands in 1989 and finished secondary school at the Christelijk Lyceum Delft (CLD) in Delft in 2004. That same year she started studying pharmacy at Utrecht University. In 2007 she obtained her bachelor's degree.

In 2009 Louise completed her Master thesis on atrial fibrillation in the Netherlands at Utrecht University and Sanofi-Aventis in Gouda (supervisors Prof.dr. A. de Boer and dr. M.M. van Riemsdijk). During her internship at the Reinier de Graaf Gasthuis in Delft she developed a growing interest in clinical pharmacy and pharmacokinetics. After completing her pharmacy master's degree in 2011, she started working as a clinical pharmacist at the Zuwe Hofpoort ziekenhuis in Woerden. The following year she obtained a position as a laboratory pharmacist at the Erasmus MC.

In 2012 Louise started working at the Erasmus Medical Centre in Rotterdam. In December that same year she started her training to become a hospital pharmacist under the supervision of Prof. dr. A.G. Vulto and Prof.dr. P.M.L.A. van den Bemt. During this training she developed an interest for academic research. In 2015 Louise was awarded a grant and given the opportunity to combine her hospital pharmacy training with a PhD project at the Erasmus MC under the supervision of Prof.dr. T. van Gelder, dr. D.A. Hesselink and dr. B.C.M. de Winter, on the dose optimisation of tacrolimus following renal transplantation in both children and adults. In May 2019 Louise received her Hospital Pharmacy degree and started working as a hospital pharmacist at the Meander Medical Centre in Amersfoort.

Louise lives with Johan and their son Simon in Bennekom.

LIST OF PUBLICATIONS

Related to this thesis

Andrews LM, de Winter BCM, Cornelissen EAM, de Jong H, Hesselink DA, Schreuder MF, Brüggemann RJM, van Gelder T, Cransberg K. *A population pharmacokinetic model does not predict the optimal starting dose of tacrolimus in pediatric renal transplant recipients in a prospective study; lessons learned and model improvement*. Clin Pharmacokinet. 2019 in press.

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PHD PORTFOLIO

Name PhD student: L.M. Andrews		**PhD period:**	1/9/2015 – 31/5/2019
Erasmus MC Department: Hospital Pharmacy		**Promotor(s):**	Prof. Dr. T. van Gelder
		Supervisors:	Dr. D.A. Hesselink
			Dr. B.C.M. de Winter

1. PhD training

	Year	Workload (ECTS)
Courses		
Research Integrity	2016	0.3
BROK ('Basiscursus Regelgeving Klinisch Onderzoek'	2016	1.0
CPO course	2016	0.3
Systematic literature search in Pubmed	2013	0.2
HESPERIS *(Rome)*	2015	1.0
CHDR Basic introduction to NONMEM	2015	1.0
PhD day Erasmus MC	2016, 2017	0.4
Nephrology Winterschool (Nierstichting Nederland)	2017	1.0
Open Clinica	2017	0.3
Seminars and workshops		
NIH Principles of Clinical Pharmacology	2014-2015	1.0
PGx workshop	2014	0.2
Excel basic and advance workshop	2016	0.6
(Inter)national conferences		
IATDMCT 2015, *Rotterdam* (poster)	2015	1.0
Research Integrity congress	2015	0.2
Figon Dutch Medicine Days, *Ede* (poster)	2016	0.6
Klinisch review symposium	2016	0.3
Bootcongres, *Zeist* (poster)	2017	0.6
ATC 2017, *Chicago* (poster)	2017	0.1
IPTA 2017, *Barcelona* (oral)	2017	1.0
PAGE 2017, *Boedapest* (poster)	2017	1.0
IATDMCT 2017, *Kyoto* (oral & poster)	2017	1.0
NVZA Ziekenhuisfarmaciedagen, *Bunnik* (oral)	2017	0.4
Bootcongres, *Rotterdam* (oral)	2018	0.6
NVZA Ziekenhuisfarmaciedagen, *Driebergen* (oral)	2018	0.4
Grants		
Grant Stichting de Merel	2015	5.0
Travel grant Vereniging Trustfonds Erasmus Universiteit	2017	0.1
Travel grant Dutch Transplantation Society	2017	0.1
Travel grant Stichting ter bevordering van TDM en toxicologie	2017	0.1

PhD Portfolio (continued)

	Year	Workload (ECTS)
Other		
Treasurer AAV (Arts Assistenten Vereniging)	2015-2018	3.0
Female Talent Class	2018	1.0
Department of Hospital Pharmacy journal club	2015-2018	2.0
Clinical Pharmacology meeting	2015-2019	1.0
NONMEM research meetings	2015-2019	2.0
2. Teaching		
Lecturing		
Teaching pharmacology to medical students	2015-2019	10.0
Teaching pharmacology to nurses	2015-2019	1.0
Supervising Master's theses		
H.L. Le (6 month research master)	2019	5.0
Other		
Deel Basis Kwalificatie Onderwijs (BKO)	2015	1.0
Organising committee Erasmus MC Wetenschapsmiddag	2018	0.3
Organising committee Erasmus MC Laboratory evening	2017	0.2

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