

# PHARMACOKINETICS

## Pretransplant 4 $\beta$ -hydroxycholesterol does not predict tacrolimus exposure or dose requirements during the first days after kidney transplantation

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### AIMS

The CYP3A metric 4 $\beta$ -hydroxycholesterol (4 $\beta$ OHC) has been shown to correlate with tacrolimus steady-state apparent oral clearance (CL/F). Recently, pretransplant 4 $\beta$ OHC was shown not to predict tacrolimus CL/F after transplantation in a cohort of renal recipients ( $n = 79$ ). The goal of the current study was determine whether these findings could be validated in a substantially larger cohort.

### METHODS

In a retrospective analysis of 279 renal recipients, tacrolimus trough concentrations (C<sub>0</sub>), daily dose, haematocrit and other relevant covariates were registered every day for the first 14 days after transplantation. 4 $\beta$ OHC and cholesterol were quantified on plasma collected immediately pretransplant using liquid chromatography tandem-mass spectrometry. Patients were genotyped for CYP3A5\*1 and CYP3A4\*22.

### RESULTS

A total of 3551 tacrolimus C<sub>0</sub> concentrations were registered. In a linear mixed model for the 14-day period, determinants of tacrolimus C<sub>0</sub> were CYP3A5 genotype, haematocrit, age and weight (overall  $R^2 = 0.179$ ). Determinants of daily dose were CYP3A5 genotype, age, methylprednisolone dose, tacrolimus formulation, ALT and estimated glomerular filtration rate (overall  $R^2 = 0.242$ ). Considering each of the first 5 days separately, 4 $\beta$ OHC had a limited effect on tacrolimus C<sub>0</sub> on day 3 only ( $-1.00 \text{ ng ml}^{-1} \text{ per ln}$ ,  $P = 0.035$ ) but not on any other day, and no effect on dose or C<sub>0</sub>/dose. During the first 5 days, haematocrit and age, which were previously established as determinants of tacrolimus disposition under steady-state conditions, never explained more than 17.7% of between-subject variability in tacrolimus C<sub>0</sub>/dose.

### CONCLUSIONS

The CYP3A metric 4 $\beta$ OHC cannot be used to predict tacrolimus dose requirements in the first days after transplantation.

## WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Tacrolimus, a substrate for cytochrome P450 enzymes 3A4 and 3A5, is characterized by high pharmacokinetic variability.
- The endogenous CYP3A metric 4β-hydroxycholesterol (4βOHC) has been shown to correlate with steady-state tacrolimus apparent oral clearance (CL/F) in renal transplant recipients beyond the third month after transplantation.
- However, a recent study found no relationship between pretransplant 4βOHC and tacrolimus CL/F in the first week after transplantation.

## WHAT THIS STUDY ADDS

- This retrospective cohort analysis of 279 renal recipients confirms that there is no significant relationship between 4βOHC and tacrolimus dose or (dose-corrected) trough concentrations on any of the first 14 days after transplantation.
- 4βOHC cannot be used to optimize the initial tacrolimus dosing strategy in the context of renal transplantation.
- This could be related to the intrinsic limitations of 4βOHC as a CYP3A metric and to a high degree of largely unexplained between- and within subject variability in tacrolimus exposure in the first days after transplantation, much of which could be independent of CYP3A4.

## Tables of Links

TARGETS	
Transporters [2]	Enzymes [3]
ABCB1 (P-gp)	CYP3A5
	CYP3A4

LIGANDS	
Midazolam	Tacrolimus

These Tables list key protein targets and ligands in this article that are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [1], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [2, 3].

## Introduction

Tacrolimus is the cornerstone of immunosuppressive therapy in solid organ transplantation, but is characterized by a narrow therapeutic window and high within- and between-subject pharmacokinetic variability, necessitating therapeutic drug monitoring (TDM). Variability in the expression and activity of the tacrolimus metabolizing enzymes cytochrome P450 (CYP) 3A4 and 3A5 contributes significantly to the variability in tacrolimus exposure. CYP3A5 activity is only meaningful in patients possessing at least one *CYP3A5\*1* allele (CYP3A5 expressers), which have 50–100% higher apparent clearance (CL/F) of tacrolimus compared with *CYP3A5\*3/\*3* patients (CYP3A5 non-expressers) [4, 5]. In spite of these significant between-subject differences in clearance, initial tacrolimus dosing after transplantation is typically based only on total body weight, followed by dose adjustments based on trough concentration monitoring. A recent prospective trial in which patients were randomized to receive a starting dose of tacrolimus based on weight or on CYP3A5 genotype showed no difference in achievement of tacrolimus target concentrations early after transplantation, or on clinical outcomes [6]. This may be related to the fact that there is considerable variability in tacrolimus exposure that is related to clinical parameters (particularly haematocrit) and CYP3A4 activity [7, 8]. The problem is that CYP3A4 activity cannot easily be predicted from genetic polymorphisms as, individually, these generally have only limited functional effects [4]. A possible exception is the *CYP3A4\*22* polymorphism, which is associated with 30–40% lower tacrolimus dose requirements [9, 10]. An

alternative approach, therefore, could be quantification of combined *in vivo* intestinal and hepatic CYP3A4 activity using a functional drug probe, midazolam being the current gold standard [11]. We have previously demonstrated that tacrolimus CL/F can be predicted using a combination of CYP3A5 genotype, CYP3A4 activity (assessed using midazolam CL/F) and haematocrit [7]. As it is impractical to perform a full 8-h midazolam area under the curve (AUC) immediately prior to transplantation, endogenous CYP3A4 metrics such as 4β-hydroxycholesterol (4βOHC) are an attractive alternative in this setting. 4βOHC is a metabolite of CYP3A4-mediated cholesterol metabolism [12] with a very long elimination half-life (~17 days), stable plasma concentrations and low intra-individual variation (4.8–13.2%) [13, 14]. We have demonstrated that a predictive model based on weight- and cholesterol-corrected 4βOHC (4βOHC/C/W) performed similarly to a model based on midazolam in predicting steady-state tacrolimus CL/F in renal recipients 3 months or more after transplantation [15]. Very recently, however, Storset *et al.* observed no relationship between pretransplant 4βOHC and tacrolimus CL/F<sub>plasma</sub> during the first days and weeks after transplantation [16]. That study included a relatively low number of patients (*n* = 79) and its external validity may be somewhat limited by the fact that target C<sub>0</sub> values were low by international standards. The goal of the current study was to determine whether pretransplant 4βOHC, alone or combined with genetic information, could be used to predict tacrolimus dose requirements and exposure immediately after transplantation in a large cohort of renal recipients with relatively high tacrolimus target concentrations.

## Methods

### Study population

This was a single-centre, retrospective analysis. Inclusion criteria were (1) age  $\geq 18$  years, (2) recipient of a single kidney allograft between 1 January 2004 and 31 October 2014, (3) treated with the combination of oral tacrolimus (once daily Advagraf<sup>®</sup> or twice daily Prograf<sup>®</sup>, Astellas Pharma Europe, Staines, UK), mycophenolic acid (MMF; either mycophenolate mofetil [Cellcept<sup>®</sup>, Roche, Basel, Switzerland] or mycophenolate sodium [Myfortic<sup>®</sup>, Novartis, Basel, Switzerland]), and methylprednisolone (Medrol<sup>®</sup>, Pfizer, New York, NY, USA), (4) availability of DNA for genotyping, and (5) availability of a plasma sample collected within 24 h before transplantation for quantification of 4 $\beta$ OHC. Exclusion criteria were (1) use of moderately potent or potent CYP3A4 inhibiting or inducing medication immediately before or in the first 14 days after transplantation, (2) admission to an intensive care unit in the first 14 days after transplantation, and (3) death or graft loss in the first 14 days after transplantation. The standard tacrolimus loading dose was 0.2 mg kg<sup>-1</sup> (single dose of twice daily formulation) administered 2 h before transplantation (the day of transplantation is referred to as 'day 0'). After this point, tacrolimus dose was adjusted to maintain C<sub>0</sub> of 12–15 ng ml<sup>-1</sup> throughout the first month. Tacrolimus twice daily was administered at 8 h and 20 h and usually switched to tacrolimus once daily between days 5 and 14. During days 0–14, MMF dose was 2000 mg (or 1440 mg of mycophenolate sodium) daily. Methylprednisolone was dosed at 500 mg IV on day 0, 40 mg IV on day 1 and 16 mg orally on day 2, followed by standardized tapering to 4 mg by month 3. Because a few patients were included in clinical trials requiring a starting dose of tacrolimus other than 0.2 mg kg<sup>-1</sup>, only patients who received 0.15–0.25 mg kg<sup>-1</sup> tacrolimus on day 0 were included in the analysis. For days 1–14 and month 3, the following parameters (where available) were retrieved from electronic patient records for every individual day: tacrolimus C<sub>0</sub>, dose and formulation, methylprednisolone dose, total body weight, haematocrit, ALT (as proxy for liver dysfunction) and serum creatinine. Fat-free mass (FFM) was estimated from weight, height, age and gender [17]. Delayed graft function was defined as the need for dialysis in the first 7 days after transplantation. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula. This study was performed according to the Declaration of Helsinki and was approved by the ethics committee of the University Hospitals Leuven, Faculty of Medicine, KU Leuven, Belgium (S53364). All study participants provided written informed consent.

### Genotyping

Genomic DNA was isolated from whole blood samples using a salting out procedure [18]. The quantity and quality of genomic DNA were verified with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) before being assayed on an OpenArray platform (Life Technologies, Carlsbad, California, USA). Participants were genotyped for single nucleotide polymorphisms (SNPs) in genes CYP3A5 (CYP3A5\*1; rs776746) and CYP3A4 (CYP3A4\*22;

rs35599367). Hardy–Weinberg equilibrium was assessed using Haploview [19].

### Quantification of tacrolimus and 4 $\beta$ -hydroxycholesterol

Pretransplant blood samples (for quantification of 4 $\beta$ OHC and genotyping) were collected in 4-ml EDTA tubes when patients presented to the hospital for transplantation, i.e. a few hours before the actual surgery. After transplantation, whole-blood samples (for quantification of tacrolimus trough concentration) were collected in 4-ml EDTA tubes between 6 and 8 a.m., prior to intake of the morning dose of TAC, on every day for the first week after transplantation. During the second week, tacrolimus trough concentrations were measured 4–7 times (unless the patient was discharged earlier). In case of tacrolimus concentrations  $<5$  ng ml<sup>-1</sup> and  $>25$  ng ml<sup>-1</sup>, medical records were manually checked to exclude errors (e.g. ingestion of tacrolimus immediately prior to quantification). Dose-corrected trough concentrations (C<sub>0</sub>/dose) were determined by dividing C<sub>0</sub> by the total dose of tacrolimus ingested on the prior day. 4 $\beta$ OHC and cholesterol were quantified on EDTA-plasma using a liquid chromatography tandem-mass spectrometry platform, as previously described [20]. Accuracy and precision were 0.6–1.5% and 8.3–13.4% for 4 $\beta$ OHC, and 5.2–9.4% and 8.5–11.2% for cholesterol. Tacrolimus C<sub>0</sub> concentrations were determined on the microparticulate enzyme immunoassay platform used by the central hospital laboratory at the time. Up to 16 December 2010 this was Dimension RxL (Siemens, Munich, Germany; analytical sensitivity 1.2  $\mu$ g l<sup>-1</sup>; coefficient of variation [CV] 2.9–6.8%; applies to 52 trough concentrations or 1.5% of total). From 17 December 2010 onwards this was Architect i2000sr (Abbott Laboratories, Illinois, USA; analytical sensitivity  $\leq 1.5$   $\mu$ g l<sup>-1</sup>; CV 8%; applies to 3499 trough concentrations or 98.5% of total).

### Statistical analysis

Data are expressed as mean  $\pm$  standard deviation except when stated otherwise. Distribution of continuous data was evaluated according to Shapiro–Wilk. The following values were not normally distributed and log-transformed for analysis: tacrolimus C<sub>0</sub>/D, 4 $\beta$ OHC and 4 $\beta$ OHC/C. Predictors of tacrolimus C<sub>0</sub> and C<sub>0</sub>/dose throughout the first 14 days were determined by constructing linear mixed models with random slopes, random intercepts and a first-order autoregressive covariance structure. Calculation of estimates was based on restricted maximum likelihoods. The following variables were considered as possible predictors of tacrolimus C<sub>0</sub>, C<sub>0</sub>/dose and dose; CYP3A4 and CYP3A5 genotype; recipient age, weight and FFM; time-varying haematocrit, eGFR, ALT, steroid dose and tacrolimus formulation; time after transplantation; delayed graft function; pretransplant 4 $\beta$ OHC and 4 $\beta$ OHC/C. We only included terms which were statistically significant using *F*-test and improved the model according to Akaike's information criterion (AIC). Pretransplant predictors of tacrolimus C<sub>0</sub>, dose or C<sub>0</sub>/dose at specific time points (e.g. day 3) were determined by multivariable analysis of variance (ANOVA) using stepwise selection of predictors (without prior univariable selection). For this analysis, only the biomarker of interest (4 $\beta$ OHC) and established determinants

of tacrolimus C0/dose (*CYP3A4* and *CYP3A5* genotype, haematocrit, age, weight) were entered into the model. Consequently, this model was run for each individual day without correction for multiple testing. All reported  $R^2$  values are semipartial. A two sided  $P$ -value  $< 0.05$  was considered statistically significant. IBM SPSS Statistics version 22 was used for all statistical analyses. Graphpad Prism version 6 was used for generation of figures.

## Results

### Study population, genetics and 4βOHC

After exclusion of 13 patients who had stayed in an ICU, died or lost their graft in the first 14 days after transplantation, a total of 279 patients contributed 3551 tacrolimus trough concentrations (mean 12.7 per patient) over the 14-day study period. For 260 patients (93%), an additional trough concentration at 3 months was also available. Baseline characteristics of the study population are presented in Table 1. Out of 33 *CYP3A5* expressers, seven were homozygous (*CYP3A5*\*1/\*1). There were no homozygous *CYP3A4*\*22 carriers, nor were there patients who were both *CYP3A5* expresser and *CYP3A4*\*22 carrier. Consequently, patients who were both *CYP3A4*\*1/\*1 and *CYP3A5*\*3/\*3 are simply referred to as *CYP3A4*\*1/\*1. 4βOHC(/C) was not correlated with weight, FFM or BMI (Pearson  $r < 0.10$  and  $P > 0.10$  for all). Furthermore, 4βOHC concentration was not different across genotype groups (median [interquartile range] was 38.9 [24.4–65.9] ng ml<sup>-1</sup>, 34.4 [24.0–48.0] ng ml<sup>-1</sup> and 30.4 [18.9–54.2] ng ml<sup>-1</sup> in *CYP3A5* expressers, *CYP3A4*\*1/\*1

**Table 1**

Baseline patient characteristics

Variable	Value	Range
Age (years)	53 ± 13	19–75
Gender (male/female)	177/102	
Weight (kg)	73.4 ± 15.2	33.5–115.5
BMI (kg m <sup>-2</sup> )	25.1 ± 4.5	11.9–41.7
Fat-free mass (kg)	49.3 ± 8.7	27.6–70.6
Diabetes mellitus	56 (20%)	
Tacrolimus dose on day 0 (mg kg <sup>-1</sup> )	0.20 ± 0.01	0.16–0.22
Induction with basiliximab	75 (27%)	
4βOHC (ng ml <sup>-1</sup> ) <sup>a</sup>	35.4 [23.7–48.7]	3.3–679.0
4βOHC/C (molar ratio × 10 <sup>4</sup> ) <sup>a</sup>	0.25 [0.18–0.34]	0.03–5.11
Delayed graft function	61 (22%)	
<i>CYP3A5</i> expresser	33 (12%)	
<i>CYP3A4</i> *22 carrier	19 (7%)	

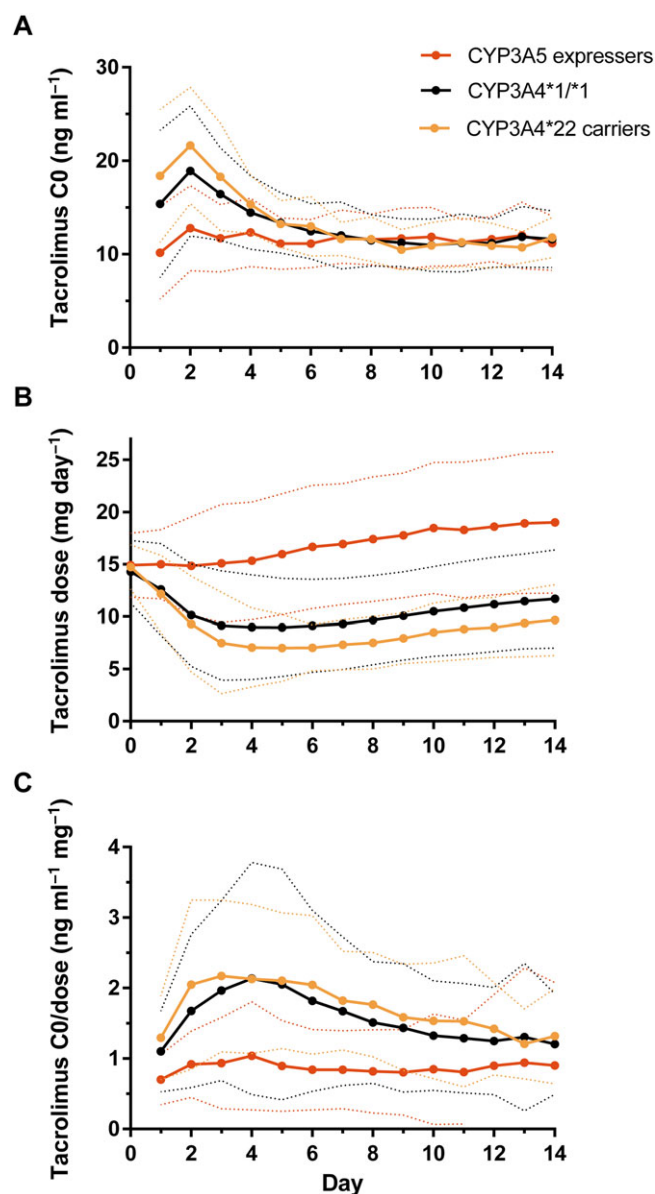
<sup>a</sup>Median [interquartile range]

4βOHC, 4β-hydroxycholesterol; 4βOHC/C, 4β-hydroxycholesterol/cholesterol; Weight, BMI and fat-free mass refer to measurement on day 0 (the day of transplantation)

patients and *CYP3A4*\*22 carriers, respectively;  $P = 0.283$  for between-group difference). These 4βOHC concentrations are broadly comparable to previously reported values in the pretransplant setting [16] and in stable renal recipients at least 3 months after transplantation [15, 21]. Mean haematocrit dropped from 36 ± 5% on day 0 to 30 ± 4% on day 1, but subsequently remained stable until day 14 (Supporting Information Figure S1).

### Tacrolimus dosing and exposure

Figure 1 shows the evolution of tacrolimus C0, dose and C0/dose over the study period, stratified by *CYP3A4/5*



**Figure 1**

Evolution of tacrolimus C0 (A), dose (B) and C0/dose (C). The solid and dotted lines represent the mean and 95% confidence interval, respectively

genotype. Mean values for these parameters and between-genotype comparisons on individual days are provided in Supporting Information Tables S1–S3. Tacrolimus C0 was significantly lower in CYP3A5 expressers compared with CYP3A4\*1/\*1 patients during the first 6 days, but was not significantly higher in CYP3A4\*22 carriers compared with CYP3A4\*1/\*1 patients. Tacrolimus dose was significantly higher in CYP3A5 expressers compared with CYP3A4\*1/\*1 patients from day 1 onwards (mean difference on day 7: 7.5 mg (83%),  $P < 0.001$ ) and lower in CYP3A4\*22 carriers compared with CYP3A4\*1/\*1 patients from day 6 onwards (mean difference on day 7: 2.1 mg (23%),  $P = 0.04$ ).

Figure 2 plots the distribution of observed tacrolimus C0 on days 1–7 stratified by broad categories of exposure, i.e. underdosed (C0 < 5 ng ml<sup>-1</sup>), somewhat underdosed (5–7.9 ng ml<sup>-1</sup>), acceptable (8–15 ng ml<sup>-1</sup>), overdosed (15.1–25 ng ml<sup>-1</sup>) and severely overdosed (>25 ng ml<sup>-1</sup>). By these definitions, 94.7% of CYP3A4\*22 carriers and 72.2% of CYP3A4\*1/\*1 patients were overdosed by day 2 using a 0.2 mg kg<sup>-1</sup> starting dose of TAC. Frank underexposure occurred mainly in CYP3A5 expressers (four out of five patients with tacrolimus C0 < 5 ng ml<sup>-1</sup> on day 1 were CYP3A5 expressers), and only during the first 2 days.

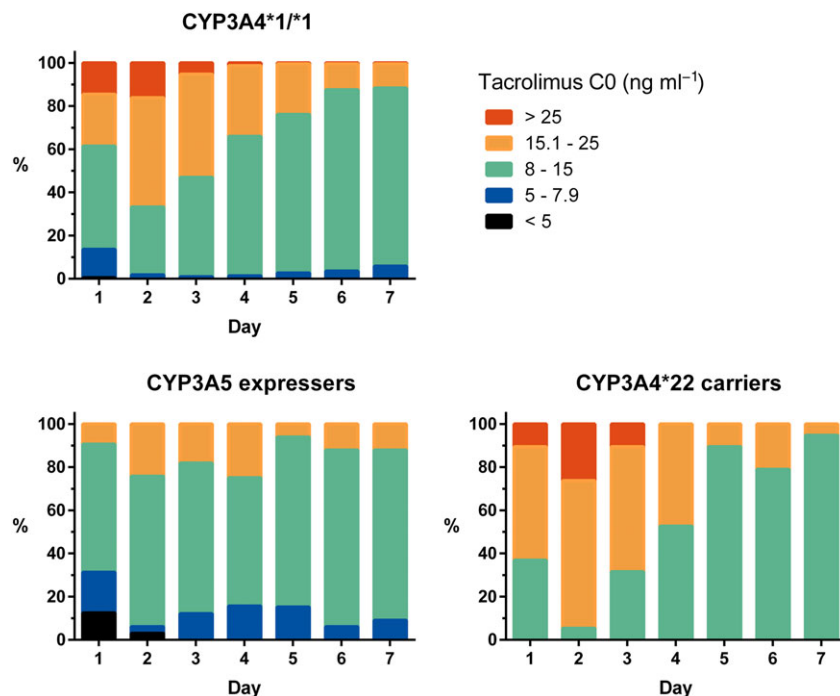
### Determinants of tacrolimus exposure

Determinants of tacrolimus C0 and C0/dose throughout the 14-day study period in linear mixed model analysis are shown in Table 2. Determinants of C0 included CYP3A5 genotype, age, weight and haematocrit; overall  $R^2$  was 0.179. Determinants of C0/dose included CYP3A5 genotype, haematocrit, age, methylprednisolone dose, tacrolimus

formulation, ALT and eGFR; overall  $R^2$  was 0.231. Determinants of tacrolimus dose were very similar to those of dose and are presented in Supporting Information Table S4. The effects of variables other than CYP3A5 genotype on C0/dose were quantitatively limited (e.g. increase in age of 30 years was predicted to increase C0/dose by 0.54 ng ml<sup>-1</sup> mg<sup>-1</sup>).

Next, separate linear regression models were constructed for tacrolimus C0 on days 1–5 using only determinants that were available before transplantation (e.g. haematocrit and weight were not used as time-varying variables, but only day 0 values were utilized). These are presented in Table 3. Haematocrit on day 0 had a clear effect on day 1 tacrolimus C0, but this effect was lost by day 3. Tacrolimus C0 on day 3, which is commonly used as the endpoint in clinical trials [6] was predicted by CYP3A5 genotype, age, weight and 4βOHC with overall  $R^2 = 0.158$ . However, 4βOHC explained only 1.1% of interpatient variability in tacrolimus C0 ( $P = 0.035$ ) and was not a significant predictor on any other day. 4βOHC(/C) was not predictive of tacrolimus C0, C0/dose, dose or tacrolimus C0 category on any day. 4βOHC(/C) was also not predictive for tacrolimus C0 and C0/dose when the analyses were repeated after exclusion of genotype information and age (as this may affect CYP3A4 activity), and not predictive in the subgroup of CYP3A4\*1/\*1 patients (data not shown). Finally, 4βOHC(/C) did not affect the probability of a C0 > 25 ng ml<sup>-1</sup> or <5 ng ml<sup>-1</sup> on days 1 and 2 ( $P > 0.600$  for all analyses).

Pretransplant (i.e. potentially available before transplantation) determinants of tacrolimus C0/dose and dose on days 1–5 are shown in Supporting Information Tables S5 and S6. By day 5, only CYP3A5 genotype and age were predictive of C0/dose ( $B = -1.149$ ,  $P < 0.001$ ,  $R^2 = 0.161$  and  $B = 0.031$ ,



**Figure 2**

Distribution of tacrolimus C0 over the first 7 days, by category of exposure

**Table 2**

Determinants of tacrolimus C0 and C0/dose on days 1–14 in multivariable linear mixed model

Determinant	Estimated effect	95% CI	P
<b>Model for tacrolimus C0 (ng ml<sup>-1</sup>)</b>			
<b>CYP3A5 expresser</b>	-1.739	-2.517 – [-0.962]	<0.001
<b>Haematocrit (%)</b>	0.249	0.194–0.303	<0.001
<b>Age (years)</b>	0.027	0.009–0.045	0.004
<b>Weight (kg)</b>	0.021	0.004–0.037	0.015
<b>Days after transplantation</b>	-0.367	-0.423 – [-0.311]	<0.001
<b>Model for tacrolimus C0/dose (ng ml<sup>-1</sup> mg<sup>-1</sup>)</b>			
<b>CYP3A5 expresser</b>	-0.675	-0.938 – [-0.412]	<0.001
<b>Haematocrit (%)</b>	0.017	0.005–0.030	0.006
<b>Age (years)</b>	0.018	0.011–0.024	<0.001
<b>Methylprednisolone dose (mg)</b>	-0.001	-0.003 – [-0.001]	0.007
<b>Tacrolimus formulation (twice daily)</b>	0.162	0.057–0.267	0.003
<b>ALT (IU ml<sup>-1</sup>)</b>	0.003	-0.006 – [-0.001]	0.004
<b>eGFR (ml min<sup>-1</sup> 1.73m<sup>-2</sup>)</b>	0.011	0.008–0.014	<0.001
<b>Days after transplantation</b>	-0.016	-0.030 – [-0.002]	0.025

Final R<sup>2</sup> was 0.179 for C0 model and 0.231 for C0/dose model.

ALT, serum alanine aminotransferase; eGFR, estimated glomerular filtration rate

**Table 3**

Pretransplant determinants of tacrolimus C0 in the first 5 days after transplantation

Determinants	Day 1			Day 2			Day 3			Day 4			Day 5		
	B value	P	R <sup>2</sup>	B value	P	R <sup>2</sup>	B value	P	R <sup>2</sup>	B value	P	R <sup>2</sup>	B value	P	R <sup>2</sup>
<b>CYP3A5 expresser</b>	-5.04	<0.001	0.043	-6.26	<0.001	0.084	-4.82	<0.001	0.095	-2.25	0.002	0.032	-2.29	<0.001	0.052
<b>CYP3A4*22 carrier</b>	3.37	0.046	0.009	—	—	—	—	—	—	—	—	—	—	—	—
<b>Haematocrit (%)</b>	0.39	<0.001	0.074	0.21	0.008	0.020	—	—	—	—	—	—	—	—	—
<b>Age (years)</b>	0.08	0.012	0.017	0.10	<0.001	0.047	0.08	<0.001	0.038	0.04	0.014	0.017	—	—	—
<b>Weight (kg)</b>	0.09	0.001	0.031	0.05	0.036	0.011	0.04	0.035	0.011	—	—	—	—	—	—
<b>4βOHC (mg ml<sup>-1</sup>)</b>	—	—	—	—	—	—	-1.00	0.035	0.011	—	—	—	—	—	—
<b>Overall R<sup>2</sup></b>	<b>0.174</b>			<b>0.162</b>			<b>0.158</b>			<b>0.049</b>			<b>0.052</b>		

Haematocrit and weight refer to the values on day 0.

When the analysis was repeated without considering CYP3A4\*22 status, 4βOHC still was not a significant predictor of tacrolimus C0 on any day except day 3.

4βOHC is log-transformed

$P < 0.001$ ,  $R^2 = 0.097$ , respectively). The effect of pretransplant haematocrit on C0/dose diminished progressively over the first 3 days and was lost by day 4. Time-dependent determinants of tacrolimus C0/dose (i.e. using haematocrit and weight values for each specific day) for days 1–5 and day 90 are shown in Supporting Information

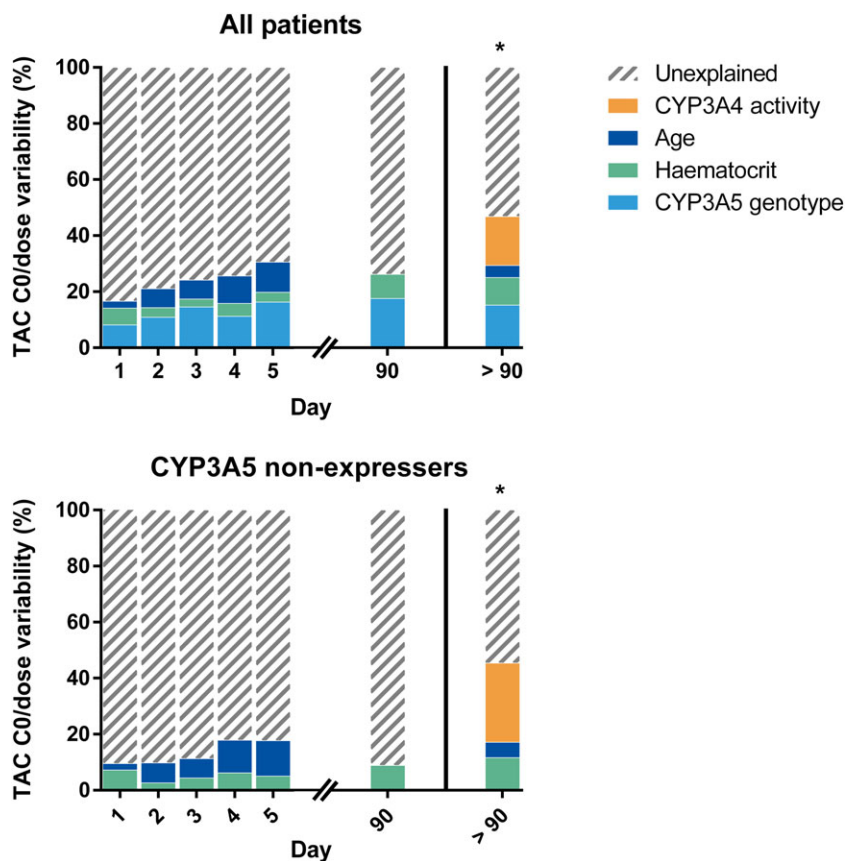
Table S7. The determinants and total R<sup>2</sup> remained similar from day 6 to day 14 and are not shown. Because patients are not in steady state on days 1–5, C0/dose does not accurately reflect clearance. Even on day 90, steady state was not guaranteed because of the retrospective nature of the data (factors such as noncompliance and diarrhoea were not

systematically registered). Therefore, the analysis for tacrolimus C<sub>0</sub>/dose was repeated on a previously published cohort of renal recipients, all of whom underwent 8-hour steady-state AUC for tacrolimus and midazolam a minimum of 90 days after transplantation as part of a prospective study [15]. We only considered patients treated with the twice daily formulation of tacrolimus and randomly selected patients from that cohort to match the *CYP3A5* genotype distribution of the current cohort (total of 94 renal recipients, including 12 (12.8%) *CYP3A5* expressers and 9 (9.6%) *CYP3A4*\*22 carriers). In this 'steady-state' cohort, *CYP3A5* genotype, midazolam CL/F, haematocrit and age together explained 46.8% of between-subject variability in tacrolimus C<sub>0</sub>/dose (Supporting Information Table S8). Figure 3 shows the relative contribution of *CYP3A5* genotype, haematocrit and age to tacrolimus C<sub>0</sub>/dose variability on days 1–5 and 90 in the current cohort as well as the added value of quantifying *CYP3A4* activity (using midazolam) in the steady-state cohort. It is likely that the predictive value of age on days 1–5 was partly due to age-related decline in *CYP3A4* activity (i.e. age was a proxy for *CYP3A4* activity), as age and midazolam CL/F were negatively correlated in the steady-state cohort ( $r = -0.269$ ,  $P = 0.009$ ).

## Discussion

In this analysis of 279 renal recipients, the only major determinant of tacrolimus dose and absolute or dose-corrected tacrolimus trough concentrations in the first 14 days after transplantation was *CYP3A5* genotype. The effect of haematocrit was intermediate and the effects of age and weight were minor. This study, although retrospective and not a formal modelling effort, suggests that the usefulness of haematocrit, age and even weight to determine the correct starting dose of tacrolimus may be limited. However, haematocrit and FFM have previously been shown to be useful in a computerized dosing algorithm to optimize tacrolimus dose adjustments early after transplantation, which is a separate issue [22]. The *CYP3A* metric 4βOHC determined immediately prior to transplantation could not be related to dose requirement or (dose-corrected) tacrolimus C<sub>0</sub> during the first 14 days.

We have previously shown that *in vivo* *CYP3A4* activity (assessed using midazolam) does not affect steady-state tacrolimus CL/F in *CYP3A5* expressers [7, 15]. The potential benefit of using a pretransplant *CYP3A4* metric for tacrolimus dose optimization would therefore likely be limited to



**Figure 3**

Determinants of between-subject variability in tacrolimus (TAC) C<sub>0</sub>/dose. The Y-axis plots  $R^2$  values (expressed as %) in multivariable linear models for each day separately in the current cohort. The right-hand panel (\*) shows the relative contribution of these factors in explaining tacrolimus C<sub>0</sub>/dose variability in a historical cohort of 94 renal recipients who were known to be in steady state and >90 days after transplantation (see text for details). *CYP3A4* activity refers to midazolam apparent oral clearance

CYP3A5 non-expressers. This also implies that the relative value of CYP3A5 genotyping and a CYP3A4 metric depend on the genetics of the transplant population. Prospective genotyping for CYP3A5 would be most informative in a population in which the allelic frequency of CYP3A5\*1 approaches 50% (e.g. African Americans [23]). The fact that the allelic frequency of CYP3A5\*1 is only 10–20% in European transplant programmes may be one of the reasons why CYP3A5 genotype-based dosing did not improve the number of patients having therapeutic tacrolimus exposure early after transplantation in a Dutch centre [6]. Conversely, the potential added value of a CYP3A4 metric should be highest in such Western populations consisting mainly of CYP3A5 non-expressers. However, we observed no relationship between 4βOHC and tacrolimus C<sub>0</sub>(/dose) even though only 12% of patients in the current study were CYP3A5 expressers, in which CYP3A4 activity explains up to 30% of steady-state tacrolimus C<sub>0</sub>/dose (Figure 3) [7].

There could be a number of reasons for the lack of an association between pretransplant 4βOHC and posttransplant tacrolimus dose requirements. Firstly, 4βOHC is a suboptimal CYP3A metric that correlates only moderately well with midazolam [15, 24–26]. Secondly, patients undergo profound physiological changes during the first days after transplantation, including rapid resolution of uraemia, a sharp drop in haematocrit on the day of transplantation and gradual recovery of intestinal motility and function. Much time-related (intra-individual) variability in tacrolimus CL/F during this period is therefore likely to be unrelated to CYP3A4 activity. For example, resolution of uraemia is likely to affect tacrolimus disposition more than it does 4βOHC, because uraemia seems to have much more profound effects on the function of transporters (such as the P-glycoprotein pump for which tacrolimus is a substrate) than on CYP3A4 [27]. Thirdly, 4βOHC has previously been shown to be related to steady-state tacrolimus CL/F, but tacrolimus is not in steady state during the first days after transplantation, when most over- and underexposure occurred. In summary, there may not only be issues with the intrinsic value of 4βOHC as a CYP3A metric but also with a high degree of variability in tacrolimus disposition immediately after transplantation that may not be related to CYP3A4, but rather to factors such as drug distribution, resolution of uraemia and intestinal motility. Whether pretransplant midazolam CL/F would have value in optimizing initial tacrolimus dosing remains to be established but, apart from being impractical (it would be as cumbersome as administering a pretransplant test dose of tacrolimus and be less informative), the abovementioned factors may pose limits on the practical use of any CYP3A4 metric.

In a recent study of 79 renal recipients, Storset *et al.* observed no correlation between pretransplant 4βOHC and tacrolimus CL/F<sub>plasma</sub> on days 2 and 7, week 4 or week 8 after transplantation [16], indicating that pretransplant 4βOHC is not a predictor of posttransplant tacrolimus clearance. The current study confirms these findings in a significantly larger cohort subject to higher tacrolimus target concentrations. We note that conversion between tacrolimus CL/F<sub>plasma</sub> (the parameter used in the study by Storset *et al.*) and CL/F<sub>whole-blood</sub> (used in clinical practice) requires haematocrit to be known. Therefore, a model that predicts CL/F<sub>plasma</sub> on day 7 cannot

be used to calculate the optimal tacrolimus dose to achieve a specific C<sub>0</sub> unless assumptions are made about what the haematocrit will be at that future point in time.

Strengths of the current study include large sample size, high frequency of tacrolimus C<sub>0</sub> measurements throughout the 14-day study period (almost daily) and determination of 4βOHC as well as 4βOHC/C. Limitations include the use of trough concentrations rather than full concentration–time profiles and the retrospective nature of the cohort. Specifically, centre-specific target trough levels for tacrolimus did not change throughout the study period, but may have been modified in specific patients depending on the clinical context.

In conclusion, CYP3A5 genotype, haematocrit and age, previously established as determinants of tacrolimus disposition under steady-state conditions, were also related to tacrolimus exposure in the first days after transplantation. However, most between-subject variability in tacrolimus exposure remained unexplained. The CYP3A metric 4βOHC cannot be used to predict tacrolimus dose requirements in the first days after transplantation.

## Competing Interests

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## Contributors

T.V. and D.K. designed the study and wrote the manuscript; M.H., P.A. and S.O. revised the manuscript; M.H. performed the 4βOHC quantification; T.V. performed statistical analyses.

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## Supporting Information

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<http://onlinelibrary.wiley.com/doi/10.1111/bcp.13343/supinfo>

**Figure S1** Evolution of haematocrit over time. Solid line represents the mean; dotted line represents 95% confidence interval

**Table S1** Tacrolimus dose (mg kg<sup>-1</sup>) on individual days

**Table S2** Tacrolimus C<sub>0</sub> (ng ml<sup>-1</sup>) on individual days

**Table S3** Tacrolimus C<sub>0</sub>/dose (ng ml<sup>-1</sup> mg<sup>-1</sup>) on individual days

**Table S4** Determinants of tacrolimus dose ( $\text{mg day}^{-1}$ ) on days 1–14 in multivariable linear mixed model

**Table S5** Pretransplant determinants of tacrolimus C<sub>0</sub>/dose in the first 5 days after transplantation

**Table S6** Pretransplant determinants of tacrolimus dose (mg) in the first 5 days after transplantation

**Table S7** Time-dependent determinants of tacrolimus C<sub>0</sub>/dose

**Table S8** Determinants of tacrolimus C<sub>0</sub>/dose in additional steady-state cohort (see panel \* in Figure 3)