Received: 17 May 2023 | Revised: 19 July 2023 | Accepted: 5 August 2023

DOI: 10.1111/bcp.15886

ORIGINAL ARTICLE

Modelling changes in the pharmacokinetics of tacrolimus during pregnancy after kidney transplantation: A retrospective cohort study

Maaike R. Schagen[1,2] | Asiye Nur Ulu[3] | Marith I. Francke[1,2] | Jacqueline van de Wetering[1] | Marleen C. van Buren[1] | Sam Schoenmakers[4] | Maja Matic[5] | Ron H. N. van Schaik[5] | Dennis A. Hesselink[1] | Brenda C. M. de Winter[1,2,3]

[1]Erasmus MC Transplant Institute, Department of Internal Medicine, Division of Nephrology and Transplantation, University Medical Center Rotterdam, Rotterdam, the Netherlands

[2]Rotterdam Clinical Pharmacometrics Group, Rotterdam, the Netherlands

[3]Department of Hospital Pharmacy, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands

[4]Department of Obstetrics and Gynaecology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands

[5]Department of Clinical Chemistry, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands

Correspondence
Maaike R. Schagen, Division of Nephrology and Transplantation, Department of Internal Medicine, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, the Netherlands.
Email: m.schagen@erasmusmc.nl

Funding information
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Aims: Pregnancy after kidney transplantation is realistic but immunosuppressants should be continued to prevent rejection. Tacrolimus is safe during pregnancy and is routinely dosed based on whole-blood predose concentrations. However, maintaining these concentrations is complicated as physiological changes during pregnancy affect tacrolimus pharmacokinetics. The aim of this study was to describe tacrolimus pharmacokinetics throughout pregnancy and explain the changes by investigating covariates in a population pharmacokinetic model.

Methods: Data of pregnant women using a twice-daily tacrolimus formulation following kidney transplantation were retrospectively collected from 6 months before conception, throughout gestation and up to 6 months postpartum. Pharmacokinetic analysis was performed using nonlinear mixed effects modelling. Demographic, clinical and genetic parameters were evaluated as covariates. The final model was evaluated using goodness-of-fit plots, visual predictive checks and a bootstrap analysis.

Results: A total of 260 whole-blood tacrolimus predose concentrations from 14 pregnant kidney transplant recipients were included. Clearance increased during pregnancy from 34.5 to 41.7 L/h, by 15, 19 and 21% in the first, second and third trimester, respectively, compared to prior to pregnancy. This indicates a required increase in the tacrolimus dose by the same percentage to maintain the prepregnancy concentration. Haematocrit and gestational age were negatively correlated with tacrolimus clearance ($P \leq 0.01$), explaining 18% of interindividual and 85% of interoccasion variability in oral clearance.

Conclusions: Tacrolimus clearance increases during pregnancy, resulting in decreased exposure to tacrolimus, which is explained by gestational age and haematocrit. To

The principal investigators of this study are Dr. D.A. Hesselink and Dr. B.C.M. de Winter.

maintain prepregnancy target whole-blood tacrolimus predose concentrations during pregnancy, increasing the dose is required.

KEYWORDS
immunosuppression, kidney transplantation, pharmacokinetics, population analysis, pregnancy

1 | INTRODUCTION

Many successful pregnancies have been reported after solid organ transplantation (SOT).[1–3] However, SOT recipients require immunosuppressive treatment to prevent graft rejection, also during gestation in transplanted women.[4,5] One of the safest immunosuppressants during pregnancy is **tacrolimus**, a calcineurin inhibitor.[6,7] Tacrolimus has a narrow therapeutic window and a high intra- and interindividual pharmacokinetic variability.[8] To maintain adequate exposure to tacrolimus, therapeutic drug monitoring (TDM) is routinely performed after transplantation.[9] However, during and due to pregnancy, physiological changes in absorption, distribution, metabolism and elimination occur which affect the pharmacokinetics of tacrolimus (reviewed in Le *et al.*) and complicate TDM.[7]

Tacrolimus is absorbed in the small intestine and colon, but this is countered by efflux mediated by the drug transporter protein **ABCB1**.[10,11] Since oestradiol and oestrogen, both increasingly produced by the placenta along the course of pregnancy, are able to induce ABCB1 in renal tissue, placental tissue and an in vitro model of human intestinal epithelial cells,[12,13] this might also occur in the intestine resulting in decreased tacrolimus bioavailability.

During pregnancy, the volume of distribution (Vd) of tacrolimus increases as a result of an increase in total blood volume of ~50%, while erythrocyte count also increases, but relatively less, with ~18% (resulting in a dilutional anaemia), and a decrease in tacrolimus binding proteins, such as albumin and α1-acid glycoprotein.[14–16] Since tacrolimus binds excessively, for 85–95%, to erythrocytes and plasma proteins, changes in Vd may lead to a decrease in whole-blood tacrolimus concentrations and an increase in the unbound tacrolimus fraction in plasma (which is normally <0.1%).[17] The erythrocyte-bound tacrolimus is thought not to be therapeutically active since only free drug molecules are able to cross cellular membranes and bind to its receptors at the target site.[18,19] Hence, the increase in the free fraction of tacrolimus (the pharmacologically active tacrolimus) during pregnancy may be of clinical importance.

The metabolism of tacrolimus is affected by the increased activity of the cytochrome P450 (CYP) enzyme system, mostly **CYP3A4** and **CYP3A5**,[20] which form the main route of tacrolimus elimination.[21] Kidney transplant recipients carrying the *CYP3A5*1* allele (CYP3A5 expressers) have a 1.5–2-fold higher apparent clearance after oral administration (CL/F), compared to recipients homozygous for the *CYP3A5*3* allele (CYP3A5 nonexpressers).[22] The *CYP3A4*22* allele is associated with reduced protein activity, requiring a 20% lower tacrolimus dose compared to recipients carrying the *CYP3A4*1* allele.[23–25] The P450 oxidoreductase (POR) enzyme also influences tacrolimus CL/F as the *POR*28* allele increases the metabolism of tacrolimus, and thus a higher dose requirement in CYP3A5

What is already known about this subject

- Physiological changes in the absorption, distribution, metabolism and elimination affect the pharmacokinetics of tacrolimus during pregnancy.
- The increase in total blood volume during pregnancy leads to a relative decrease in tacrolimus binding sites, e.g. erythrocytes and plasma proteins, which may lead to a decrease in whole-blood tacrolimus concentrations and an increase in the unbound tacrolimus fraction in plasma.
- Pregnancy after a solid organ transplantation is realistic, but comes with an increased risk for many adverse reproductive outcomes. Appropriate management of tacrolimus therapy during pregnancy is therefore of utmost importance and a better understanding of the changes in tacrolimus pharmacokinetics may allow for more tailored treatment.

What this study adds

- The biggest decrease in whole-blood tacrolimus concentrations is observed in the first trimester, and the tacrolimus concentration decreases a bit further during the second trimester. This lower concentration remains stable during the last trimester and rapidly increases to normal concentrations postpartum.
- Haematocrit and gestational age are inversely correlated with tacrolimus apparent oral clearance.
- To maintain tacrolimus whole-blood concentrations in the target range, a dose increase during pregnancy is required.
- Since the greatest change in apparent clearance occurs in the first trimester, more frequent monitoring during this period and whenever changes in haematocrit occur is advised.

expressers.[26–29] Williams *et al.*[30] demonstrated that high oestrogen concentrations decrease CYP3A4 activity and increase CYP3A5 activity throughout gestation. Zheng *et al.*[16] reported that the mean tacrolimus CL/F during pregnancy increases by 39% compared with postpartum.

Finally, an increase in renal plasma flow of 50–85% as a result of renal vasodilatation during pregnancy, leads to an increased glomerular filtration rate. This results in a higher renal excretion of nonmetabolized tacrolimus.[21]

Although pregnancy after SOT is a realistic option, these pregnancies have a substantially increased risk for many adverse reproductive outcomes, such as pre-eclampsia, gestational hypertension, renal graft impairment, preterm birth, lower birth weight and intrauterine growth restriction.[31–35] In addition, to prevent graft rejection, appropriate management of tacrolimus therapy during pregnancy is of utmost importance. Data on changes in tacrolimus pharmacokinetics and its exposure in pregnant kidney transplant recipients are scarce. A better understanding of the changes in tacrolimus pharmacokinetics that occur during pregnancy may allow for more personalized treatment during this critical phase of the life course of female transplant recipients. The aim of this study was to describe the pharmacokinetics of tacrolimus in pregnant kidney transplant recipients with a population pharmacokinetic (popPK) model.

2 | METHODS

2.1 | Patient selection and data collection

This was a retrospective study, which was approved by the Medical Ethical Review Board of the Erasmus MC, University Medical Center, Rotterdam, the Netherlands (MEC number 2021-0817). This study was conducted in accordance with the Declaration of Helsinki. All women aged >18 years, who had a successful pregnancy after a kidney transplantation from the year 2000 onwards (tacrolimus was introduced as the primary immunosuppressant after kidney transplantation in 2000) and were followed at the outpatient clinic of the Erasmus MC, and used the twice-daily oral tacrolimus formulation Prograf (Astellas Pharma, Leiden, The Netherlands) throughout gestation, were eligible for enrolment. If a woman had multiple pregnancies, only the first was taken into account.

Demographic and clinical data were collected from the patients' electronic files. All administered tacrolimus doses and all available tacrolimus whole-blood predose concentrations were collected. The data was collected from the 6-month period prior to conception, throughout gestation and up to 6 months postpartum.

Body composition parameters were calculated according to the following formulas[36]:

- Body mass index = (weight in kg) / (height in m)^2;
- Lean body weight (LBW) = 1.07 × weight in kg − 148 × (weight in kg / height in cm)^2;
- Body surface area = √(height in cm × weight in kg / 3600).

2.2 | Tacrolimus dosing regimen

Whole-blood predose concentrations (C_0) were measured at the attending physician's discretion aiming for a tacrolimus C_0 of 5–8 ng/mL.

2.3 | Tacrolimus concentration measurement

Tacrolimus concentrations in whole-blood were measured utilizing 3 different methods: antibody conjugated magnetic immunoassay (ACMIA); enzyme multiplied immunoassay (EMIT); or validated liquid chromatography–tandem mass spectrometry (LC–MS/MS). Samples from 2000 to 2012 were measured with ACMIA, from 2013 to 2015 with EMIT and from 2016 on with LC–MS/MS. Quantification ranges differ for the 3 analytical methods. For ACMIA, it was set between 1.5 and 30 ng/mL, EMIT between 2.0 and 30 ng/mL, and LC–MS/MS between 1.0 and 35 ng/mL.[37] All concentrations below the lower limit of quantification were excluded from the analysis.

2.4 | Genotyping

All patients were genotyped as part of the ongoing Biobanking programme of the division of Nephrology and Transplantation, which includes DNA isolation and pharmacogenetic genotyping (MEC number 2010-022). Genotyping of *CYP3A4*22* 15389C>T (rs35599367), *CYP3A5*3* 6986G>A (rs776746) and *POR*28* 1508C>T (rs1057868) single-nucleotide polymorphisms (SNPs) was performed according to standard laboratory procedures in an ISO15189-certified laboratory. *CYP3A4*22* 15389C>T and *CYP3A5*3* 6986G>A genotyping was performed using Autogenomics INFINITI CYP450 3A4-3A5 Assay (Autogenomics, Carlsbad, CA, USA) on the INFINITI High Throughput System (HTS), according to the instructions of the manufacturer. While *POR*28* 1508C>T was determined with the TaqMan allelic discrimination assay (C___8890131_30; ThermoFisher Scientific).

2.5 | PopPK modelling

Pharmacokinetic analysis was conducted by nonlinear mixed effects modelling using NONMEM version 7.5.1 (FOCE, ICON Development Solutions, Ellicott City, MD, USA). PsN (version 5.0.0), Pirana (version 3.0.0), R (version 4.2.1) and Xpose (version 4) were used for data management and graphical diagnostics.

2.6 | Base model development

A popPK model describing the pharmacokinetics of tacrolimus in de novo adult kidney transplant recipients, based on whole-blood concentrations (predose and full pharmacokinetic profiles) in $n = 337$ patients, was developed earlier.[37] This popPK model was

prospectively tested in $n = 59$ de novo kidney transplant recipients, demonstrating a higher proportion of patients within the tacrolimus target range at Day 3 post-transplantation compared to a historic control group with a bodyweight-based starting dose (58 *vs.* 37.4%, respectively).[38] This two-compartment model with first-order absorption and linear elimination from the central compartment was used as a reference model. Five parameters, lag-time (t_{lag}), absorption rate constant (K_a), central volume of distribution (V_1), peripheral volume of distribution (V_2) and intercompartmental clearance (Q) could not be estimated since only C_0 were available. These parameters where therefore fixed according to the reference model.[37] Allometric weight scaling for both bodyweight and LBW was tested on these 5 parameters, but this did not improve the model significantly. CL/F is the only estimated parameter, including its variability. The interindividual variability (IIV) and interoccasion variability (IOV) were modelled with an exponential error model. For the analytical methods—LC–MS/MS, immunoassay ACMIA and EMIT—a separated residual variability was described using an additive and proportional error model. The judgement criteria for model selection were based on parameter estimation, shrinkage values (<25%), inspection of residual and goodness-of-fit plots, objective function values (OFV) and standard errors of estimated parameters.

2.7 | Covariate model development

Covariates were selected based on their known or theoretical relationship with tacrolimus pharmacokinetics. The following demographic, clinical and genetic characteristics were evaluated as potential model covariates: age of the mother, height, bodyweight before pregnancy, bodyweight during follow-up, the calculated body composition parameters (body mass index, LBW, body surface area), gestational age (in weeks), interacting comedication (described in Table S1), use of glucocorticoids, prednisolone dose, haematocrit, serum albumin and serum creatinine concentrations, estimated glomerular filtration rate (eGFR; CKD-EPI),[39] and the women's *CYP3A4*, *CYP3A5* and *POR* genotype.

Continuous covariates (CO) were calculated according to the following equation:

$$\mathrm{CL/F} = \theta_{CL/F} \times (\mathrm{CO}/\mathrm{median\,CO})^{\theta_{COV}}.$$

Categorical covariates were transformed to binary covariates (BI) and calculated as

$$\mathrm{CL/F} = \theta_{CL/F} \times (\theta_{COV})^{BI}.$$

In which $\theta_{CL/F}$ is the typical value of clearance for a patient with the median covariate or most common BI and θ_{COV} describes the effect of the covariate.

Gestational age was modelled according to the following equation:

$$\mathrm{CL/F} = \theta_{CL/F} + \left(\theta\Delta \times e^{-\theta\mathrm{rate} \times \mathrm{time}}\right),$$

where $\theta\Delta$ is the relative change in CL/F over gestational age compared to its stabilized value prior to pregnancy, θrate is a first-order rate constant describing the increase rate of CL/F throughout pregnancy, time equals the gestational age in weeks.

The relationship between covariates and IIV in clearance was first investigated graphically and then further tested in a univariate analysis. Covariates that significantly improved the model ($P \leq 0.05$; decrease in OFV of >3.84) were added stepwise to the model. When no significant covariates could be added, a backward elimination process was performed, with statistical significance indicated by $P \leq 0.01$ (increase in OFV of >6.64).

2.8 | Model validation

The validity of the final model was evaluated using bootstrap analysis (by simulating 1000 datasets) and visual predictive checks (VPCs, by simulating 1000 datasets) and then generating the 95% prediction intervals from these simulations. VPCs were computed based on covariates that were included in the final model and pregnancy duration.

2.9 | Simulations

Simulations were performed to evaluate the effect of the significant covariates during the preconception period, each trimester and the postpartum period. The dose for each patient was set to 5 mg tacrolimus twice daily with a dose interval of 12 h. Final estimates were fixed, the covariates of interest varied during the simulation.

2.10 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22.[11,20]

3 | RESULTS

3.1 | Patient characteristics

A total of 43 women with 55 successful pregnancies after a kidney transplantation from 2000 and onwards were identified. Women who did not use tacrolimus (23 women, 30 pregnancies) and women who used a once-daily tacrolimus formulation (6 women, 10 pregnancies), were excluded. One woman had 2 pregnancies, and only the first was taken into account. This led to the inclusion of 14 women with a successful pregnancy using the twice-daily oral tacrolimus formulation after a kidney transplantation. Their median age at conception was 32 years (total range 22–40 years), with a median time after

transplantation of 4.5 years (total range 1–13 years). All patient and clinical characteristics are presented in Table 1.

The pregnancy duration in these 14 women varied from 30 weeks and 4 days to 41 weeks and 1 day. Only 3 women experienced an uncomplicated pregnancy with regard to kidney function and hypertension. One woman had increased proteinuria during pregnancy (no pre-eclampsia criteria), and 5 other women experienced pre-eclampsia. Another 5 women experienced worsening of their kidney function, of whom 1 was treated for presumed kidney transplant rejection postpartum with pulse dose corticosteroids, and another woman was treated for biopsy-proven kidney transplant rejection postpartum with pulse dose corticosteroids and intravenous immunoglobulin.

A total of 263 whole-blood tacrolimus C_0 was collected, of which 3 samples were excluded from analysis since they were below the lower limit of quantification for the ACMIA assay. The number of samples per patient varied from 11 to 30. The median tacrolimus C_0 prior to pregnancy was 5.3 ng/mL (interquartile range [IQR] 4.2–6.5), with a median daily tacrolimus dose of 6.0 mg/day (IQR 4.0–7.0). As presented in Table 1, the median tacrolimus C_0 decreased in the first and second trimester (median 4.5 and 3.9 ng/mL, respectively), and returned to baseline during the third trimester and postpartum (median 4.5 and 5.8 ng/mL, respectively). Daily doses of tacrolimus ranged between 2 and 22 mg/day throughout the follow-up. The change in tacrolimus concentrations corrected for the corresponding daily dose (concentration/dose ratio) for each stage of follow-up is presented in Table 1 and displayed in Figure 1A. The median concentration/dose ratio prior to pregnancy was 0.98 ng/mL/mg (IQR 0.61–1.33). The observed decrease in concentration/dose ratio in the first and second trimester (median 0.70 and 0.52 ng/mL/mg, respectively), demonstrates the higher dose requirement to obtain the tacrolimus target range, and indicates an increase in tacrolimus CL/F. Haematocrit decreases during pregnancy (median 0.39, 0.36, 0.31, 0.31 and 0.34 L/L, for prior to pregnancy, first, second and third trimester, and postpartum, respectively), and this was observed for serum albumin concentrations as well (median 45, 44, 38, 35 and 43 g/L, for prior to pregnancy, first, second and third trimester, and postpartum, respectively). The change over time for haematocrit and serum albumin concentrations, considered important covariates, are presented in Figure 1B,C.

3.2 | Base model

A two-compartment model was used to describe the data. K_a, t_{lag}, V_1/F, V_2/F and Q/F were fixed and CL/F was estimated (OFV = 605.894). Including IIV and IOV on CL/F improved the model fit and goodness-of-fit plots. Residual error for the analytical methods (LC–MS/MS, EMIT, ACMIA) was best described with a proportional and additive error model. The proportional error for both immunoassays could be estimated together without influencing the results. The base model estimates are shown in Table 2.

3.3 | Covariate analysis

After univariate analysis, forward inclusion and backward elimination, haematocrit (ΔOFV −86.46) and gestational age in weeks (ΔOFV −77.36) remained in the final model (for a full list of the covariates tested, see Table 3). Haematocrit and gestational age (in weeks) were both negatively correlated with CL/F:

$$\mathrm{CL/F} = 41.8 \times \left(\frac{\text{haematocrit}}{0.33}\right)^{-0.791} + (-7.29 \times \exp(-0.109 \times \text{gestational age in weeks}))$$

The mean predicted CL/F increased during pregnancy from 34.5 to 41.7 L/h, with the highest change already in the first trimester (Figure 2). Compared to CL/F values prior to pregnancy, gestational age increased the CL/F by 15% in the first trimester, by 19% in the second trimester and by 21% in the third trimester. The IIV on CL/F decreased from 26.5 to 24% and the IOV on CL/F from 19.4 to 7.5%. In total, these covariates could explain 18% of IIV and 85% of IOV. Estimations of the parameters in the final model are presented in Table 2. The population predictions (PRED) and individual predictions (IPRED) were normally distributed around the line of unity. The conditional weighted residuals (CWRES) were normally distributed and no trend was observed in the residuals (Figure S1).

3.4 | Model validation

The VPCs of the final model demonstrates an adequate fit and a good predictive performance of the final model as illustrated in Figure 3A (Haematocrit) and Figure 3B (Gestational age in weeks). Only small deviations in variability could be seen, which could be explained by the low number of patients included in the study. The medians of the estimated parameters from the bootstrap analysis were in range of the 95% confidence interval as demonstrated in Table 2.

3.5 | Simulations

The whole-blood tacrolimus concentrations were simulated for each stage of follow-up for the covariates that were included in the final model. The gestational age per stage of the follow-up was correlated to mean haematocrit values of that period. The mean haematocrit was 0.38, 0.35, 0.31, 0.31 and 0.33 L/L for the stages prior to pregnancy, first, second and third trimester, and postpartum respectively. As can be seen in Figure 4, there is a clear distinction between nonpregnant state and pregnancy in CL/F, with the biggest effect during the first trimester. The tacrolimus concentrations decreased further during the second trimester and stayed stable during the last trimester. The tacrolimus concentrations increased postpartum.

TABLE 1 Baseline characteristics.

Baseline characteristics	Prior to pregnancy	First trimester (1–12 weeks)	Second trimester (13–26 weeks)	Third trimester (27–42 weeks)	Postpartum
Age at conception (years)	32 (28.2–36.8)	-	-	-	-
Transplantation information					
Time after transplantation (years)	4.5 (2–8.5)	-	-	-	-
Number of kidney transplantations (*n*)					
First	11 (79%)	-	-	-	-
Second	3 (21%)	-	-	-	-
Donor type (*n*)					
Living	12 (86%)	-	-	-	-
Deceased	2 (14%)	-	-	-	-
HLA mismatch (*n*)					
0	1 (7%)	-	-	-	-
1	0 (0%)	-	-	-	-
2	5 (36%)	-	-	-	-
3	4 (29%)	-	-	-	-
4	2 (14%)	-	-	-	-
5	2 (14%)	-	-	-	-
Body composition					
Height (cm)	163.7 ± 8.2	-	-	-	-
Bodyweight (kg)	66.1 (59.25–71.25)	69.4 (58.1–80)	71.0 (59.6–84.7)	79.1 (64–88.3)	67.5 (55.9–80.9)
BMI (kg/m^2)	24.09 (22.07–28.08)	25.74 (23.12–28.39)	25.54 (23.78–29.91)	28.77 (26.02–31.23)	24.73 (22.66–28.70)
BSA (m^2)	1.75 (1.63–1.83)	1.81 (1.60–1.91)	1.83 (1.61–1.99)	1.90 (1.71–2.00)	1.74 (1.58–1.94)
LBW (kg)	47.54 (43.34–49.90)	49.00 (42.55–50.18)	49.24 (42.83–53.12)	49.51 (45.05–50.61)	44.61 (42.10–52.47)
Laboratory measurements					
Haematocrit (L/L)	0.39 (0.37–0.40)	0.36 (0.34–0.37)	0.31 (0.29–0.33)	0.31 (0.29–0.33)	0.34 (0.31–0.36)
Serum creatinine (μmol/L)	103 (84–123)	89 (71–112)	88 (71–117)	109 (87–141)	125 (90–178)
eGFR (mL/min/1.73 m^2)	64 (54.5–76)	76 (58–90)	76 (54–90)	57 (42–73)	56 (32–72)
Serum albumin (g/L)	45 (43–46)	44 (40–47)	38 (34–40)	35 (30–37)	43 (40–45)
Tacrolimus measurements					
Tacrolimus C_0 (ng/mL)	5.3 (4.2–6.5)	4.5 (3.2–5.6)	3.9 (3.2–4.5)	4.5 (3.6–5.6)	5.8 (4.5–7.6)
Tacrolimus daily dose (mg/day)	6.0 (4.0–7.0)	6.0 (4.0–8.0)	6.0 (4.0–10.0)	8.0 (6.0–14.0)	6.0 (5.0–8.0)
Tacrolimus C_0/D ratio (ng/mL/mg)	0.98 (0.61–1.33)	0.70 (0.47–1.12)	0.52 (0.38–0.93)	0.56 (0.40–0.71)	0.86 (0.64–1.15)
Genotype[a] (*n*)					
CYP3A4					

TABLE 1 (Continued)

Baseline characteristics	Prior to pregnancy	First trimester (1–12 weeks)	Second trimester (13–26 weeks)	Third trimester (27–42 weeks)	Postpartum
*1/*1	12 (100%)	-	-	-	-
CYP3A5					
*1/*3	5 (42%)	-	-	-	-
*3/*3	7 (58%)	-	-	-	-
POR					
*1/*1	5 (42%)	-	-	-	-
*1/*28	6 (50%)	-	-	-	-
*28/*28	1 (8%)	-	-	-	-
Comedication[b] (*n* of women)					
Corticosteroids					
Prednisolone	3	4	4	4	4
Prednisolone dose (range)	7.5 (5–7.5)	5.0 (5–7.5)	5.0 (2.5–5.0)	5.0 (2.5–5.0)	5.0 (5–7.5)
Antibiotics	None	None	None		None
Flucloxacillin				1	
Antiepileptics	None	None	None	None	None
Antihypertensive and antiarrhythmic agents					
Amlodipine	None	None	None	None	1
Barnidipine	1	None	None	None	1
Nicardipine	None	None	None	None	2
Nifedipine	3	1	2	5	6
Antimycotic drugs	None	None	None	None	None
Other	None	None	None	None	None

Note: Baseline characteristics in 14 pregnant women. Data are shown as median (interquartile range).

Abbreviations: BMI, body mass index; BSA, body surface area; C_0, whole-blood predose concentration; CYP, cytochrome P450; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; LBW, lean body weight; POR, P450 oxidoreductase.

[a]The *CYP3A4*, *CYP3A5* and *POR* genotyping of 2 patients were not taken into the analysis since they did not sign consent for the Biobanking programme (MEC-2010-022).

[b]Drugs having clinically relevant pharmacokinetic interactions with tacrolimus, as specified in Table S1.

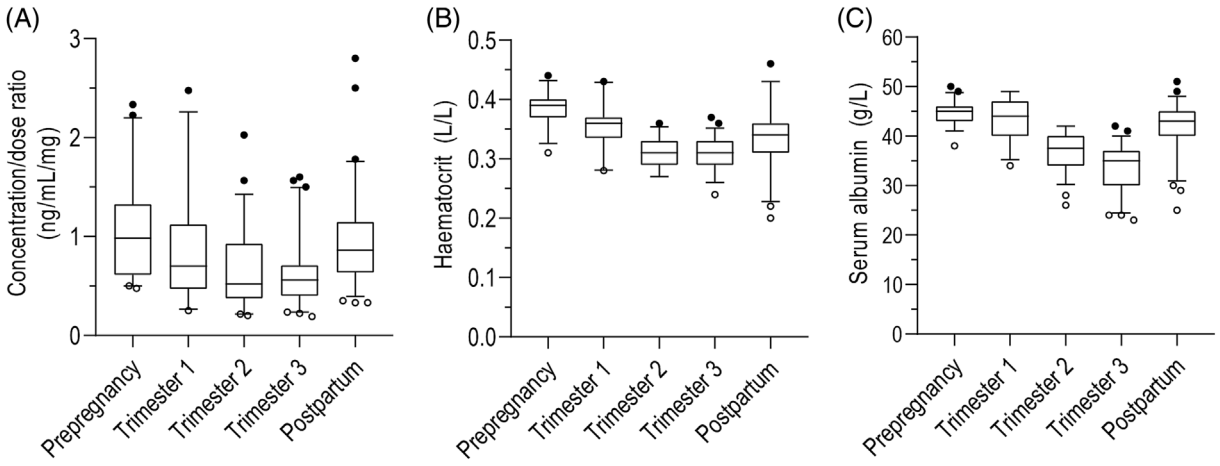


FIGURE 1 (A) Tacrolimus concentration/dose ratio (ng/mL/mg) plotted per stage of follow-up. (B) Haematocrit (L/L) plotted per stage of follow-up. (C) Serum albumin (g/L) plotted per stage of follow-up. Box plots showing the quartiles, the 5th and 95th percentiles (whiskers) and extreme values to the minimum (○) and maximum (●).

4 | DISCUSSION

This study demonstrates that haematocrit and gestational age are associated with the pharmacokinetics of tacrolimus during pregnancy. Together, these 2 covariates explain 18% of the IIV and 85% of the IOV in tacrolimus CL/F. Oral clearance increased during pregnancy from 34.5 to 41.7 L/h, with the largest change occurring in the first trimester. Subsequently, a rapid decrease in tacrolimus whole-blood predose concentrations was observed, also during the first trimester.

Compared to CL/F values prior to pregnancy, gestational age increased the CL/F by 15% in the first trimester, by 19% in the second trimester and by 21% in the third trimester, which could be attributed to a combination of physiological changes during pregnancy. For instance, haematocrit was inversely correlated with CL/F, in other words, a decrease in haematocrit results in an increase in CL/F. This in turn results in reduced tacrolimus whole-blood concentrations. The correlation between tacrolimus CL/F and haematocrit is consistent with previous findings in pregnant kidney transplant recipients.[16] Haematocrit measures the volume of erythrocytes compared to the total blood volume.[40] The ratio of tacrolimus distribution to whole-blood: plasma is 20:1 and >85% of tacrolimus measured in whole blood is located inside erythrocytes or plasma protein-bound, indicating inactive tacrolimus.[17] Tacrolimus has a low hepatic extraction ratio with a ratio equivalent to ~3% of liver blood flow.[41] Thus, for highly-bound, low-extraction-ratio drugs such as tacrolimus, one would expect CL/F to be affected by changes in haematocrit and plasma proteins. The decrease in haematocrit during pregnancy, observed in this study, can be explained as a result of a greater increase in plasma volume in relation to erythrocytes.[42]

Other known physiological changes during pregnancy were investigated, but no association with CL/F was found. For instance, a decrease in serum albumin concentrations during pregnancy was observed, but this did not correlate significantly with CL/F. We are uncertain of the exact reason this covariate did not affect the CL/F, but the assumption can be made that correction of whole-blood tacrolimus concentrations to albumin concentrations is not sufficient since the majority of tacrolimus is associated with erythrocytes.[43] Previous studies utilizing other substrates suggested that CYP3A activity may be increased by 25–100% during pregnancy.[13,44,45] Thus partly explaining the variability in CL/F between the nonpregnant state and pregnancy. However, in this study the effect of the *CYP3A* and *POR* genotype did not significantly influence tacrolimus pharmacokinetics. This might be explained by the fact that we took the genotype into account and not the protein activity, of which the latter can be influenced by oestrogen activity. The effect of oestrogen on CYP3A protein activity during pregnancy is potentially greater than the effect of genetics on CYP3A protein activity, resulting in the nullification of the genotype effect.[46–48] Another explanation might be the absence of *CYP3A5*1* homozygotes (CYP3A5 expressers) in this small, mostly Caucasian population. We observed a decrease in median serum creatinine and increase in median eGFR in our population (Table 1), which is consistent with previous findings.[49] However, both covariates were not significantly correlated to CL/F, which can be explained by the fact that tacrolimus practically undergoes no renal clearance. None of the body size indicators contributed to variability, which may be explained by the fact that the formulas that were used to estimate these were not specifically developed for pregnancy. A previous study did find a correlation between bioimpedance spectroscopy-derived phase angle, calculated as the arc tangent of reactance over resistance, and tacrolimus CL/F.[36] Possibly, despite the absence of a correlation with the estimated values, there may be a correlation when measuring body composition in pregnant kidney transplant recipients, which could explain a part of the changes in tacrolimus CL/F, considering the significant alterations in body composition that occur during pregnancy.

The covariates mentioned above, except for *POR* genotype, had a significant effect on the univariate analysis but not on the multivariate

TABLE 2 Estimates of the base model, final model and bootstrap analysis.

Parameter	Base model	Final model	Bootstrap (95% CI)
Typical parameters			
t_{lag} (h)[a]	0.38	0.38	-
K_a (L/h)[a]	3.58	3.58	-
V_1/F (L)[a]	692	692	-
V_2/F (L)[a]	5340	5340	-
Q/F (L/h)[a]	11.6	11.6	-
CL/F	39.5	41.8	41.99 (35.61–52.20)
Covariate effect on CL/F			
Haematocrit (L/L)			
θ_{12}	NA	−0.791	−0.734 (−1.34 to −0.40)
Gestational age (weeks)			
θ_{13}	NA	−7.29	−7.523 (−13.22 to −2.05)
θ_{14}	NA	0.109	0.107 (0.059–0.182)
IIV			
CL/F	0.068	0.0559	0.0452 (0.0051–0.1204)
V_1/F[a]	0.242	0.242	-
V_2/F[a]	0.281	0.281	-
Q/F[a]	0.619	0.619	-
IOV			
CL/F	0.0368	0.00555	0.00561 (0.00035–0.01955)
Residual variability			
Proportional			
LC–MS/MS	0.186	0.204	0.198 (0.133–0.249)
Immunoassay EMIT/ACMIA	0.254	0.319	0.276 (0.079–0.353)
Additive			
LC–MS/MS[a]	0	0	-
Immunoassay EMIT	0.881	0.644	0.988 (0.302–1.514)
Immunoassay ACMIA	1.11	0.633	0.803 (0.322–2.317)

Note: Estimates of the base model, final model and bootstrap analysis from 260 whole-blood tacrolimus predose concentrations in 14 pregnant women. Formula for clearance: $CL/F = 41.8 \times (\text{haematocrit} / 0.33)^{-0.791} + (-7.29 \times \exp(-0.109 \times \text{gestational age in weeks}))$.
Abbreviations: ACMIA, antibody conjugated magnetic immunoassay; CI, confidence interval; CL, clearance; EMIT, enzyme multiplied immunoassay; IIV, interindividual variability; IOV, interocassion variability; K_a, absorption rate constant; LC–MS/MS, liquid chromatography–tandem mass spectrometry; Q, intercompartmental clearance; t_{lag}, lag time; V_1, central compartment; V_2, peripheral compartment.
[a]Fixed parameter. Therefore, bootstrap estimates are not reported.

analysis (Table 3). These are physiological changes known to occur in every woman during pregnancy and could thus be part of the total effect of pregnancy duration on tacrolimus CL/F. Another explanation could be the low power of our study population. Therefore, a potential effect, albeit small, of these covariates cannot be excluded. There still is a possibility that a combination of these covariates, the unknown effects of pregnancy hormones, and other changes in body composition influence the CL/F on gestational age.

The information obtained from this popPK model can help physicians in the interpretation of tacrolimus whole-blood predose concentrations during pregnancy. As the greatest change in CL/F occurs in the first trimester, we advise more frequent monitoring during this period and whenever changes in haematocrit occur, which is usually in the third trimester. Since the CL/F increases, a dose increase is needed to maintain tacrolimus whole-blood concentrations in the target concentration rage during pregnancy. The dose should be increased compared to the preconception dose in each of the 3 trimesters by 15, 19 and 21%, respectively. Furthermore, this popPK model can be potentially implemented to guide tacrolimus dosing in future pregnant kidney transplant recipients.

Although the tacrolimus dose needs to be increased to keep the predose concentration stable, one should realize that the pharmacologically active moiety is the unbound tacrolimus fraction in plasma.[43] Pregnancy duration and haematocrit may decrease tacrolimus whole-blood concentrations but are not expected to affect the

TABLE 3 Covariate analysis.

Covariate	ΔOFV	Covariate effect	Included after forward inclusion	Included after backward elimination
Haematocrit	−86.46	−1.39	**Yes ($P \leq 0.05$)**	**Yes ($P \leq 0.01$)**
Serum albumin	−37.74	−0.82	No	No
Serum creatinine	−6.18	−0.26	No	No
eGFR	−6.01	0.21	No	No
Age	−1.48	0.53	No	No
Gestational age in weeks	−77.36	−12.7	**Yes ($P \leq 0.05$)**	**Yes ($P \leq 0.01$)**
		0.12		
Weight before pregnancy	−0.57	0.27	No	No
Weight during pregnancy	−21.04	1.35	No	No
Height	−1.13	−1.67	No	No
BMI	−27.74	1.54	No	No
LBW	−11.76	2.48	No	No
BSA	−16.98	2.4	No	No
Genotype				
CYP3A5 nonexpressor (*3/*3)	−6.742	1.47	No	No
POR*28 intermediate metabolizer (*1/*28)	−0.35	1.05	No	No
Comedication				
Antibiotics	−0.44	3.33	No	No
Antihypertensive and antiarrhythmic agents	−0.65	1.01	No	No
Corticosteroid use	−16.45	1.47	No	No
Prednisone dose	−16.66	−0.04	No	No
No use of prednisone		0.69		

Abbreviations: BMI, body mass index; BSA, body surface area; eGFR, estimated glomerular filtration rate; LBW, lean body weight; OFV, objective function value.

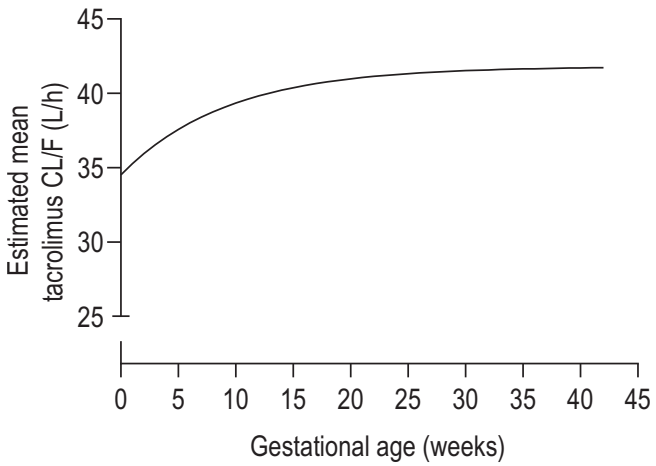


FIGURE 2 Mean predicted clearance of tacrolimus (L/h) throughout pregnancy.

unbound concentrations.[16,43,50–52] Consequently, unbound tacrolimus concentrations might be a better surrogate for tacrolimus dosing. Nevertheless, bioanalysis of unbound tacrolimus plasma concentration is challenging and time consuming.[43,53] Moreover, no target concentrations have been established for unbound tacrolimus.[43] Therefore, tacrolimus dose titration targeting the lower range of the *therapeutic window* during pregnancy seems reasonable.

The main limitation of this study is the small number of included patients. Second, no external validation was performed, caused by the lack of suitable datasets in this study field. Third, the tacrolimus concentrations were analysed by 3 different methods (ACMIA, EMIT, LC–MS/MS), which could potentially cause variability. However, by adding a residual error model, which calculates residuals errors for the different methods, this variability was accounted for. Fourth, only predose tacrolimus concentrations were available, which required fixing of the pharmacokinetic parameters and provided less pharmacokinetic information. However, these predose concentrations are most informative for CL/F, which is the most important pharmacokinetic parameter for clinical use. Lastly, this popPK model is developed for twice-daily tacrolimus and cannot be extrapolated to once-daily tacrolimus formulations. To improve this popPK model, further research is required in pregnant kidney transplant recipients by measuring area under the concentration–time curves, and by determining the effect of unbound tacrolimus and intralymphocyte tacrolimus concentrations.[54] Currently, we are setting up such a (nationwide) study.

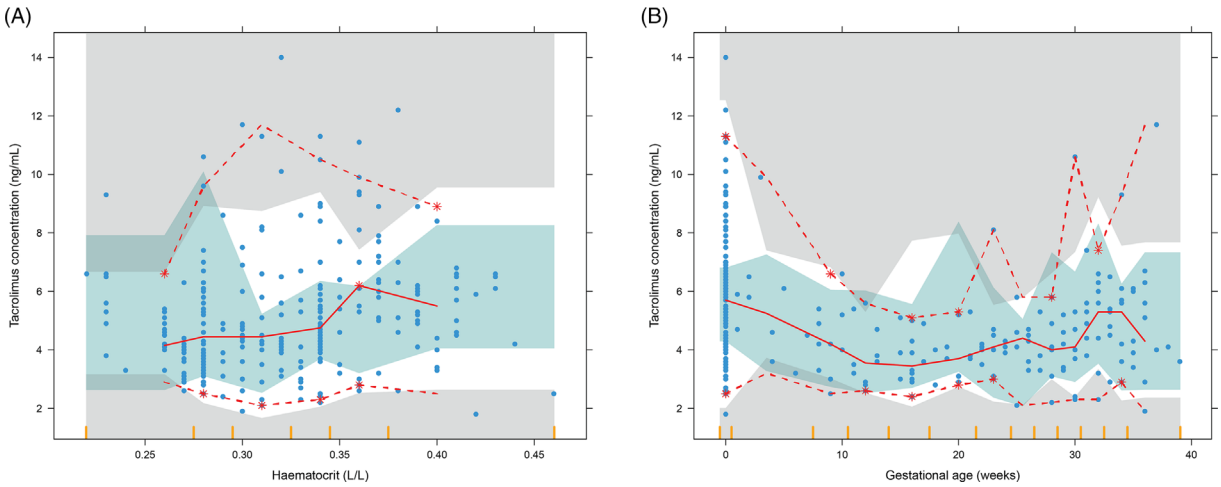
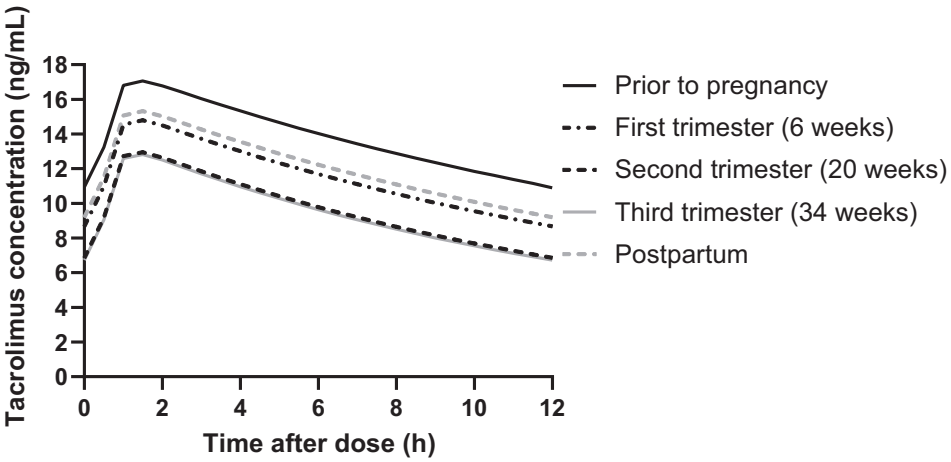


FIGURE 3 (A) Visual predictive check of haematocrit. (B) Visual predictive check of gestational age. Visual predictive checks showing how well the mean observed tacrolimus concentration (*red line*) versus the haematocrit (A) and the gestational age in weeks (B) falls within the 95% confidence interval for the predicted ($n = 1000$) mean tacrolimus concentration (*green area*) and how well the variability of the observed tacrolimus concentration (*red dotted line*) falls within the 95% confidence interval for the predicted variability of the tacrolimus concentration (*grey area*).

FIGURE 4 Simulations. Simulated tacrolimus concentrations for each stage of follow-up for gestational age and haematocrit. The gestational age was correlated to mean haematocrit values of that period. The mean haematocrit value was 0.38 L/L (prior to pregnancy; *black line*), 0.35 L/L (first trimester; *black dotted line*), 0.31 L/L (second trimester; *black dashed line*), 0.31 L/L (third trimester; *grey line*) and 0.33 L/L (postpartum; *grey dashed line*).



5 | CONCLUSION

Tacrolimus clearance increases throughout pregnancy, up to 21% at the end of gestation. Haematocrit and gestational age are associated with the tacrolimus clearance during pregnancy in kidney transplant recipients. Together, these 2 covariates explained 18% of the IIV and 85% of the IOV in oral clearance. Our study shows that increased dosing is required during pregnancy to maintain target whole-blood concentrations. In future, this popPK model can be used in pregnant kidney transplant recipients for dose adjustments based on the target tacrolimus whole-blood concentration.

AUTHOR CONTRIBUTIONS

Dennis A. Hesselink and Brenda C.M. de Winter designed the study. Asiye Nur Ulu and Marith I. Francke collected the data. Maaike R. Schagen, Asiye Nur Ulu and Brenda C.M. de Winter analysed the data. Maja Matic and Ron H.N. van Schaik performed the pharmacogenetic genotyping. Dennis A. Hesselink, Jacqueline van de Wetering, Marleen C. van Buren and Sam Schoenmakers had clinical responsibility for the patients. Maaike R. Schagen wrote the first draft of this manuscript, and all authors were involved in writing and review of this manuscript and approved the final version.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST STATEMENT

D.A. Hesselink has received grant support (paid to his institution) from Astellas Pharma, Chiesi Farmaceutici SpA and Bristol Myers-Squibb, as well as lecture and consulting fees from Astellas Pharma, Chiesi Farmaceutici SpA, Novartis Pharma and Vifor Pharma.

The other authors have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Maaike R. Schagen https://orcid.org/0000-0002-8759-9866
Marith I. Francke https://orcid.org/0000-0001-5641-8375

REFERENCES

1. Murray JE, Reid DE, Harrison JH, Merrill JP. Successful pregnancies after human renal transplantation. *N Engl J Med*. 1963;269(7):341-343. doi:10.1056/NEJM196308152690704
2. Jain AB, Shapiro R, Scantlebury VP, et al. Pregnancy after kidney and kidney-pancreas transplantation under tacrolimus: a single center's experience. *Transplantation*. 2004;77(6):897-902. doi:10.1097/01.TP.0000117564.50117.FB
3. Gutiérrez MJ, Acebedo-Ribó M, García-Donaire JA, et al. Pregnancy in renal transplant recipients. *Transplant Proc*. 2005;37(9):3721-3722. doi:10.1016/j.transproceed.2005.09.175
4. Armenti VT, Moritz MJ, Cardonick EH, Davison JM. Immunosuppression in pregnancy: choices for infant and maternal health. *Drugs*. 2002;62(16):2361-2375. doi:10.2165/00003495-200262160-00004
5. Claeys E, Vermeire K. Immunosuppressive drugs in organ transplantation to prevent allograft rejection: mode of action and side effects. *J Immunol Sci*. 2019;3(4):14-21. doi:10.29245/2578-3009/2019/4.1178
6. Neuwirt H, Rudnicki M, Schratzberger P, Pirklbauer M, Kronbichler A, Mayer G. Immunosuppression after renal transplantation. *memo*. 2019;12(3):216-221. doi:10.1007/s12254-019-0507-4
7. Le HL, Francke MI, Andrews LM, de Winter BCM, van Gelder T, Hesselink DA. Usage of tacrolimus and mycophenolic acid during conception, pregnancy, and lactation, and its implications for therapeutic drug monitoring: a systematic critical review. *Ther Drug Monit*. 2020;42(4):518-531. doi:10.1097/FTD.0000000000000769
8. Thongprayoon C, Hansrivijit P, Kovvuru K, et al. Impacts of high intra- and inter-individual variability in tacrolimus pharmacokinetics and fast tacrolimus metabolism on outcomes of solid organ transplant recipients. *J Clin Med*. 2020;9(7):9. doi:10.3390/jcm9072193
9. Brunet M, van Gelder T, Åsberg A, et al. Therapeutic drug monitoring of tacrolimus-personalized therapy: second consensus report. *Ther Drug Monit*. 2019;41(3):261-307. doi:10.1097/FTD.0000000000000640
10. Kanado Y, Tsurudome Y, Omata Y, et al. Estradiol regulation of P-glycoprotein expression in mouse kidney and human tubular epithelial cells, implication for renal clearance of drugs. *Biochem Biophys Res Commun*. 2019;519(3):613-619. doi:10.1016/j.bbrc.2019.09.021
11. Alexander SPKE, Mathie A, Peters JA, Veale EL, et al. The concise guide to pharmacology 2021/22: transporters. *Br J Pharmacol*. 2021;178(Suppl 1):S412-S513. doi:10.1111/bph.15543
12. Vanhove T, Annaert P, Kuypers DR. Clinical determinants of calcineurin inhibitor disposition: a mechanistic review. *Drug Metab Rev*. 2016;48(1):88-112. doi:10.3109/03602532.2016.1151037
13. Hebert MF, Easterling TR, Kirby B, et al. Effects of pregnancy on CYP3A and P-glycoprotein activities as measured by disposition of midazolam and digoxin: a University of Washington specialized center of research study. *Clin Pharmacol Ther*. 2008;84(2):248-253. doi:10.1038/clpt.2008.1
14. Venkataramanan R, Swaminathan A, Prasad T, et al. Clinical pharmacokinetics of tacrolimus. *Clin Pharmacokinet*. 1995;29(6):404-430. doi:10.2165/00003088-199529060-00003
15. Hytten F. Blood volume changes in normal pregnancy. *Clin Haematol*. 1985;14(3):601-612. doi:10.1016/S0308-2261(21)00496-3
16. Zheng S, Easterling TR, Umans JG, et al. Pharmacokinetics of tacrolimus during pregnancy. *Ther Drug Monit*. 2012;34(6):660-670. doi:10.1097/FTD.0b013e3182708edf
17. Undre NA, Stevenson P, Schäfer A. Pharmacokinetics of tacrolimus: clinically relevant aspects. *Transplant Proc*. 1999;31(7A):21S-24S. doi:10.1016/S0041-1345(99)00788-5
18. Iwasaki K, Miyazaki Y, Teramura Y, et al. Binding of tacrolimus (FK506) with human plasma proteins re-evaluation and effect of mycophenolic acid. *Res Commun Mol Pathol Pharmacol*. 1996;94(3):251-257.
19. Nagase K, Iwasaki K, Nozaki K, Noda K. Distribution and protein binding of FK506, a potent immunosuppressive macrolide lactone, in human blood and its uptake by erythrocytes. *J Pharm Pharmacol*. 1994;46(2):113-117. doi:10.1111/j.2042-7158.1994.tb03752.x
20. Alexander SPFD, Kelly E, Mathie A, Peters JA, Veale EL, et al. The concise guide to pharmacology 2021/22: enzymes. *Br J Pharmacol*. 2021;178(Suppl 1):S313-S411. doi:10.1111/bph.15542
21. Hebert MF, Zheng S, Hays K, et al. Interpreting tacrolimus concentrations during pregnancy and postpartum. *Transplantation*. 2013;95(7):908-915. doi:10.1097/TP.0b013e318278d367
22. Hesselink DA, Bouamar R, Elens L, van Schaik RH, van Gelder T. The role of pharmacogenetics in the disposition of and response to tacrolimus in solid organ transplantation. *Clin Pharmacokinet*. 2014;53(2):123-139. doi:10.1007/s40262-013-0120-3
23. Bandur S, Petrasek J, Hribova P, Novotna E, Brabcova I, Viklicky O. Haplotypic structure of ABCB1/MDR1 gene modifies the risk of the acute allograft rejection in renal transplant recipients. *Transplantation*. 2008;86(9):1206-1213. doi:10.1097/TP.0b013e318187c4d1
24. Hesselink DA, van Schaik RH, van der Heiden IP, et al. Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther*. 2003;74(3):245-254. doi:10.1016/S0009-9236(03)00168-1
25. Kuypers DR, de Jonge H, Naesens M, Lerut E, Verbeke K, Vanrenterghem Y. CYP3A5 and CYP3A4 but not MDR1 single-nucleotide polymorphisms determine long-term tacrolimus disposition and drug-related nephrotoxicity in renal recipients. *Clin Pharmacol Ther*. 2007;82(6):711-725. doi:10.1038/sj.clpt.6100216
26. Elens L, Hesselink DA, Bouamar R, et al. Impact of POR*28 on the pharmacokinetics of tacrolimus and cyclosporine A in renal transplant patients. *Ther Drug Monit*. 2014;36(1):71-79. doi:10.1097/FTD.0b013e31829da6dd
27. Lunde I, Bremer S, Midtvedt K, et al. The influence of CYP3A, PPARA, and POR genetic variants on the pharmacokinetics of tacrolimus and cyclosporine in renal transplant recipients. *Eur J Clin Pharmacol*. 2014;70(6):685-693. doi:10.1007/s00228-014-1656-3
28. Oneda B, Crettol S, Jaquenoud Sirot E, Bochud M, Ansermot N, Eap CB. The P450 oxidoreductase genotype is associated with CYP3A activity in vivo as measured by the midazolam phenotyping test. *Pharmacogenet Genomics*. 2009;19(11):877-883. doi:10.1097/FPC.0b013e32833225e7
29. Zhang JJ, Zhang H, Ding XL, Ma S, Miao LY. Effect of the P450 oxidoreductase 28 polymorphism on the pharmacokinetics of tacrolimus in Chinese healthy male volunteers. *Eur J Clin Pharmacol*. 2013;69(4):807-812. doi:10.1007/s00228-012-1432-1
30. Williams ET, Leyk M, Wrighton SA, et al. Estrogen regulation of the cytochrome P450 3A subfamily in humans. *J Pharmacol Exp Ther*. 2004;311(2):728-735. doi:10.1124/jpet.104.068908
31. McKay DB, Josephson MA. Pregnancy in recipients of solid organs—effects on mother and child. *N Engl J Med*. 2006;354(12):1281-1293. doi:10.1056/NEJMra050431
32. Shah S, Venkatesan RL, Gupta A, et al. Pregnancy outcomes in women with kidney transplant: metaanalysis and systematic review. *BMC Nephrol*. 2019;20(1):24. doi:10.1186/s12882-019-1213-5

33. Gosselink ME, van Buren MC, Kooiman J, et al. A nationwide Dutch cohort study shows relatively good pregnancy outcomes after kidney transplantation and finds risk factors for adverse outcomes. *Kidney Int*. 2022;102(4):866-875. doi:10.1016/j.kint.2022.06.006
34. Moritz MJ. Transplant Pregnancy Registry International, 2020 Annual Report. Philadelphia: Gift of Life Institute, 2020: 25.
35. van Buren MC, Gosselink M, Groen H, et al. Effect of pregnancy on eGFR after kidney transplantation: a national cohort study. *Transplantation*. 2022;106(6):1262-1270. doi:10.1097/TP.0000000000003932
36. Francke MI, Visser WJ, Severs D, de Mik-van Egmond AME, Hesselink DA, de Winter B. Body composition is associated with tacrolimus pharmacokinetics in kidney transplant recipients. *Eur J Clin Pharmacol*, submitted. 2022;78(8):1273-1287. doi:10.1007/s00228-022-03323-0
37. Andrews LM, Hesselink DA, van Schaik RHN, et al. A population pharmacokinetic model to predict the individual starting dose of tacrolimus in adult renal transplant recipients. *Br J Clin Pharmacol*. 2019;85(3):601-615. doi:10.1111/bcp.13838
38. Francke MI, Andrews LM, Le HL, et al. Avoiding tacrolimus underexposure and overexposure with a dosing algorithm for renal transplant recipients: a single arm prospective intervention trial. *Clin Pharmacol Ther*. 2021;110(1):169-178. doi:10.1002/cpt.2163
39. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612. doi:10.7326/0003-4819-150-9-200905050-00006
40. Billett HH. Hemoglobin and hematocrit. In: Walker HK, Hall WD, Hurst JW, eds. *Clinical Methods: The History, Physical, and Laboratory Examinations*. 3rd ed. Boston: Butterworths; 1990. Chapter 151. https://www.ncbi.nlm.nig.gov/books/NBK259/
41. Zhu L, Wang H, Sun X, et al. The population pharmacokinetic models of tacrolimus in Chinese adult liver transplantation patients. *J Pharm (Cairo)*. 2014;2014:713650. doi:10.1155/2014/713650
42. Khoigani MG, Goli S, Hasanzadeh A. The relationship of hemoglobin and hematocrit in the first and second half of pregnancy with pregnancy outcome. *Iran J Nurs Midwifery Res*. 2012;17(2 Suppl 1):S165-S170.
43. Sikma MA, Van Maarseveen EM, Hunault CC, et al. Unbound plasma, Total plasma, and whole-blood tacrolimus pharmacokinetics early after thoracic organ transplantation. *Clin Pharmacokinet*. 2020;59(6):771-780. doi:10.1007/s40262-019-00854-1
44. Tracy TS, Venkataramanan R, Glover DD, Caritis SN, National Institute for Child Health and Human Development Network of Maternal-Fetal-Medicine Units. Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A activity) during pregnancy. *Am J Obstet Gynecol*. 2005;192(2):633-639. doi:10.1016/j.ajog.2004.08.030
45. Hirt D, Treluyer JM, Jullien V, et al. Pregnancy-related effects on nelfinavir-M8 pharmacokinetics: a population study with 133 women. *Antimicrob Agents Chemother*. 2006;50(6):2079-2086. doi:10.1128/AAC.01596-05
46. Choi SY, Koh KH, Jeong H. Isoform-specific regulation of cytochromes P450 expression by estradiol and progesterone. *Drug Metab Dispos*. 2013;41(2):263-269. doi:10.1124/dmd.112.046276
47. Mlugu EM, Minzi OM, Kamuhabwa AAR, Diczfalusy U, Aklillu E. Pregnancy increases CYP3A enzymes activity as measured by the 4β-hydroxycholesterol/cholesterol ratio. *Int J Mol Sci*. 2022;23(23):15168. doi:10.3390/ijms232315168
48. Papageorgiou I, Grepper S, Unadkat JD. Induction of hepatic CYP3A enzymes by pregnancy-related hormones: studies in human hepatocytes and hepatic cell lines. *Drug Metab Dispos*. 2013;41(2):281-290. doi:10.1124/dmd.112.049015
49. Maynard SE, Thadhani R. Pregnancy and the kidney. *J Am Soc Nephrol*. 2009;20(1):14-22. doi:10.1681/ASN.2008050493
50. Piletta-Zanin A, De Mul A, Rock N, Lescuyer P, Samer CF, Rodieux F. Case report: low hematocrit leading to tacrolimus toxicity. *Front Pharmacol*. 2021;12:717148. doi:10.3389/fphar.2021.717148
51. Schijvens AM, van Hesteren FHS, Cornelissen EAM, et al. The potential impact of hematocrit correction on evaluation of tacrolimus target exposure in pediatric kidney transplant patients. *Pediatr Nephrol*. 2019;34(3):507-515. doi:10.1007/s00467-018-4117-x
52. Størset E, Holford N, Midtvedt K, Bremer S, Bergan S, Åsberg A. Importance of hematocrit for a tacrolimus target concentration strategy. *Eur J Clin Pharmacol*. 2014;70(1):65-77. doi:10.1007/s00228-013-1584-7
53. Trull AK. Therapeutic monitoring of tacrolimus. *Ann Clin Biochem*. 1998;35(Pt 2):167-180. doi:10.1177/000456329803500201
54. Udomkarnjananun S, Francke MI, Dieterich M, et al. P-glycoprotein, FK-binding protein-12, and the intracellular tacrolimus concentration in T-lymphocytes and monocytes of kidney transplant recipients. *Transplantation*. 2022;107(2):382-391. doi:10.1097/TP.0000000000004287

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Schagen MR, Ulu AN, Francke MI, et al. Modelling changes in the pharmacokinetics of tacrolimus during pregnancy after kidney transplantation: A retrospective cohort study. *Br J Clin Pharmacol*. 2023;1-13. doi:10.1111/bcp.15886