THE CYP3A BIOMARKER 4β-HYDROXYCHOLESTEROL DOES NOT IMPROVE TACROLIMUS DOSE PREDICTIONS EARLY AFTER KIDNEY TRANSPLANTATION

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Running head: 4βOHC and tacrolimus dosing
Key words: Tacrolimus, 4β-hydroxycholesterol, kidney transplantation, population pharmacokinetics

Word count: 3,357
Number of tables: 1
Number of figures: 3, + 2 Supplementary Figures
**STRUCTURED SUMMARY**

**Aims.** Tacrolimus is a cornerstone in modern immunosuppressive therapy after kidney transplantation. Tacrolimus dosing is challenged by considerable pharmacokinetic variability, both between patients and over time after transplantation, partly due to variability in cytochrome P450 3A (CYP3A) activity. The aim of this study was to assess the value of the endogenous CYP3A marker 4β-hydroxycholesterol (4βOHC) for tacrolimus dose individualization early after kidney transplantation.

**Methods.** Data were obtained from 79 adult kidney transplant recipients who contributed a total of 625 4βOHC measurements and 1999 tacrolimus whole blood concentrations during the first two months after transplantation. The relationships between 4βOHC levels and individual estimates of tacrolimus apparent plasma clearance (CL/F\textsubscript{plasma}) at different time points after transplantation were investigated using scatterplots and population pharmacokinetic modeling.

**Results.** There was no significant correlation between pre-transplant 4βOHC levels and tacrolimus CL/F\textsubscript{plasma} the first week (r=0.20 [95% CI −0.11-0.47]) or between 4βOHC and tacrolimus CL/F\textsubscript{plasma} one week (r=0.20 [−0.11-0.47]), four weeks (r=0.21 [−0.11-0.47]) or two months (r=0.24 [−0.03-0.48]) after transplantation (p≥0.06). In the population analysis, time-varying 4βOHC was not a statistically significant covariate on tacrolimus CL/F\textsubscript{plasma}, neither in terms of absolute values (p=0.11) nor in terms of changes from baseline (p=0.17). 4βOHC values increased between one week and two months after transplantation (median change +57% [IQR +22-83%], p<0.001), indicating increasing CYP3A activity. Contradictorily, tacrolimus CL/F\textsubscript{plasma} decreased over the same period (median change −13% [IQR −3-26%], p<0.001).

**Conclusions.** 4βOHC does not appear to have a clinical potential to improve individualization of tacrolimus doses early after kidney transplantation.
What is known about this subject

- Tacrolimus dosing is challenged by highly variable pharmacokinetics, and there is a need for additional clinically useful biomarkers for dose individualization.
- $4\beta$-hydroxycholesterol ($4\beta$OHC) is a potential endogenous biomarker of CYP3A activity.
- The value of $4\beta$OHC in tacrolimus dose individualization early after kidney transplantation is unknown.

What this study adds

- There was no significant or clinically relevant correlations between $4\beta$OHC levels and tacrolimus apparent clearance at any time during the first two months after kidney transplantation.
- $4\beta$OHC seems to have no clinical value in the prediction of tacrolimus initial dose or dose changes early after kidney transplantation.

INTRODUCTION

Tacrolimus is a widely used immunosuppressant that prevents rejection after kidney transplantation [1]. Tacrolimus has a narrow therapeutic index, and the pharmacokinetics vary considerably between and within individuals [2]. Furthermore, tacrolimus pharmacokinetics change systematically with time after transplantation [3,4]. This variability challenges not only prediction of the initial dose, but also the continuous dose adaptations during the first months after kidney transplantation. Tacrolimus concentrations outside the therapeutic range are frequent [5,6]. Numerous studies have developed initial dose algorithms [7] and population pharmacokinetic models [8] identifying patient factors that can be used in tacrolimus dose...
individualization. Consistently identified factors include cytochrome P450 (CYP) 3A5 genotype, hematocrit and glucocorticoid dose [8]. Still, a substantial part of tacrolimus variability remains unexplained. There is a need for additional clinically useful factors to aid in prediction of tacrolimus dose requirement early after transplantation.

Tacrolimus is metabolized by intestinal and hepatic CYP3A enzymes [9]. The expression and activity of these enzymes vary extensively between individuals [10]. Thus, markers of CYP3A activity may be valuable to guide tacrolimus dosing. Traditional CYP3A markers, such as midazolam clearance and erythromycin breathing test results, are based on exogenous drug administration and relatively laborious analyses [11]. This limits their clinical value. The endogenous compound 4β-hydroxycholesterol (4βOHC), which is synthesized in vivo from cholesterol via CYP3A enzymes and slowly eliminated [12], has been suggested as a clinically relevant biomarker of CYP3A activity [13]. 4βOHC is more readily assessed in the clinical setting because it can be determined from a single blood sample. Vanhove et al. recently reported a significant correlation between 4βOHC:cholesterol/bodyweight and tacrolimus CL/F/bodyweight in stable kidney recipients after a mean of 2.5 years after transplantation [14]. However, the greatest need for predictors of tacrolimus dose requirement is at treatment initiation, which usually takes place at the time of transplantation, and during the early post-transplant phase, which is associated with significant time-varying pharmacokinetics. We therefore performed the present study to assess the value of pre-transplant and early post-transplant 4βOHC levels in prediction of tacrolimus initial dose and dose requirement the first two months after kidney transplantation.

METHODS

Patients

This study included patients enrolled in a previously described randomized controlled trial that evaluated computerized dosing of tacrolimus after kidney transplantation [15] (www.ClinicalTrials.gov, NCT02010320). Patient characteristics are presented in Table 1. All patients were >18 years and received a single kidney transplant at Oslo University Hospital Rikshospitalet between January and June 2014. None of the
patients included in the present analysis received drugs that are known to induce or inhibit CYP3A. The study was approved by the Regional Committee for Medical Research Ethics. All participants gave written informed consent.

**Immunosuppression**

The patients received oral tacrolimus (Prograf®, Astellas Pharma, Killorglin, Ireland) twice daily. Treatment was initiated on the day of transplantation (day 0) with a dose of 0.04 mg/kg in standard risk patients and 0.05 mg/kg in high-risk patients (defined as presence of donor-specific antibodies, panel reactive antibodies > 20% and/or ABO incompatibility between donor and recipient), followed by dose adjustments to achieve trough concentrations between 3 and 7 µg/L in standard risk patients and between 8 and 12 µg/L in high-risk patients (6-10 µg/L after day 30). All patients also received 0.75-1 g mycophenolate mofetil twice daily and oral prednisolone once daily, starting with a daily dose of 20 mg (80 mg in high-risk patients) that was tapered gradually to 10 mg daily during the first two months after transplantation.

Induction therapy consisted of 20 mg basiliximab on day 0 and day 4 after transplantation and 250 mg (standard risk) or 500 mg (high-risk) intravenous methylprednisolone on day 0. High-risk patients also received 500 mg/kg intravenous human immune globulins daily from day 0 to 4 and 375 mg/m² rituximab on day 0.

**Tacrolimus measurements**

Tacrolimus trough concentrations were measured on day 1, 2 and 3 after transplantation, followed by 2-5 measurements per week throughout the first two months after transplantation. The exact times of dose intake were recorded by the patients and available for the population pharmacokinetic analysis. Tacrolimus concentrations were determined in whole blood using a chemiluminescent microparticle immunoassay (CMIA, analyzed on the Architect Instrument; Abbott Laboratories, Abbott Park, IL [16]), with a lower limit of quantification of 1.0 mcg/L. Values reported below this limit were to be discarded from the analysis if they represented <1% of the total observations {Keizer:2015ia}.

**4βOHC quantification**

Residual whole blood samples originally drawn for tacrolimus trough concentration determination were centrifuged at 1,800 g for ten minutes, and serum was transferred...
to new tubes and stored at –80°C until the time of analysis (range of storage durations 13-24 months; within the time frame 4βOHC maintains acceptable stability [17]).

4βOHC levels were quantified using ultra-performance liquid chromatography tandem mass spectrometry (UPLS-MS/MS) at Center for Psychopharmacology, Diakonhjemmet Hospital, as previously described in detail [18]. An additional filtration step was added after liquid-liquid-extraction to remove lipid precipitations. A quality control (QC) sample produced at the same time as the oldest samples (January 2014) was included for each series to ensure that the concentration did not change during storage. The 4βOHC concentration of the QC sample in an analytical run was required to be within +/- 15% of the original value for 4βOHC concentrations to be used in the analyses.

Duplicate measurements were performed for each sample, and the mean value across the duplicates was used in this analysis. Intra- and inter-day precision was <8% at 10 ng/mL and <4% at 644 ng/mL. The corresponding accuracies were <15% and <2%, respectively (n=6). The lower limit of quantification was 10 ng/mL. Values below the formal quantification limit, i.e. the lowest validated concentration, were still reported by the laboratory and included as continuous data in the analysis.

4βOHC was typically measured once weekly for each patient during the first two months after transplantation. For each tacrolimus concentration without an accompanying 4βOHC measurement, the 4βOHC level was imputed by linear interpolation between each two consecutive values.

**Genotyping analyses**

Cytochrome P450 3A4 and 3A5 genotypes (CYP3A4*22; rs35599367 and CYP3A5*3; rs776746) were determined using real-time polymerase chain reaction and melting curve analysis with hybridization probes on the LightCycler 480 instrument (Roche Applied Science, Penzberg, Germany).

**Population pharmacokinetic analysis**

**Original model**

Population pharmacokinetic analysis was based on a previously published population pharmacokinetic model, updated using immunoassay equivalent tacrolimus.
concentrations [19]. In brief, this model was developed using data from 242 kidney-
transplanted adults and described tacrolimus pharmacokinetics using a two-
compartment model with first-order absorption and a lag-time. The model included
plasma-based pharmacokinetic parameters (e.g. CL/F_{plasma}) rather than whole blood
parameters. These were estimated based on tacrolimus plasma concentrations that
were predicted from whole blood concentrations, individual hematocrit levels and
literature values for the parameters that describe binding of tacrolimus to red blood
cells (B_{max}, K_D) [20]. Fat-free mass, CYP3A5 genotype, prednisolone dose and early
time after transplantation were identified as covariates. The model has been externally
evaluated in 72 independent patients and demonstrated satisfying predictive
performance [19]. Because mainly trough concentrations were available, absorption
parameters and disposition parameters except CL/F_{plasma}, with their associated
variability parameters, were fixed to the previous model final estimates. CYP3A4
genotype was not evaluated in the original model due to missing data and was
therefore considered as an additional covariate in the present analysis. Model fit was
assessed using standard goodness-of-fit plots and prediction-corrected visual
predictive checks (pcVPCs) [21].

In the population analysis, the population pharmacokinetic parameters of
tacrolimus were re-estimated based on the present data. Pre-transplant 4βOHC level
was evaluated as a covariate on CL/F on day 2 and on day 7 after transplantation.
Time-varying 4βOHC values were modeled as a covariate on CL/F as shown in
Equation 1:
TVCL = θCL × [1 + θ4βOHC × (4βOHC – 4βOHC_{median})],  \hfill (1)  

where TVCL is the typical value of CL/F_{plasma}, θCL is the estimated CL/F_{plasma} in a patient with the median value of 4βOHC, and θ4βOHC is the estimated fractional change in TVCL for each unit of change in 4βOHC. In addition, due to the time-varying nature of the data, an extended model was evaluated to allow different effects of 4βOHC changes between patients and within patients [22]:

TVCL = θCL × [1 + θ_{B_{4βOHC}} × (B_{4βOHC} – 4βOHC_{median}) + θ_{D_{4βOHC}} x D_{4βOHC}]  \hfill (2)  

where B_{4βOHC} is the baseline 4βOHC level (i.e. pretransplant level) and D_{4βOHC} is the individual change in 4βOHC from baseline. θ_{B_{4βOHC}} and θ_{D_{4βOHC}} are estimable parameters for the effect of baseline values between patients and the effect of changes from baseline within an individual, respectively. Models were also tested with θ_{B_{4βOHC}} or θ_{D_{4βOHC}} fixed to zero. Because 4βOHC is expected to correlate with established covariates, such as CYP3A genotypes, 4βOHC was also evaluated as a covariate in the structural model without covariates.

Statistics and software
Modeling and individual parameter estimation was performed with NONMEM v. 7.2 (Icon Development Solutions, Ellicott City, MD, USA) using the first-order conditional method with interaction. Nested models were compared using the likelihood ratio test. Remaining statistical analyses were carried out using R v. 3.0.2 [23]. Xpose ([24], v. 4.4.1) in R was used for data exploration and model diagnostics. 4βOHC levels were compared between groups using the Mann Whitney U test. Changes in variables within patients were compared using the Wilcoxon signed rank test. Correlations were evaluated using Pearson’s r. P-values less than 0.05 were considered to represent statistical significance.

RESULTS
Patients and data
Seventy-nine patients were included in this study and contributed a total of 2003 tacrolimus whole blood concentrations. Four concentrations (<0.2%) were reported as below the lower limit of quantification and were discarded, leaving 1999 concentrations for analysis (median 25 per patient, range 9-41). Of these, 85 (4%) were taken within the dose interval, whereas 96% were trough concentrations.

Seventy-three patients contributed tacrolimus data throughout the study period (from the day of transplantation until two months after transplantation). All patients had 4βOHC measured immediately prior to transplantation. Sixteen patients did not provide additional 4βOHC samples after transplantation and were included only in the analysis regarding pre-transplant 4βOHC levels. The remaining 63 patients contributed a median of 10 (range 2-12) 4βOHC samples during the first two months after transplantation and were included in all analyses. The total number of samples collected for 4βOHC determination was 631. Three of these samples were missing at the time of analysis and three samples (of which two were pre-transplant samples) did not have enough serum, leaving 625 samples for the present analyses. Of these, 3.7% had at least one of the duplicate measurements below the lower limit of quantification.

4βOHC levels and tacrolimus CL/F
The 4βOHC quality control sample concentrations remained stable (all within +/- 15% of the original value). All 4βOHC patient samples were therefore assumed to have maintained stability during storage and were included in the analysis.

Prior to transplantation, the median [interquartile range] 4βOHC level was 23.3 [15.2-32.4] ng/mL. One week after transplantation the median level was slightly lower at 20.3 [14.8-25.4] ng/mL (p=0.02). Then, 4βOHC generally increased with time after transplantation towards a median value of 31.5 [22.7-40.4] ng/mL two months after transplantation (p<0.001). The median individual increase was +57% [interquartile range (IQR) +22-83%], with considerable variability between patients in the extent of change (range –12% to +246%).

One week after transplantation, there was a 7-fold variation in tacrolimus CL/F. Median whole blood-based CL/F (CL/F<sub>wb</sub>) at this time was 29.0 L/h (range 11.5-72.2 L/h), and median plasma based CL/F (CL/F<sub>plasma</sub>) was 1025 L/h (range 322-2204 L/h). CL/F<sub>wb</sub> decreased with time after transplantation towards a median of 21.6
L/h after two months (median individual change –28%, p<0.001). This decrease could partly be attributed to increases in hematocrit over the same period (median 30% after one week, median 37% after two months, p<0.001). After accounting for hematocrit variability, CL/F_{plasma} still decreased over the same period towards a median of 865 L/h (median individual change –13% [IQR –3-26%], p<0.001).

**Exploratory analyses**

There was no significant correlations between pre-transplant 4βOHC levels and estimated individual tacrolimus CL/F_{plasma} after one week (Figure 1), four weeks or two months after transplantation, neither in the overall data (|r|≤0.27, p≥0.09) or when analyzed separately in CYP3A5 expressors and CYP3A5 nonexpressors. Likewise, there were no significant correlations between simultaneously assessed 4βOHC levels and CL/F_{plasma} after one week, four weeks or two months (|r|≤0.33, p≥0.06, Figure 2). In 70% of the 57 patients with tacrolimus data and 4βOHC measurements available both prior to transplantation and two months after transplantation, tacrolimus CL/F_{plasma} and 4βOHC changed in “opposite” directions than theoretically expected (assuming that increasing 4βOHC reflects increasing CYP3A activity and decreasing tacrolimus CL/F_{plasma} reflects decreasing CYP3A activity). Supplementary Figure 1 shows the individual change in CL/F_{plasma} together with the individual change in 4βOHC for each individual.

**Population pharmacokinetic analyses**

The pcVPC indicated that the population model described the present data well (Figure 3). Pre-transplant 4βOHC was not found as a significant covariate on early post-transplant CL/F_{plasma} on day 2 (difference in objective function value (ΔOFV) –0.93, p=0.33) or day 7 (ΔOFV –2.4, p=0.12). Time-varying 4βOHC did not show a significant effect on CL/F (ΔOFV –2.6, p=0.11). The fit was not significantly improved by allowing different effects of baseline values and individual changes in 4βOHC as described in Equation 2 (ΔOFV –3.6, p=0.17). θ_{B,4βOHC} and θ_{D,4βOHC} were estimated to 0.0041 and 0.0012, respectively, indicating a trend towards a relatively more important effect of baseline values than of individual changes from baseline [22]. When evaluating 4βOHC as a covariate in the structural model, 4βOHC remained insignificant as a covariate with ΔOFV values associates with p-values.
higher than described above for the full model (ΔOFV –0.4 to –2.04, p>0.12).

*CYP3A4* genotype was not identified as a significant covariate on tacrolimus CL/F (ΔOFV –0.02, p=0.88).

**DISCUSSION**

This is the first evaluation of the potential value of the CYP3A activity marker 4βOHC in tacrolimus dose individualization early after kidney transplantation. We found that the pre-transplant 4βOHC levels were not predictive of post-transplant tacrolimus pharmacokinetics. Furthermore, individual changes in 4βOHC over the first two months after kidney transplantation did not correlate with the changes in tacrolimus pharmacokinetics over the same period. Based on these results, 4βOHC seems to have no clinical value in prediction of tacrolimus dose requirement early after kidney transplantation.

Tacrolimus is metabolized by CYP3A enzymes with negligible renal elimination [25], and 4βOHC is synthesized by the same enzymes [12]. The lack of correlation between tacrolimus 4βOHC and tacrolimus CL/F is therefore surprising. Tacrolimus bioavailability (F) is generally low (average 25%), mainly due to first-pass metabolism by intestinal CYP3A and P-glycoprotein. It shows considerable variability between patients and between dosing occasions (4-89%), and is affected by disease status and concomitant food intake [2]. Bioavailability variability may be diluting a potential underlying correlation between 4βOHC and tacrolimus clearance in our data. Data from intravenous tacrolimus administration would be interesting to further elucidate the relationship between 4βOHC and tacrolimus clearance.

However, even with a detectable relationship, the usefulness of 4βOHC measurements in tacrolimus individualization would probably not improve significantly for the standard oral dosage form as long as the oral bioavailability represents the major source of unexplained variability for tacrolimus.

The proposed applicability of 4βOHC as a CYP3A biomarker is mostly based on theory and its significantly altered levels after administration of CYP3A inducers [26] and inhibitors [27]. Only weak to moderate correlations have been demonstrated between 4βOHC and the established CYP3A activity metric midazolam clearance in
patients not treated with CYP3A-interacting drugs [14,28]. It has therefore been suggested that 4βOHC may not be suitable to reflect differences in basal or temporal variations in CYP3A activity [28]. Moreover, the long elimination half-life of 4βOHC (62 hours-17 days [13,29]) limits the ability of 4βOHC to reflect rapid changes in CYP3A activity. The present study confirms the failure of 4βOHC to describe differences in basal tacrolimus pharmacokinetics as well as time-related changes in tacrolimus pharmacokinetics early after transplantation.

The correlations between 4βOHC and tacrolimus CL/F in our data were insignificant, but generally slightly positive and remained relatively stable as time after transplantation evolved (Figure 2). It is currently unknown whether the correlation may increase at later time points after transplantation when tacrolimus pharmacokinetics stabilize. Vanhove et al. recently reported that 4βOHC:cholesterol:bodyweight levels are predictive of tacrolimus weight-adjusted apparent clearance in stable kidney transplant recipients after a mean of 2.5 years [14]. This correlation, however, may be biased by bodyweight-adjustment of both variables [30]. Indeed, when analyzing the present data in the same manner as Vanhove et al., strong and significant correlations appear (e.g. r=0.46, p=0.002 one week post-transplant). Regardless, if a more pronounced correlation exists later after transplantation, it would hardly have clinical implications, because therapeutic drug monitoring is routinely performed for tacrolimus, and the individual dose is typically well established at these later time points. The most clinically interesting predictors of tacrolimus dose are those that improve prediction of the initial dose or time-varying dose requirement during the first months after transplantation. Unfortunately, 4βOHC does not seem useful for this purpose. Still, 4βOHC may potentially be useful to predict tacrolimus dose to patients switching to tacrolimus treatment in the stable post-transplant phase or who start using CYP3A inhibitors or inducers.

For tacrolimus, CYP3A5 genotype is an established covariate that has been recommended for clinical implementation [31]. CYP3A4 genotype has also received attention lately for its influence on tacrolimus pharmacokinetics [32,33]. It was, however, not identified as a significant covariate in the present analysis, possibly due to a small number of CYP3A4*22 expressers in the dataset (n=8, 10%). The continuous nature of CYP3A biomarkers values, such as 4βOHC, may theoretically provide more valuable information than genotype status by reflecting
pharmacokinetic variability also within each genotype group. However, 4βOHC could not effectively replace CYP3A5 genotype in our population pharmacokinetic structural model. Our data do not support that 4βOHC provide valuable information for tacrolimus dosing neither in the presence nor in the absence of CYP3A genotype information.

Tacrolimus dosing is complicated by its time-varying pharmacokinetics. Decreasing tacrolimus dose requirement with time after kidney transplantation to maintain stable whole blood exposure has been consistently reported [3,6,34]. This phenomenon has been partly attributed to increasing hematocrit (due to additional erythrocytes available for tacrolimus accumulation in whole blood) and partly to decreasing tacrolimus unbound CL/F [19,35]. The underlying cause of decreasing CL/F of tacrolimus has not been established because of the number of clinical and physiologic factors that change in a collinear manner during the time course of recovery after kidney transplantation. The most common hypothesis is that tacrolimus CL/F decreases due to the use of glucocorticoids [2,36], because glucocorticoids are inducers of CYP3A/P-glycoprotein expression [37], and because glucocorticoid doses are typically tapered with time after transplantation. It has remained unclear whether CYP3A or P-glycoprotein induction is the dominating factor and whether the time-decreasing tacrolimus CL/F is due to decreasing clearance, increasing bioavailability or changes in both parameters [36]. In this study, 4βOHC levels increased with time after transplantation, which contradictorily indicates accelerating CYP3A activity. A similar pattern has been described in Japanese kidney transplant recipients [38]. If tacrolimus clearance increases with time after kidney transplantation as suggested by the increasing 4βOHC levels, tacrolimus bioavailability must theoretically increase simultaneously to a more pronounced extent, counteracting increasing clearance and leading to a net decrease in CL/F. Furthermore, 4βOHC values in this study were lower than or comparable to values reported in healthy Caucasians (average 30, range 10-60 ng/mL) [12], although all patients were treated with glucocorticoids (initially 20 mg in 95% of the patients). The use of potent CYP3A inducers is known to increase the 4βOHC by ten-fold compared with normal values [26], and simulation studies have suggested that 4βOHC would increase markedly also with the use of a weak inducer after two weeks of treatment [39]. This indicates that CYP3A is not induced in these patients and supports the theory that the time-varying
pharmacokinetics of tacrolimus is due to changes in bioavailability, potentially through glucocorticoid-related P-glycoprotein induction [40,41]. More studies are needed to characterize the distinct time courses of CYP3A and P-glycoprotein activity after kidney transplantation.

4βOHC is synthesized from cholesterol, and it is currently debated whether the 4βOHC:cholesterol-ratio may be a better CYP3A activity marker than 4βOHC values alone. In the present study, in which none of the study participants received statins, patient serum cholesterol values were only available two months after transplantation. In an additional analysis, we calculated the 4βOHC:cholesterol ratio at this time point. It correlated well with 4βOHC (r=0.91, p<0.001) and its correlation with tacrolimus CL/F was similar to that of 4βOHC alone (r=0.18, p=0.18, Supplementary Figure 2). This is in agreement with other studies that have indicated only minor differences in the performance of the two metrics [12,28]. Thus, it is not expected that the use of the 4βOHC:cholesterol ratio in this study would lead to different conclusions.

A limitation of our study was that tacrolimus plasma clearance was predicted from whole blood concentrations, hematocrit and literature average values of the erythrocyte binding parameters, thus introducing uncertainty to the plasma parameter estimates. Tacrolimus plasma concentrations, however, are not routinely measured, making this limitation challenging to overcome. Another limitation was that >95% of the tacrolimus data were trough concentrations, thus we could not distinguish between variability in clearance and bioavailability. Furthermore, the individual pharmacokinetic parameters are estimated closer to the population means in case of uninformative sampling such as the use of mostly trough concentrations [42], and the true distribution of tacrolimus CL/F is therefore expected to be somewhat wider than depicted in the figures. This limits the inferences that can be made from our exploratory analyses based on individual parameter estimates [42]. However, it should not influence the results of the population pharmacokinetic analyses or the conclusions of the study. Notably, none of the participants used drugs known to inhibit or induce CYP3A, and we could therefore not evaluate whether 4βOHC may help to predict the impact of interacting drugs on tacrolimus dose requirement. The main strength of our study is the availability of data from the pre-transplant and early
post-transplant period, which is the most relevant time period for the potential clinical use of tacrolimus dose prediction models.

In conclusion, we have evaluated the ability of the CYP3A activity marker 4βOHC to predict tacrolimus pharmacokinetics early after kidney transplantation. Our results indicate that 4βOHC has no or limited clinical value as a biomarker for individualized tacrolimus dosing early after transplantation.

ACKNOWLEDGMENTS

The authors wish to thank the medical laboratory technologists, transplant physicians, nurses and patients at Oslo University Hospital for excellent collaboration during the conduction of this study. The study was supported by a grant from the South-Eastern Norway Regional Health Authority.

COMPETING INTERESTS

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no competing interests.

AUTHOR CONTRIBUTIONS

ES contributed to acquisition of data, analysed and interpreted data, participated in study design and drafted the manuscript.

KH, KM, EM and AÅ contributed to acquisition of data, participated in study design and revised the manuscript.

SB performed pharmacological analyses and revised the manuscript.

All authors approved the final version to be published.

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27. Lütjohann D, Marinova M, Schneider B, Oldenburg J, Bergmann von K,


### TABLE 1. Demographic and clinical characteristics of the participants

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Values are shown as numbers or median (range) unless stated otherwise.

CYP3A5, Cytochrome P450 3A5; CYP3A4, Cytochrome P450 3A4;
**FIGURE LEGENDS**

1. **Figure 1.** Pre-transplant 4β-hydroxycholesterol vs. tacrolimus estimated individual apparent plasma clearance (CL/F_{plasma}) one week after kidney transplantation, stratified by cytochrome P450 3A5 (CYP3A5) genotype.

2. **Figure 2.** 4β-hydroxycholesterol vs. tacrolimus estimated individual apparent plasma clearance (CL/F_{plasma}) A) one week, B) four weeks and C) two months after kidney transplantation. The figures are stratified by cytochrome P450 3A5 (CYP3A5) genotype.

3. **Figure 3.** Prediction-corrected visual predictive check of the model’s description of the present data. *Red solid line* median observed concentration; *red dashed lines* 5\(^{th}\) and 95\(^{th}\) percentiles of the observed concentrations; *black solid line* median predicted concentration; *black dashed lines* 5\(^{th}\) and 95\(^{th}\) percentiles of the predicted concentrations. The red- and blue-shaded areas represent 95% confidence intervals of the prediction percentiles.

4. **Supplementary Figure 1.** Individual percentage changes in tacrolimus apparent clearance (red) and 4β-hydroxycholesterol (black) from baseline over time after transplantation.

5. **Supplementary Figure 2.** Left: 4β-hydroxycholesterol:cholesterol ratio vs. tacrolimus estimated individual apparent plasma clearance (CL/F_{plasma}), and right: 4β-hydroxycholesterol vs. 4β-hydroxycholesterol:cholesterol ratio; both two months after kidney transplantation, stratified by cytochrome P450 3A5 (CYP3A5) genotype.