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ORIGINAL ARTICLE

The impact of tacrolimus exposure on extrarenal adverse effects in adult renal transplant recipients

Correspondence Kathleen M. Tornatore, PharmD, FAST, FCCP; 219 Kapoor Hall, School of Pharmacy and Pharmaceutical Sciences, Buffalo, NY 14203, USA. Tel.: +1 716 645 2828; Fax: +1 716 645 2886; E-mail: tornator@buffalo.edu

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Olivia Campagne[1,2], Donald E. Mager[1], Daniel Brazeau[3], Rocco C. Venuto[4] and Kathleen M. Tornatore[4,5]

[1]Department of Pharmaceutical Sciences, University at Buffalo, SUNY, Buffalo, NY, USA, [2]Faculty of Pharmacy, Universités Paris Descartes-Paris Diderot, Paris, France, [3]Department of Pharmaceutical Sciences, College of Pharmacy, University of New England, Portland, ME, USA, [4]Erie County Medical Center, Division of Nephrology; Department of Medicine: Nephrology Division; School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY, USA, and [5]Department of Pharmacy Practice, School of Pharmacy and Pharmaceutical Sciences, Immunosuppressive Pharmacology Research Program, University at Buffalo, Buffalo, NY, USA

Keywords adverse effects, area under the curve, AUC, limited sampling, tacrolimus; pharmacokinetics

AIMS

Tacrolimus has been associated with notable extrarenal adverse effects (AEs), which are unpredictable and impact patient morbidity. The association between model-predicted tacrolimus exposure metrics and standardized extrarenal AEs in stable renal transplant recipients was investigated and a limited sampling strategy (LSS) was developed to predict steady-state tacrolimus area under the curve over a 12-h dosing period ($AUC_{ss,0–12h}$).

METHODS

All recipients receiving tacrolimus and mycophenolic acid ≥6 months completed a 12-h cross-sectional observational pharmacokinetic–pharmacodynamic study. Patients were evaluated for the presence of individual and composite gastrointestinal, neurological, and aesthetic AEs during the study visit. The associations between AEs and tacrolimus exposure metrics generated from a published population pharmacokinetic model were investigated using a logistic regression analysis in NONMEM 7.3. An LSS was determined using a Bayesian estimation method with the same patients.

RESULTS

Dose-normalized tacrolimus $AUC_{ss,0–12h}$ and apparent clearance were independently associated with diarrhoea, dyspepsia, insomnia and neurological AE ratio. Dose-normalized tacrolimus maximum concentration was significantly correlated with skin changes and acne. No AE associations were found with trough concentrations. Using limited sampling at 0, 2h; 0, 1, 4h; and 0, 1, 2, 4h provided a precise and unbiased prediction of tacrolimus AUC (root mean squared prediction error < 10%), which was not well characterized using trough concentrations only (root mean squared prediction error >15%).

CONCLUSIONS

Several AEs (i.e. diarrhoea, dyspepsia, insomnia and neurological AE ratio) were associated with tacrolimus dose normalized $AUC_{ss,0–12h}$ and clearance. Skin changes and acne were associated with dose-normalized maximum concentrations. To facilitate clinical implementation, a LSS was developed to predict $AUC_{ss,0–12h}$ values using sparse patient data to efficiently assess projected immunosuppressive exposure and potentially minimize AE manifestations.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Adverse effects (AEs) from tacrolimus immunosuppression are common and significantly contribute to morbidity in renal transplant patients. In addition, minimal investigations have reported use of standardized extrarenal AE criteria in stable transplant recipients.
- The association between AEs and tacrolimus exposure is poorly understood in relation to trough blood concentrations, the most commonly measured exposure metric used for routine therapeutic drug monitoring.
- Innovative therapeutic drug monitoring strategies are needed to predict and prevent tacrolimus AEs in the clinical arena.

WHAT THIS STUDY ADDS

- Several extrarenal AEs were associated with dose-normalized tacrolimus area under the curve and maximum concentration as well as apparent clearance. These extrarenal AEs were not associated with tacrolimus trough concentrations.
- The developed limited sampling methods enabled accurate tacrolimus exposure predictions, represented by area under the curve, and may improve individualized therapeutic drug monitoring. Poor predictive performance was found when using trough concentrations only.

Introduction

Tacrolimus and mycophenolic acid have become the immunosuppressant regimen of choice to prevent allograft rejection in renal transplant recipients [1–3]. Tacrolimus has a narrow therapeutic range from 4 to 15 ng ml^{-1} [4]. This drug exhibits notable inter- and intraindividual variability in pharmacokinetics and clinical response [4–7]. Therapeutic drug monitoring of trough concentrations is the standard of care to help minimize individual treatment inefficacy, adverse effects, and allograft rejection [8]. Tacrolimus pharmacokinetic variability has been well characterized in numerous population-based pharmacokinetic analyses, which aim to describe interpatient variability as well as identify the covariate contribution [9]. However, there is lack of pharmacodynamic modelling endeavours directed at tacrolimus efficacy and adverse effects as clinical endpoints [9].

Tacrolimus treatment is associated with various adverse effects (AEs) including acute and chronic nephrotoxicities as well as extrarenal manifestations including neurotoxicities, hypertension, post-transplant diabetes mellitus, gastrointestinal (GI) manifestations, aesthetic alterations and hyperlipidaemia [10–12]. Whereas acute nephrotoxicity is a limiting factor in the clinical use of tacrolimus and may be associated with trough concentrations, extrarenal AEs must also be carefully monitored to prevent medication noncompliance and avoid long term consequences such as increased cardiovascular risks that impact long-term allograft survival [13–16]. Identifying pharmacokinetic factors contributing to AE manifestations is critical to guide tacrolimus dosing adjustment, improve treatment tolerance and reduce risk potential among these patients. A validated and standardized immunosuppressive extrarenal AE scoring system that includes physical findings, laboratory results and concomitant medications was aimed to minimize reporting bias of these manifestations [17, 18]. Previous associations between extrarenal AEs, sex and **ABCB1** haplotypes have been reported, but the relationship between tacrolimus pharmacokinetics and these validated adverse events has not been investigated [18]. Several studies reported that tacrolimus-related AEs were more frequent or severe at higher tacrolimus exposures [12, 19, 20]. By reducing the targeted trough concentration ranges as recommended with tacrolimus minimization post-transplant [14], the frequency of specific AE events, such as neurotoxicities, has improved [19]. A recent study reported increased donor specific antibody formation with allograft failure as tacrolimus troughs decrease below the target range. These findings create a therapeutic dilemma requiring verification of tacrolimus drug exposure as dose minimization protocols are prescribed [6, 21].

The area under the tacrolimus concentration–time curve (AUC) is considered an essential objective marker for drug exposure and predictive of pharmacological response [19]. However, therapeutic drug monitoring of tacrolimus trough concentrations at steady-state is the current clinical practice, and this surrogate marker is not consistently correlated with dose or AUC [5, 7]. The European Consensus recommends a tacrolimus AUC range targeted at 120 and 200 ng h ml^{-1} to achieve efficacy based upon prior studies using Bayesian analysis [19, 22]. As a result, 12-h intensive sampling over dosing intervals is the ideal means for accurately determining tacrolimus AUC [19]. However, collecting multiple time-points over a dosing interval is inconvenient for patients, costly, and time-consuming for clinicians. To overcome these issues, limited sampling strategies (LSS) have been developed to predict tacrolimus AUC using the minimal number of concentrations at sampling times in the early periods after drug administration [23]. These analyses can be conducted using a *maximum a posteriori* (MAP) Bayesian approach, underpinned by a population pharmacokinetic model [24]. LSS may offer an efficient and accurate surrogate tool to quantify tacrolimus AUC in clinical practice rather than using a single trough concentration to monitor drug efficacy and adverse effects. Ideally, specific correlations between tacrolimus AUC and AE manifestations would support the use of this drug exposure metric. However, minimal investigations have been conducted with tacrolimus AUC and associated AEs post-transplant [9].

The first aim of this study is to investigate whether standardized extrarenal AEs are associated with tacrolimus exposure metrics, generated using a previously published population pharmacokinetic model [25], in stable renal transplant recipients. The second aim is to develop a LSS to enable therapeutic drug monitoring strategies in the clinic using model-predicted tacrolimus exposure metrics.

Methods

Ethics Statement

This study was approved by the University at Buffalo Health Sciences Institutional Review Board (IRB# PHP0599703–4). All patients provided informed consent with adherence to the Declaration of Helsinki data, which was reported according to the *Declaration of Istanbul on Organ Trafficking and Transplant Tourism*.

Study design

Sixty-seven stable African American and Caucasian renal transplant recipients, receiving oral tacrolimus (Prograf) and enteric-coated mycophenolate sodium (ECMPS; Myfortic) for ≥6 months, were included in a cross-sectional, open-label, 12-h clinical pharmacokinetic-pharmacodynamic study. The study was conducted at the UB Renal Research Unit at the Erie County Medical Center. Study design and methods have been previously described [18, 25]. Clinical stability was based on physical examination, comprehensive metabolic panel, fasting lipid panel and complete blood count at enrolment and on study morning. Tacrolimus doses were administered twice daily and adjusted to 4–9 ng ml^{-1} trough concentrations using a minimization dosing protocol, time post-transplant and clinical response. ECMPS dose was adjusted based upon clinical response. Medication adherence was verified at enrolment and prior to the study by research personnel. The inclusion criteria were: (i) ≥ 6 months post-renal transplant; (ii) age 25–70 years; (iii) first or second time deceased-donor or living allograft recipient; (iv) stabilized on same dose of immunosuppressive drugs for ≥7 days prior to study; (v) serum creatinine ≤3.25 mg dl^{-1} with no change in serum creatinine >0.25 mg dl^{-1} during prior 2 visits; and (vi) leucocyte count ≥3000 cells μl^{-1} and haemoglobin ≥8.0 g dl^{-1}. Exclusion criteria were: (i) infection within 2 weeks; (ii) acute rejection within 2 weeks; (iii) concomitant drugs interfering with tacrolimus or MPA absorption; (iv) concomitant cytochrome P450 3A4/3A5 or P-gp inhibitors or inducers within 4 weeks; and (v) significant cardiovascular, GI, haematological, psychiatric, and neurological or oncological diseases that would limit participation. Patients were enrolled only if they had received the same dose of tacrolimus and ECMPS for ≥7 days prior to study. This was assumed to be sufficient to approach steady-state concentrations. Patients took immunosuppressives between 5:30 and 6:30 PM followed by a 12-h fast prior to study. At 6:00 AM of study day, patients were admitted, vital signs were documented and an intravenous angiocatheter inserted. Serial blood samples were collected at times 0 (predose) and 1, 2, 3, 4, 6, 8, 10 and 12 h after drug administration. Whole blood samples were aliquoted immediately and stored at –70°C until analysis.

Adverse effect assessment

Patients were evaluated during the pharmacokinetic study for the severity and/or frequency of extrarenal adverse effects using a validated rating system summarized in the Supplemental Data: Table S1 [17, 18]. The nephrologists assessed each patient for all AEs during the morning of the pharmacokinetic study as a change from pre-transplant status. Each AE was scored as 0 (absence), +1, +2 and + 3 for severity and frequency. Individual AEs were included to determine a composite system AE including: GI (vomiting, diarrhoea, dyspepsia and acid suppressive therapy); neurological (headache, tremor and insomnia); and aesthetic (acne, skin changes, hirsutism and gingival hyperplasia) [18]. Myopathy, post-transplant diabetes and fasting lipids (total cholesterol, high-density lipoprotein, low-density lipoprotein and triglycerides) were also evaluated. A ratio of the sum of individual AE scores divided by the maximum score was generated as the adverse effect ratio for interpatient comparisons [18]. A cumulative AE ratio represented the summation of GI, neurological and aesthetic AE ratios plus myopathy and post-transplant diabetes [18]. No AEs were re-evaluated or immunosuppressive dosage adjustments were made in these stable patients during this study.

Bioanalysis

Tacrolimus concentrations were measured using the *ARCHITECT* chemiluminescent microparticle immunoassay system (Abbott, Abbott Park, IL). The lower limit of detection was 0.5 ng ml^{-1} and intraday assay variability was <7%. The calibration standard curve ranged from 1 to 30 ng ml^{-1} and quality controls were 3.0, 12.0, 25 ng ml^{-1} (Bio-Rad, Hercules, CA, USA). The interday coefficient of variation for each quality control was <4% and intraday coefficient of variation was <5%.

Blood was also collected in Cell Preparation Tubes (CPT-BD Vacationer) with sodium citrate predose for separation of peripheral blood mononuclear cells according to processing protocol at 25°C. These samples were all viable and analysed in a genomics laboratory (University of New England's Genomics Analytical Core). The genotypes were determined using TaqMan allelic discrimination assays (Applied Biosystems, Foster City, CA, USA) with a *CFX96* Real-Time Polymerase Chain Reaction Detection System (Bio-Rad). Personnel de-identified to patient demographics assayed for *ABCB1*: *1236C > T (rs1128503), 2677G > T/A (rs2032582), 3435C > T (rs1045642)* and **CYP3A5***3 (rs776746), *CYP3A5*6 (rs10264272),* and *CYP3A5*7 (rs41303343)*. Allele frequencies were confirmed in Hardy–Weinberg equilibrium when adjusted for race. A metabolic composite *CYP3A5*3*6*7* was generated based upon the combined allelic status for each single-nucleotide polymorphism (SNP). Patients were classified as extensive, intermediate and poor *CYP3A5*3*6*7* metabolizers [25, 26].

Population pharmacokinetic model

A population pharmacokinetic analysis for tacrolimus post renal transplant has been published [25]. Briefly, a two-compartment model was developed with first-order absorption and elimination with a lag-time for the absorption process to describe tacrolimus concentration–time profiles using NONMEM 7.3.0 (ICON Development Solutions, Ellicott City, MD, USA). The volume of distribution was allometrically scaled to total body weight. The *CYP3A5*3*6*7* metabolic composite was a significant covariate for tacrolimus apparent clearance (CL/F). The mean CL/F was 19.7 l h^{-1} for poor, 28.6 l h^{-1} for intermediate and 44.3 l h^{-1} for extensive metabolizers. In this study, the

published population pharmacokinetic model [25] was used to estimate for each patient, the maximum tacrolimus concentrations (C_{MAX}) and tacrolimus AUC between 0 and 12 h, at steady-state ($AUC_{ss,0-12h}$), calculated based upon the individual study dose and CL/F as follows:

$$AUC_{ss,0-12hr} = \frac{Dose}{CL/F} \quad (1)$$

Exposure–response modelling

The AE events (composite ratio or individual AE) were treated as ordered categorical data, and the AE events were modelled using a logistic regression approach [27]. The analysis was conducted with NONMEM 7.3.0 using the first-order conditional estimation with interaction method to estimate the parameters. The logit transform of the probability of an adverse effect event occurring in an individual (p_i) was defined as:

$$Logit(p_i) = \ln\left(\frac{p_i}{1-p_i}\right) = \beta + \theta \cdot X_i \quad (2)$$

with β as an underlying baseline parameter, and X represents one or several covariates associated with a scale factor (θ). For binary adverse effects (absence or presence), the probabilities were calculated as follows: the probability of an adverse effect occurring equalled p_i (1), and the probability of an adverse effect not occurring was defined as $1 - p_i$ (1). For adverse effects with several possible outcomes and levels of severity (e.g. 0, absence; 1, low; 2, high), the logit was re-defined for ordered categorical variables:

$$g[P(Y_i \geq m] = \ln\left(\frac{p}{1-p}\right) = \sum_{k=1}^{m}\beta_k + \theta \cdot X_i \quad (3)$$

with Y_i as the observation from the i^{th} individual, m represents the number of severity grade categories, and β_k is the underlying baseline probability for each category. Cumulative probabilities of categories not greater or lower than a certain value were calculated as follows:

- Probability of an adverse effect grade 2 occurring: p_i (2)
- Probability of an adverse effect grade 1 occurring: p_i (1) – p_i (2)
- Probability of an adverse effect grade not occurring (grade 0): 1 – p_i (1)

The AE events were evaluated one time per patient, and between-subject variability could not be estimated. Individual drug exposure metrics obtained from the population pharmacokinetic model were investigated as potential continuous covariates, such as: tacrolimus exposure at steady-state including absolute and dose-normalized $AUC_{ss0-12h}$ and $AUC_{ss0-4\ h}$, C_{MAX} with trough concentrations, and CL/F. Dose-normalized AUC and C_{MAX} were derived from the ratio of the AUC_{0-12h} (ng h ml^{-1}) or C_{MAX} (ng ml^{-1}) and the tacrolimus dose (mg), and the units reported for these metrics were ng h ml^{-1} mg^{-1} and ng ml^{-1} mg^{-1}. Categorical patient variables that were investigated included: sex, race, *CYP3A5*3*6*7* and *ABCB1* polymorphisms. Covariates were tested by forward inclusion and were selected if a significant decrease of at least 3.84 (χ^2-test, $P < 0.05$) in the objective function from the base model without any covariate was demonstrated [28]. To visually assess the impact of covariates on the adverse effects, bar plots were produced. For continuous covariates such as tacrolimus exposure metrics, probabilities of each event were calculated for the 5^{th}, 50^{th} and 95^{th} percentiles of the variable observed in the population. For categorical covariates, probabilities of each event were calculated for each value of the variable. The precision of the parameter estimates was evaluated using a nonparametric bootstrap resampling method [29]. Two hundred replicates of the analysis dataset were generated by bootstrapping and run with the final model to obtain the median and 95% confidence interval of all parameter estimates, which were compared to those of the final model.

LSS

The LSS determined the optimal limited combination of sampling times to calculate an accurate tacrolimus $AUC_{ss0-12h}$ using a Bayesian forecasting approach [24]. The published population pharmacokinetic model [25] was developed originally from intensive serial samples and determined individual tacrolimus $AUC_{ss,0-12h}$ values, which were used as the reference AUC_{int}. In the absence of an external validation group, data from these patients were used for the LSS. New datasets were built with the same patient characteristics (dose, weight and genotype) and feedback concentrations were used for different combinations of sampling times. Final estimates of typical pharmacokinetic parameters and between patient variabilities from the population pharmacokinetic model were used as *prior* information and computed with the new datasets in NONMEM without the estimation step (MAXEVAL = 0 approach). With this approach, the model used the available concentration–time profiles at different sampling times with the fixed population pharmacokinetic parameters and their between-subject variabilities to obtain individualized pharmacokinetic parameter estimates or so-called MAP Bayesian estimates. The individual estimates were then used to calculate the projected $AUC_{ss,0-12h}$ or AUC_{lss} (Eq.(1)), which were compared to the reference AUC_{int} in terms of precision and bias [30]. For precision, the relative root mean squared error (RMSE) and the mean absolute prediction error (MAPE) were calculated:

$$RMSE\ (\%) = \sqrt{\frac{1}{N}\cdot\sum_{i=1}^{N}\left(\frac{AUC_{lss}-AUC_{int}}{AUC_{int}}\right)^2} \quad (4)$$

$$MAPE\ (\%) = \frac{1}{N}\cdot\sum_{i=1}^{N}\left(\left|\frac{AUC_{lss}-AUC_{int}}{AUC_{int}}\right|\cdot 100\right) \quad (5)$$

For bias, the mean prediction error (MPE) and the mean percentage prediction error (MPPE) were calculated:

$$MPE\left(hr\ ng\ ml^{-1}\right) = \frac{1}{N}\cdot\sum_{i=1}^{N}(AUC_{lss}-AUC_{int}) \quad (6)$$

$$MPPE\ (\%) = \frac{1}{N}\cdot\sum_{i=1}^{N}\left(\frac{AUC_{lss}-AUC_{int}}{AUC_{int}}\cdot 100\right) \quad (7)$$

The acceptable criteria for RMSE, MAPE and MPPE are <15% [31]. With a narrow therapeutic index drug such as

tacrolimus, stricter criteria for predictive performance have been reported for MAPE as below 10% and MPPE between –5 and 5% [23, 32]. The coefficient of determination (r^2) assessed the correlation between the projected AUC_{lss} and reference AUC_{int}. The percentage of AUC_{lss} estimated within 15% of the reference AUC_{int} was also reported. To visually evaluate the overall predictive performance, correlation plots, Bland–Altman plots, and scatter plots of the absolute mean prediction error *vs*. reference AUC_{int} were produced. Combinations of sampling times were selected based upon the intensive specimen schedule. The LSS was restricted to the first 6 h after drug administration with inclusion of one to four time points with troughs incorporated to reflect the required parameter for tacrolimus therapeutic drug monitoring.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [33], and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 [34, 35].

Results

Patient characteristics and extrarenal adverse effects

Sixty-seven patients participated in the 12-h clinical pharmacology study. Demographics, laboratory parameters, and SNPs are summarized in Table 1. The distribution of *CYP3A5*3*6*7* metabolic composite was correlated with race: 91% of Caucasians were poor metabolizers, and 89% of African Americans were intermediate or extensive metabolizers. Table 2 summarizes the frequency and percentage of the composite and individual AEs with fasting lipids using the validated criteria summarized in Table S1 [17, 18]. Approximately 76% of recipients exhibited low to high frequency and/or severity for composite GI or neurological AEs. Lipid profiles were generally well-controlled attributed to statin therapy [36].

Exposure–response modelling

Logistic regression identified tacrolimus dose-normalized $AUC_{ss0–12h}$ and dose-normalized C_{MAX} with apparent clearance (CL/F) to be significant predictors for adverse events. Dose-normalized $AUC_{ss0–12h}$ and C_{MAX} distributions across all patients and *CYP3A5* variants are represented in Figure 1. Diarrhoea, dyspepsia, insomnia and composite neurological ratio were associated with dose-normalized $AUC_{ss0–12h}$ depicted in Figures 2A-D. Acne and skin changes were related to dose-normalized C_{MAX} (Figures 2E, F). For diarrhoea and acne, the scores of 0, 1 and 2 were treated as ordered categorical data. Due to a low incidence of dyspepsia, insomnia and skin changes, each AE was treated as a binary category, which included absence (0) and presence (1, 2 or 3). For composite neurological ratio, data were pooled in three categories: 0 for absence, 1 for low ratio (0.14) and 2 for high ratios (0.28,

Table 1

Patient characteristics

Parameters	
Tacrolimus 12-h study dose (mg)	3.0 (1.0–8.0)
Tacrolimus trough (ng ml^{-1})	7.2 (3.3–14)
MPA daily dose (mg)	1260 (360–2160)
MPA trough (ng ml^{-1})	4.0 (0.3–10)
Race	
African-American, *n* (%)	35 (52.2%)
Caucasian, *n* (%)	32 (47.8%)
Sex	
Male, *n* (%)	38 (56.7%)
Female, *n* (%)	29 (43.3%)
Age (years)	46 (24–76)
Total body weight (kg)	86 (52–125)
Body mass index (kg m^{-2})	30.1 (18.7–44.4)
Albumin (g dl^{-1})	4.2 (3.4–4.7)
Triglycerides (mg dl^{-1})	117 (45–950)
Total cholesterol (mg dl^{-1})	154 (82–388)
HDL (mg dl^{-1})	45 (24–122)
LDL (mg dl^{-1})	73 (15–172)
eGFR (ml min^{-1} 1.73 m^{-2})	54 (22–98)
Serum creatinine (mg dl^{-1})	1.4 (0.8–3.1)
Haemoglobin (g dl^{-1})	12 (10–16)
Haematocrit (%)	37 (31–53)
Platelet (cells mm^{-3})	195 (93–428)
Leukocytes (cells mm^{-3})	4.9 (2.3–13)
ABCB1 1236C > T	29/25/13
CC/CT/TT, *n* (%)	(43%/37%/20%)
ABCB1 2677G > T/A	35/25/6
GG/GT/TT, *n* (%)	(53%/38%/9%)
ABCB1 3435C > T	30/28/9
CC/CT/TT, *n* (%)	(45%/42%/13%)
***CYP3A5*3*6*7* metabolic composite**	35/24/8
poor/intermediate/extensive *n* (%)	(52%/36%/12%)
Time post-transplant (years)	2.2 (0.6–14)
Diabetes presence, *n* (%)	25 (36)
Prednisone use, *n* (%)	14 (20.9)
Statin use, *n* (%)	23 (34.3)

Data represented as median (range) or frequency (percentage)
MPA, mycophenolic acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein;
eGFR estimated glomerular filtration rate
Allele frequencies were confirmed to be in Hardy–Weinberg equilibrium when adjusted for race

Table 2

Frequency/(%) of patients exhibiting severity scores for tacrolimus adverse effects [18]

Adverse effects ratio	Absence	Low	Moderate	High
Cumulative	5/7.5	18/26.9	36/53.7	8/11.9
Range of ratio score (0–0.34)	(0)	(0.03–0.09)	(0.125–0.22)	(0.25–0.34)
Gastro-intestinal	16/23.9	13/19.4	31/46.3	7/10.4
Range of ratio score (0–0.7)	(0)	(0.1–0.2)	(0.1–0.4)	(0.5–0.7)
Neurological	15/22.4	20/30	21/31.3	11/16.4
Range of ratio score (0–0.57)	(0)	(0.14)	(0.28)	(0.43–0.57)
Cosmetic	38/56.7	21/31.3	4/6.0	4/6.0
Range of ratio score (0–0.5)	(0)	(0.08)	(0.16)	(0.25–0.5)
Adverse effects	**0**	**1+**	**2+**	**3+**
Vomiting	60/89.6	7/10.4	0/0.0	NA
Diarrhoea	44/65.7	15/22.4	8/11.9	NA
Dyspepsia	25/37.3	3/4.5	34/50.7	5/7.5
Acid suppressive therapy	28/41.8	35/52.2	4/6.0	NA
Tremor	33/49.3	31/46.3	3/4.5	0/0.0
Headache	55/82.1	12/17.9	NA	NA
Insomnia	34/50.7	16/23.9	16/23.9	1/1.5
Acne[a]	56/83.6	9/13.4	2/3.0	0/0.0
Skin changes	54/80.6	11/16.4	1/1.5	1/1.5
Hirsutism	58/86.6	8/11.9	0/0.0	1/1.5
Gingival hyperplasia	62/92.5	5/7.5	0/0.0	NA
Myopathy	54/80.6	12/17.9	1/1.5	0/0.0
Post-transplant diabetes mellitus	64/95.5	3/4.5	NA	NA
Lipids	**Normal values**		**High values**[b]	
Total cholesterol (mg dl^{-1})	< 200	64/95.5	> 200	3/4.5
LDL (mg dl^{-1})	<100	52/77.6	>100	15/22.4
HDL (mg dl^{-1})	>60	16/23.9	<60	51/76.1
Triglycerides (mg dl^{-1})	<200	57/85.1	>200	10/14.9

NA, not applicable since no rating
HDL, high-density lipoprotein; LDL, low-density lipoprotein
[a]No patients exhibited a 4+ for acne
[b]Lipids were categorized based upon the values considered as potential risk: > 200 mg/dl from total cholesterol and triglycerides, > 100 mg dl^{-1} for LDL and < 60 mg dl^{-1} for HDL [36]
Permission granted for reproduction of Table 1 from Venuto R, Meaney C, Chang S, Leca N, Consiglio J, Wilding G, *et al*. Association of extrarenal adverse effects of posttransplant immunosuppression with sex and ABCB1 haplotypes. Medicine 2015; 94: e1315

0.42 or 0.57). All parameters were estimated with acceptable precision and summarized in the logit scale in Tables 3 and 4. The drug exposure metrics showed the most impact on the composite neurological ratio ($P < 0.005$) and the skin changes ($P < 0.02$). The greater the dose-normalized $AUC_{ss0–12h}$ or $C_{MAX,}$ the more frequent or severe diarrhoea, insomnia, composite neurological ratio, acne, and skin changes is predicted. Dyspepsia was inversely correlated with dose-normalized $AUC_{ss0–12h}$. The 5th, 50th and 95th percentiles for dose-normalized tacrolimus $AUC_{ss0–12h}$ were 19, 42 and 88 ng h ml^{-1} mg^{-1}, whereas dose-normalized C_{MAX} were 2.4, 6.4 and 12.8 ng ml^{-1} mg^{-1}, respectively. These percentiles were defined as low, median and high, and used with the exposure–response model parameter estimates to predict the occurrence of each AE (Figure 2A–F). For example, to compare AE in patients with a low dose-normalized $AUC_{ss0–12h,}$ the probability of exhibiting insomnia (score 1, 2 or 3) is 31.1% compared to 71.7% at high dose-normalized $AUC_{ss0–12h}$ (Figure 2C). This dose normalization enables comparison between different stable regimens. No significant trends were identified with individual and/or composite AEs or fasting lipids with tacrolimus trough concentrations (absolute or

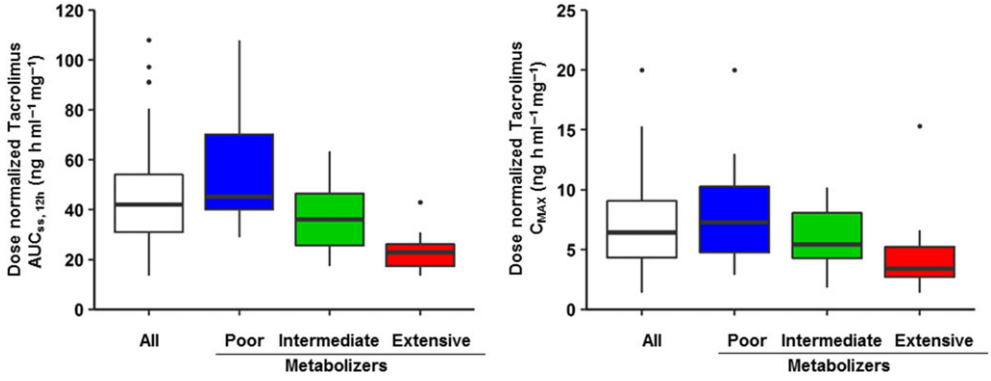

Figure 1

Dose-normalized tacrolimus exposure (left) and maximum concentration (right) in the study population. Tacrolimus exposure is represented as the dose normalized area under the concentration curve at steady-state between 0 and 12 h ($AUC_{ss0-12h}$), and on the right as the dose normalized maximum concentration (C_{MAX}). Both exposure metrics were obtained from the population pharmacokinetic model previously developed [25]. In each graph, the distribution of the exposure metrics is shown for all patients (white) and stratified by *CYP3A5*3*6*7* metabolic composite as poor (blue), intermediate (green) and extensive (red) metabolizers. Extensive metabolizers, who are African Americans only, exhibit less interpatient variability in dose normalized tacrolimus $AUC_{ss0-12h}$ and C_{MAX} with a reduction in tacrolimus exposure of approximately 50% compared to poor metabolizers who are primarily Caucasians

dose-normalized). Sex was found as a notable predictor for the composite AE ratios and fasting lipids (Supporting Information Table S2). Females exhibited more frequent and/or severe composite GI, neurological and aesthetic AE ratio, whereas males showed greater probabilities of exhibiting low high-density lipoprotein and high triglycerides. *CYP3A5* and *ABCB1* polymorphisms and demographics were not significant covariates on AEs.

LSS

Twenty-six different combinations of sampling times were evaluated, which included the maximum of four timed concentrations collected from 0 (trough) to 6 h after drug administration to generate tacrolimus $AUC_{ss,0-12h}$ (AUC_{lss}) using a Bayesian forecasting approach. The optimal predictive performance of these combinations using one, two, three or four timed concentrations from 0 to 4 h of the dosing interval is summarized in Table 5. The timed combinations provided low bias of <5%. The combination of four timed samples at 0, 1, 2 and 4 h exhibited the best predictive performance of RMSE 5.1% and MAPE 4.2% followed by the three timed sample combination at 0, 1 and 4 h. The coefficient of determination greater than 0.95 was achieved for both these timed concentration combinations (Table 5). For clinical feasibility in predicting AUC, two timed collections at 0 and 2 h provide good estimates with r^2 = 0.89. The tacrolimus reference AUC_{int} compared to AUC_{lss} predicted by LSS were well below the clinically accepted criteria [23, 32]. For comparison, the predictive performances for all combinations are summarized in Table S3. When troughs only were used for LSS, the projected AUC_{lss} values did not achieve acceptable precision (i.e. RMSE 19.5% and MAPE 20.4%) compared to reference AUC_{int} with a coefficient of determination of 0.61. The correlation, Bland–Altman and scatter plots of the absolute mean prediction error *vs*. reference AUC_{int} for selected timed concentration combinations are presented in Supporting Information Figures S1–S3.

Discussion

This is the first report linking tacrolimus exposure metrics (i.e. clearance and dose normalized AUC) to extrarenal adverse effects in renal transplant patients using an exposure–response logistic regression approach. This method can be used to analyse one or several occurrences of adverse effects, and a similar approach using total AUC has been frequently utilized for safety assessment of antineoplastic agents [27, 37, 38]. This method could also be used to account for past events when predicting future AE occurrences by including a transition (Markov) model [39–41]. The model and limited sampling analysis in the present study describes important clinical links using tacrolimus exposure metrics, such as dose-normalized $AUC_{ss0-12h}$ and C_{MAX}, as well as apparent CL/F, as predictors of standardized extrarenal AEs and may provide a method to minimize these manifestations. This predictive relationship of tacrolimus exposure metrics to adverse effects offers well-timed therapeutic alternatives to empirical dose minimization of this immunosuppressive that often results in sub-therapeutic trough concentrations and donor specific antibody formation compounding the renal allograft rejection process [21]. Once validated using a longitudinal assessment, this report will enable the prospective evaluation of dose-normalized AUC as a tool for minimizing adverse effects. Using dose-normalized AUC or apparent clearance to compare different dosing regimens may offer an improved clinical approach compared to the therapeutic drug monitoring of trough concentrations [19].

The regimen of tacrolimus and mycophenolic acid has been found to be associated with notable GI AEs [18, 42]. These GI AEs may reduce medication adherence, patient tolerability, and therapeutic drug exposure impacting allograft survival. This report utilized a tacrolimus population pharmacokinetic model to predict exposure metrics and establish standardized GI AEs relationships, which are poorly defined [19, 42]. Dose-normalized tacrolimus $AUC_{ss0-12h}$ and CL/F were associated with diarrhoea and dyspepsia. The more

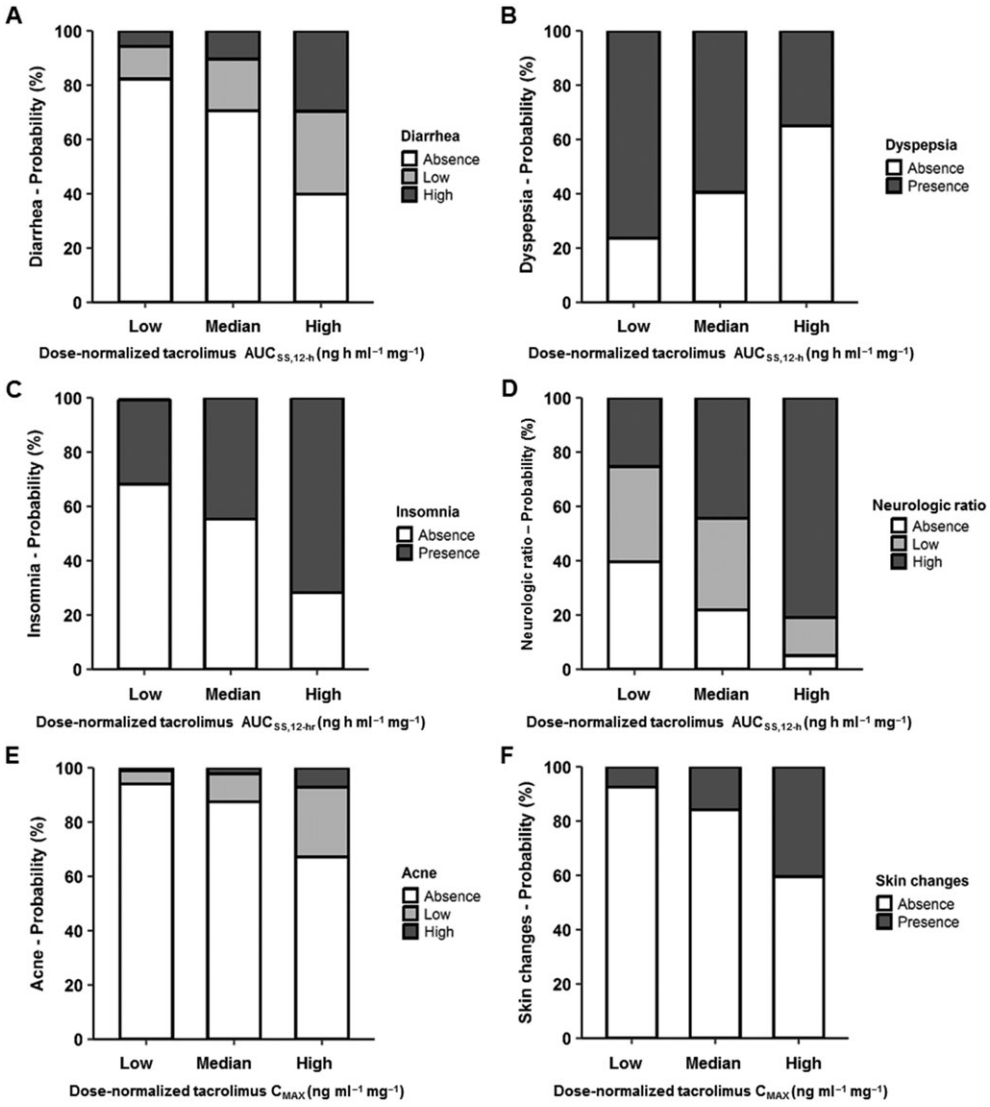

Figure 2

Probabilities of the occurrence of adverse effects after tacrolimus administration in renal transplant recipients according to dose-normalized tacrolimus exposure $AUC_{ss0-12h}$ and maximum concentration (C_{MAX}). Tacrolimus exposure is represented as the area under the concentration curve at steady-state between 0 and 12 h ($AUC_{ss0-12h}$; A–D). The 5th, 50th, and 95th percentiles of the distribution of dose-normalized tacrolimus exposure and maximum concentration obtained from the population pharmacokinetic model for all patients were used to calculate the probabilities of adverse effect occurrence. For dose-normalized tacrolimus $AUC_{ss0-12h}$, the 5th, 50th and 95th percentiles were 18 (low), 42 (median), and 88 (high) ng h ml^{-1} mg^{-1}. Note that at high dose-normalized $AUC_{ss0-12h}$, the severity and/or frequency of the AE including diarrhoea, insomnia and neurological ratio are greater. For dose-normalized tacrolimus C_{MAX}, the 5th, 50th, and 95th percentiles were 2.4 (Low), 6.4 (Median) and 12.8 (High) ng ml^{-1} mg^{-1}. The frequency or severity of AEs is greater at higher dose-normalized C_{MAX} (E, F)

frequent or severe diarrhoea was observed at higher dose-normalized $AUC_{ss0-12h}$. In clinical trials, diarrhoea was reported in 22–72% of recipients receiving tacrolimus with no objective AE quantification provided [42]. In contrast, the more frequent or severe dyspepsia was observed at the lower dose-normalized $AUC_{ss0-12h}$ with an unclear mechanism. However, the combined inter-relationship of the pharmacokinetics of MPA, MPA glucuronide and tacrolimus contribute to the overall GI AE observed in these patients [43, 44]. Due to the limited patient number, joint contributions of both immunosuppressive drugs were not evaluated.

Dose-normalized tacrolimus $AUC_{ss0-12h}$ and CL/F were positively associated with insomnia and the composite neurological AE ratio consisting of tremor, headache and insomnia [17, 18]. Insomnia was reported in 24–64% in major clinical trials [42]. Our analysis revealed that more frequent or severe insomnia was observed at the higher dose-normalized $AUC_{ss0-12h}$. Previous reports of subjective associations of headaches and tremor with tacrolimus dose conflict with the absence of these AEs [45, 46]. The pathophysiology of tacrolimus-induced neurotoxicity could be attributed to notable drug accumulation in the central nervous system due to high lipophilicity [47, 48].

Table 3

Summary of association of tacrolimus exposure metrics with extrarenal adverse effects: model parameter estimates

Adverse effect: categories			PK predictor	Parameter estimates (95% CI)		OR	*P*-value[†]
Gastrointestinal							
Diarrhoea	0	Absence	Dose-normalized $AUC_{ss0-12h}$ (ng h ml^{-1} mg^{-1})	$\beta_1 = -2$	(–2.97, –0.86)	1.03	<0.05
	1	Low		$\alpha_2 = -1.28$	(–1.92, –0.68)		
	2	High		$\theta_{AUCN} = 0.027$	(0.003, 0.05)		
Dyspepsia	0	Absence	Dose-normalized $AUC_{ss0-12h}$ (ng h ml^{-1} mg^{-1})	$\beta_1 = 1.73$	(0.69, 2.77)	0.96	< 0.05
	1/2/3	Presence		$\theta_{AUCN} = -0.032$	(–0.063, –0.005)		
Neurological							
Insomnia	0	Absence	Dose-normalized $AUC_{ss0-12h}$ (ng h ml^{-1} mg^{-1})	$\beta_1 = -1.21$	(–1.92, –0.52)	1.02	< 0.05
	1/2/3	Presence		$\theta_{AUCN} = 0.024$	(0.004, 0.04)		
Neurological ratios	0	Absence	Dose-normalized $AUC_{ss0-12h}$ (ng h·ml^{-1} mg^{-1})	$\beta_1 = -0.18$	(–0.97, –0.04)	1.03	< 0.005
	0.14	Low		$\alpha_2 = -1.5$	(–1.96, –0.97)		
	>0.28	High		$\theta_{AUCN} = 0.027$	(0.02, 0.05)		
Cosmetic							
Skin changes	0	Absence	Dose-normalized C_{MAX} (ng ml^{-1} mg^{-1})	$\beta_1 = -3$	(–3.94, –1.76)	1.23	< 0.02
	1/2/3	Presence		$\theta_{CmaxN} = 0.208$	(0.02, 0.32)		
Acne	0	Absence	Dose-normalized C_{MAX} (ng ml^{-1} mg^{-1})	$\beta_1 = -3.24$	(–3.92, –1.78)	1.22	< 0.05
	1	Low		$\alpha_2 = -1.86$	(–2.93, –1.15)		
	2	High		$\theta_{CmaxN} = 0.201$	(0.03, 0.3)		

PK, pharmacokinetic; $AUC_{ss0-12h}$, tacrolimus area under the concentration curve at steady-state between 0 and 12 h; CL/F, tacrolimus apparent oral clearance; θ_{CL}, slope for tacrolimus CL/F effect; OR, odds ratio

θ_{AUCN} slope for AUCss0-12 h dose-normalized effect; C_{MAX} maximum tacrolimus concentration;

θ_{CmaxN} slope for dose-normalized C_{MAX} effect

The probability p_i of an adverse effect event is defined as follows: $p_i = \frac{e^{\beta+\theta \cdot X_i}}{1+e^{\beta+\theta \cdot X_i}}$

For binary adverse effect events (dyspepsia, skin changes, insomnia), β_1 represents the underlying baseline for p(1), the probability of an adverse effect occurring. The probability of an adverse effect not occurring is defined as 1 – p(1)

For ordered categorical variables (diarrhoea, acne, neurological ratios), β_1 represents the underlying baseline for p(1), the sum of β_1 and α_2 represents the underlying baseline p(2). The probability of an adverse effect grade 2 occurring is p(2). The probability of an adverse effect grade 1 occurring is p(1) – p(2). The probability of an adverse effect not occurring (grade 0) is 1 – p(1)

Neurological adverse effect ratio represents a composite of individual rated adverse effects: tremors, headaches, insomnia [17, 18]

The odds of interest increase by $e^{\theta AUCN}$ or $e^{\theta CmaxN}$ fold for every one unit increase in dose-normalized AUC or maximum concentration. [†]The *P*-value indicates the statistical significance of the PK predictor on the adverse effect model developed in NONMEM. The PK predictor was considered as significant if it showed a decrease of at least 3.84 points (χ^2-test, $P < 0.05$) in the objective function value from the initial logistic model

P-glycoprotein may also play an important role in modulating tacrolimus metabolism and cellular distribution at the blood brain barrier [49, 50]. Variations in *ABCB1* genotypes, which encodes for this transporter, can result in loss of function of P-glycoprotein and could lead to drug-brain accumulation. However, no clinically meaningful associations between these genotypes and tacrolimus-induced neurotoxicity have been identified [47, 48].

Interestingly, dose-normalized tacrolimus C_{MAX} was associated with two aesthetic AEs of acne and skin changes. More frequent or severe aesthetic AEs were reported at higher tacrolimus C_{MAX}, but remained globally rare since <20% of patients exhibited either manifestation. These side effects are generally less reported adverse reactions [42], with reduced frequency in tacrolimus regimens compared to other immunosuppressive drugs [51].

The prior population pharmacokinetic model highlighted the significant impact of the metabolic *CYP3A5*3*6*7* composite on tacrolimus CL/F [25]. As shown in Figure 1, subsequent distributions in the dose-normalized $AUC_{ss0-12h}$ and C_{MAX} stratified by *CYP3A5* genotype were observed. This suggests that patients with poor, intermediate and extensive metabolism would have different probabilities in the occurrence of extrarenal AEs. However, the *CYP3A5*3*6*7* genotypes were not identified as a significant predictor for AEs in our analysis. Although genotype explains a significant amount of the variability in CL/F, it is not the only factor contributing to this variability. Therefore, the entire population model may provide a better tool for identifying individual CL/F values than using genotypes alone. Another investigation explored the impact of *CYP3A5*3* genotypes only on tacrolimus renal and extrarenal AEs across time using a Markov chain model and concluded that the probability of an adverse event occurrence in the first 3 months in the *CYP3A5* nonexpressers is 2-fold greater compared to the *CYP3A5* expressers [52]. Another important genomic contribution to AE manifestations are the *ABCB1* SNPs, *1236C > T* (rs1128503), *2677G > T/A* (rs2032582), and *3435C > T* (rs1045642) variants that encode for P-glycoprotein and modulates cellular distribution of tacrolimus. This model found no association of these individual *ABCB1* genotypes as an AE predictor. This finding conflicts

Table 4

Tacrolimus apparent clearance CL/F association with extrarenal adverse effects: model parameter estimates

Adverse effect: categories			PK predictor	Parameter estimates (95% CI)		OR	*P*-value[a]
Gastrointestinal							
Diarrhea	0	Absence	Tacrolimus CL/F (l h^{-1})	β_1 = 0.745	(0.019, 0.99)	0.94	<0.02
				α_2 = 1.29	(–2.57, –0.74)		
	1	Low		θ_{CL} = –0.059	(–0.093, –0.018)		
	2	High					
Dyspepsia	0	Absence	Tacrolimus CL/F (l h^{-1})	β_1 = 0.208	(0.020, 0.69)	1.03	<0.05
	1/2/3	Presence		θ_{CL} = 0.027	(0.0008, 0.065)		
Neurological							
Insomnia	0	Absence	Tacrolimus CL/F (l h^{-1})	β_1 = –0.947	(–1.84, –0.18)	0.95	<0.05
	1/2/3	Presence		θ_{CL} = –0.053	(–0.10, –0.019)		
Neurological ratios	0	Absence	Tacrolimus CL/F (l h^{-1})	β_1 = 0.391	(0.109, 1.53)	2.74	<0.05
	>0.14	Presence		θ_{CL} = 1.01	(0.25, 1.91)		

PK, pharmacokinetic; CL/F, tacrolimus apparent oral clearance; θ_{CL}, slope for tacrolimus CL/F effect; OR, odds ratio
The probability p_i of an adverse effect event is defined as follows: $p_i = \frac{e^{\beta+\theta \cdot X_i}}{1+e^{\beta+\theta \cdot X_i}}$
For binary adverse effect events (dyspepsia, insomnia, neurological ratios), β_1 represents the underlying baseline for p(1), the probability of an adverse effect occurring. The probability of an adverse effect not occurring is defined as 1 – p(1)
For ordered categorical variables (diarrhoea), β_1 represents the underlying baseline for p(1), the sum of β_1 and α_2 represents the underlying baseline p(2). The probability of an adverse effect grade 2 occurring is p(2). The probability of an adverse effect grade 1 occurring is p(1) – p(2). The probability of an adverse effect not occurring (grade 0) is 1 – p(1)
Neurological adverse effect ratio represents a composite of individual rated adverse effects: tremors, headaches, insomnia [17, 18]
[a]The *P*-value indicated the statistical significance of the exposure metric on the adverse effect model developed in NONMEM. The exposure metric was considered as significant if it showed a decrease of at least 3.84 points (χ^2-test, $P < 0.05$) in the objective function value from the initial logistic model
The odds of interest increases by $e^{\theta CL}$ fold for every one unit increase in tacrolimus CL/F

Table 5

Predictive performance of most accurate selected limited sampling strategies predicting tacrolimus exposure in adult kidney transplant recipients

Sampling times	Precision		Bias		% AUC_{lss} within 15% AUC_{int}	r^2
	RMSE (%)	MAPE (%)	MPE (ng h ml^{-1})	MPPE (%)		
0 h	19.5	20.4	–0.9	0.5	59.7	0.61
0, 2 h	9.2	7.3	–3.2	–2.4	92.5	0.89
0, 1, 4 h	6.0	5.2	–2.3	–1.5	100.0	0.95
0, 1, 2, 4 h	5.1	4.2	–2.5	–1.8	100.0	0.96

RMSE, relative root mean squared prediction error; MAPE, mean absolute percentage prediction error; MPE, mean prediction error; MPPE, mean percentage prediction error; AUC_{lss}, tacrolimus area under the concentration curve predicted by Bayesian forecasting; AUC_{int}, tacrolimus area under the concentration curve calculated from the final pharmacokinetic model with intensive serial sampling; r^2, coefficient of determination between AUC_{lss} and AUC_{int}

with our previous report of notable associations between extrarenal AEs and *ABCB1* haplotypes (i.e. *TTT*) determined by *Theasis* computation [18]. Future models should include *ABCB1* haplotypes with *CYP3A5*3*6*7* genotypes that may provide more comprehensive mechanistic insights into the inter-relationship between CYP3A5 enzymes and P-glycoprotein in transplant subpopulations that include sex and race. [47, 48, 53].

To apply these results in clinical practice, tacrolimus $AUC_{ss0–12h}$ must be generated for each individual. An LSS was developed using the population pharmacokinetic model and a Bayesian forecasting method to predict tacrolimus $AUC_{ss0–12h}$, and different combinations of minimum timed samples were evaluated. Using one to four samples including troughs within the first 6 h after drug administration, the most accurate and convenient LSS strategies selected were within the first 4 h after tacrolimus administration and included 0–2 h, 0–1-4 h and 0–1–2-4 h, which provided good precision and low bias. This outcome was expected since the inclusion of more timed concentrations provided greater reliance on the measured timed concentrations than on the population model predictive power [22, 31]. Our results showed no major differences between the selected strategies using three or four timed concentrations with RMSE<6% and

MAPE<5%, compared to the limited strategy including only two timed concentrations (Table 5). Based on these results, the LSS with three time-points at 0, 1 and 4 h might be considered an optimal estimate for use in clinical practice to estimate an accurate and reliable tacrolimus $AUC_{ss0–12h}$. In addition, for clinical feasibility and cost, the use of two timed specimens at trough and 2 h postdrug administration provides a reasonable $AUC_{ss0–12h}$.

Our LSS is consistent with a previous review of different tacrolimus analyses conducted during early and late post-transplant periods using a Bayesian estimator [9]. The majority of the studies concluded that a combination of a trough concentration with at least one timed concentration 2 h postdose is required to predict an accurate and reliable tacrolimus $AUC_{ss0–12h}$ using RMSE and MAPE [31]. The selected LSS across these studies were 0–1-3 h, 0–1-4 h, 0–2-3 h and 0–2-4 h [22, 54–57]. Instead of testing all possible combinations of timed concentrations collected during the 12-h intensive sampling schedule, several studies were based on the D-optimality criterion for selection of the optimal sampling design [58]. This approach resulted in the following LSS including: 0.3–2-4 h, 1–3-6 h, 1–3-8 h, 0–1.5-4 h and 0–1-3 h and is consistent with the other analyses [59–62]. The inclusion of at least one timed concentration after 2 h post-dose is in concordance with the absorption process of tacrolimus, in which C_{MAX} is generally achieved within the first 3 h post-dose [3, 8, 12]. Numerous tacrolimus LSS were developed using a multiple linear regression method [31]. Although this approach is easily applied, it does not allow for any flexibility. Unlike with the Bayesian forecasting approach, exact sampling times must be collected to assure the predictive performance of the equation developed, which may not be feasible in clinical practice [63]. In our study, using only a trough concentration to predict tacrolimus AUC resulted in low precision with RMSE = 19.5% and MAPE = 20.4%, compared to the acceptable criteria of RMSE<15% and MAPE<10%. A low coefficient of correlation (r^2 = 0.61) in spite of low bias (0.5%) also resulted. These results are consistent with previous studies that reported unacceptable precision and bias and poor correlation between tacrolimus troughs and AUC (range r^2: 0.27–0.79), which is concerning with a narrow therapeutic range drug [22, 31, 57, 64–66]. The interpatient variability in the relationship between tacrolimus trough concentration and AUC has been described and remains controversial [19]. Therapeutic drug monitoring based on trough measurements is justified in the clinical setting due to patient ease and is economical but does not consistently predict an accurate AUC. As a result, the LSS represents an easy and clinically applicable approach to predict a reliable estimate of drug exposure to elicit the desired pharmacological response for tacrolimus. It is important to emphasize that a qualified population pharmacokinetic model is a prior requirement to use this method [67].

A few limitations do exist with this report. One limitation is the cross-sectional study design that incorporates simultaneous evaluation of drug exposure and adverse effects in stable recipients. No longitudinal follow-up studies were conducted to serve as comparators for adverse effects manifested as tacrolimus dosing regimens were adjusted. Ideally, the LSS should be validated using an external patient group separate from the cohort used to build the pharmacokinetic model [67]. In our analysis, due to a limited number of stable patients included, no randomization into an index and validation group was possible. This limitation may explain the consistent low bias observed in the different combinations of timed concentrations evaluated. However, employing this configuration, our analysis showed a poor prediction when using troughs only to project tacrolimus exposure accurately. This finding may raise concerns with relying only on monitoring trough concentrations. The drug exposure associations to chronic AE in stable patients are preliminary findings and require confirmation with repeated studies. Another study limitation that may be addressed more comprehensively in a larger patient population are the associations of *CYP3A5*3*6*7* metabolic composite and *ABCB1* haplotypes to tacrolimus exposure and AE manifestations.

Conclusions

An exposure–response logistic regression approach was used to identify the impact of dose-normalized tacrolimus $AUC_{ss0–12h}$ and C_{MAX} or with CL/F for several extrarenal AEs such as diarrhoea, dyspepsia, insomnia, acne, skin changes, and the composite neurological ratio. A Bayesian estimator based on a population pharmacokinetic model and LSS was developed to provide an efficient tool for clinicians to predict tacrolimus exposure with accuracy and maintain patient convenience to facilitate individualized monitoring and minimize adverse effects. Our study identified a notable correlation of tacrolimus dose-normalized $AUC_{ss0–12h}$ and CL/F with extrarenal AEs. In addition, the LSS analysis confirmed precise estimation of AUC using multiple timed concentrations collected from 0 to 4 h, which was an improvement over the use of trough concentrations. Future research may be aimed at confirming these results in larger study cohorts with collection of serial AEs.

Competing Interests

There are no competing interests to declare.

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Supporting Information

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Table S1 Immunosuppressive adverse effects scoring system [18]

Table S2 Sex association with composite adverse effect ratio: model parameter estimates

Table S3 Predictive performance of all tested limited sampling strategies predicting tacrolimus exposure in adult kidney transplant recipients

Figure S1 Correlation plots A–D between the reference AUC_{int} and the projected AUC_{lss} for the selected limited sampling strategies. AUC_{int} is the tacrolimus area under the curve between 0 and 12 h estimated with the full intensive sampling strategy. AUC_{lss} is the projected area under the curve obtained from the different combinations of sampling times using a Bayesian forecasting approach. Continuous lines represent the linear regression lines. Dashed lines represent the 95% confidence interval for each linear regression line. The limited sampling strategies including three and four time-point (panels C and D) exhibited the best correlation between reference AUC_{int} and projected AUC_{lss} with r^2 of 0.947 and 0.961, respectively. Patients were categorized as poor (blue dots), intermediate (green dots) or extensive (red dots) metabolizer based on a *CYP3A5*3*6*7* metabolic composite

Figure S2 Scatter plots A–D of the absolute percentage prediction error *vs*. the reference AUC_{int} for the selected limited sampling strategies. The AUC_{int} represents the tacrolimus area under the curve between 0 and 12 h estimated using the full intensive sampling strategy. The absolute percentage prediction error was calculated between AUC_{int} and AUC_{lss}, which is the projected area under the curve obtained from the different combinations of sampling times using a Bayesian forecasting approach, as following: $\frac{|AUC_{int}-AUC_{lss}|}{AUC_{int}} \cdot 100$ Accuracy within 15%, which is the clinical acceptable level criteria, is represented by the dashed line for each combination of sampling times. 100% of the projected AUC_{lss} obtained with the limited sampling strategies including three time-points (C, D) fell within 15% of reference AUC_{int}, as opposed to 59.7% for the limited sampling strategy including tacrolimus trough concentration (A). Patients were categorized as poor (blue dots), intermediate (green dots), or extensive (red dots) metabolizers based on a *CYP3A5*3*6*7* metabolic composite

Figure S3 Bland–Altman graphs (A–D) comparing the difference between individual AUC_{int} and AUC_{lss} (y-axis) and the average of individual AUC_{int} and AUC_{lss} (x-axis) for the selected limited sampling strategies. Dashed lines represent the 5^{th}, 50^{th} and 95^{th} percentiles. Reference AUC_{int} is the tacrolimus area under the curve between 0 and 12 h estimated using the 12-h intensive sampling strategy. AUC_{lss} is the projected area under the curve obtained from the different combinations of sampling times using a Bayesian forecasting approach. The limited sampling strategies including three or four timed concentrations (C, D) showed the best precision with the narrowest agreement interval between the 5^{th} and 95^{th} percentiles. No systematic bias was depicted in the four graphs with no consistent pattern to the direction of bias, with AUC_{lss} both under- and overestimating AUC_{int}. Patients were categorized as poor, intermediate or extensive metabolizers based on a *CYP3A5*3*6*7* metabolic composite, and are represented by blue, green and red dots, respectively