

# Evaluation of limited sampling methods for estimation of tacrolimus exposure in adult kidney transplant recipients

Katherine A. Barraclough,<sup>1</sup> Nicole M. Isbel,<sup>1</sup> Carl M. Kirkpatrick,<sup>2</sup> Katie J. Lee,<sup>2</sup> Paul J. Taylor,<sup>3</sup> David W. Johnson,<sup>1</sup> Scott B. Campbell,<sup>1</sup> Diana R. Leary<sup>1</sup> & Christine E. Staatz<sup>2</sup>

<sup>1</sup>Department of Nephrology, University of Queensland at the Princess Alexandra Hospital, Brisbane, QLD 4102, Australia, <sup>2</sup>School of Pharmacy, University of Queensland, St Lucia, Brisbane, QLD 4072, Australia and <sup>3</sup>Department of Clinical Pharmacology, Princess Alexandra Hospital, Brisbane, QLD 4102, Australia

## WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Tacrolimus pre-dose ( $C_0$ ) concentrations are currently used to guide tacrolimus dosing.
- However, conflicting data exist regarding the relationship of  $C_0$  with tacrolimus area under the concentration–time curve from 0 to 12 h post-dose ( $AUC_{0-12}$ ) and clinical outcomes.
- Previous literature suggests that limited sampling methods, such as multiple linear regression-derived limited sampling strategies or maximum *a posteriori* (MAP) Bayesian analyses, may provide more reliable estimations of tacrolimus exposure.

## WHAT THIS STUDY ADDS

- For the first time, the predictive performances of all published limited sampling methods for tacrolimus are compared in an independent cohort of adult kidney transplant recipients.
- Limited sampling methods better predict tacrolimus exposure compared with measurement of  $C_0$ .
- However, the predictive power of the methods is highly variable, highlighting the importance of validating any method prior to applying it to an alternative population.

## Correspondence

Dr Katherine Barraclough, Department of Nephrology, Level 2, ARTS Building, Princess Alexandra Hospital, Ipswich Road, Woolloongabba, Brisbane, QLD 4102, Australia.

Tel.: +61 7 3240 7488

Fax: +61 7 3240 5480

E-mail:

katherine\_barraclough@health.qld.gov.au

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## AIMS

To examine the predictive performance of limited sampling methods for estimation of tacrolimus exposure in adult kidney transplant recipients.

## METHODS

Twenty full tacrolimus area under the concentration–time curve from 0 to 12 h post-dose ( $AUC_{0-12}$ ) profiles (AUCf) were collected from 20 subjects. Predicted tacrolimus  $AUC_{0-12}$  (AUCp) was calculated using the following: (i) 42 multiple regression-derived limited sampling strategies (LSSs); (ii) five population pharmacokinetic (PK) models in the Bayesian forecasting program TCIWorks; and (iii) a Web-based consultancy service. Correlations ( $r^2$ ) between  $C_0$  and AUCf and between AUCp and AUCf were examined. Median percentage prediction error (MPPE) and median absolute percentage prediction error (MAPE) were calculated.

## RESULTS

Correlation between  $C_0$  and AUCf was 0.53. Using the 42 LSS equations, correlation between AUCp and AUCf ranged from 0.54 to 0.99. The MPPE and MAPE were <15% for 29 of 42 equations (62%), including five of eight equations based on sampling taken  $\leq 2$  h post-dose. Using the PK models in TCIWorks, AUCp derived from only  $C_0$  values showed poor correlation with AUCf ( $r^2 = 0.27-0.54$ ) and unacceptable imprecision (MAPE 17.5–31.6%). In most cases, correlation, bias and imprecision estimates progressively improved with inclusion of a greater number of concentration time points. When concentration measurements at 0, 1, 2 and 4 h post-dose were applied, correlation between AUCp and AUCf ranged from 0.75 to 0.93, and MPPE and MAPE were <15% for all models examined. Using the Web-based consultancy service, correlation between AUCp and AUCf was 0.74, and MPPE and MAPE were 6.6 and 9.6%, respectively.

## CONCLUSIONS

Limited sampling methods better predict tacrolimus exposure compared with  $C_0$  measurement. Several LSSs based on sampling taken 2 h or less post-dose predicted exposure with acceptable bias and imprecision. Generally, Bayesian forecasting methods required inclusion of a concentration measurement from >2 h post-dose to adequately predict exposure.

## Introduction

Tacrolimus is an immunosuppressive drug that is one of the cornerstones in the prevention of rejection following solid organ transplantation. In 2006, 82.4% of kidney transplant recipients reported to the US Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients were prescribed this agent at hospital discharge [1].

Tacrolimus has a narrow therapeutic index [2], making dosing difficult. Adequate exposure is imperative for the prevention of rejection, while overexposure risks serious toxicities that reduce tolerability and impact long-term allograft and patient survival [3, 4]. Tacrolimus also displays considerable between- and within-subject pharmacokinetic (PK) variability, with a poor correlation between dose and blood concentration [2]. Multiple factors have been identified as contributors to PK variability (Table 1) [3, 5–37]. As a consequence, therapeutic drug monitoring is mandatory [38].

While full dose interval area under the concentration–time curve ( $AUC_{0-12}$ ) is generally considered the best marker of drug exposure [38], the requirement for collection of multiple samples over a 12 h period makes this approach impractical for routine clinical use. Subsequently, largely for reasons of practicality and convenience, most transplant centres use pre-dose ( $C_0$ ) concentrations to guide tacrolimus dosing. However, evidence regarding the relationship of  $C_0$  with  $AUC_{0-12}$  is conflicting (correlation,  $r^2 = 0.04–0.91$ ) [39–50], and there are minimal prospective data relating  $C_0$  values to clinical outcomes [51]. A feasible alternative is the use of limited sampling methods, such as multiple linear regression or maximum *a posteriori* (MAP) Bayesian analyses [52]. These may offer a better means of estimating tacrolimus exposure, yielding greater accuracy

than  $C_0$  measurements, while being less cumbersome than full  $AUC_{0-12}$  measurements [52, 53]. The multiple linear regression method uses an equation derived from multiple linear regression analysis to estimate tacrolimus  $AUC_{0-12}$  from a limited number of concentrations measured at pre-defined times after dosing [52]. Such equations are relatively easy for the clinician to use and do not require specialist software. However, there is heavy reliance on exact sampling times, such that deviation of timing of sample collection may compromise equation predictive power.

Alternatively, Bayesian analysis uses information from a population PK model for tacrolimus. The model provides population PK parameter estimates (such as mean drug clearance and volume of distribution) and expected associated variability, and allows the opportunity to consider the influence of patient variables (covariates) on tacrolimus exposure. Tacrolimus  $AUC_{0-12}$  is determined from individualized PK parameter estimates by combining concentration measurements and data from that individual (such as patient weight or genotype) with available population data. The more individual data provided, the less the reliance on population data [52, 54]. Major advantages of this method include more flexible timing of blood sampling and improved prediction in patients with unusual pharmacokinetics. Disadvantages include reliance on the existence of an appropriate PK model, and a more complex calculation requiring specialist software and user training [38, 52].

It is likely that both multiple regression-derived limited sampling strategies (LSSs) and Bayesian analysis can only be applied with any accuracy to a population similar to the one in which they were developed, as defined by graft type, time post-transplant and analytical technique used for tacrolimus measurement. To ensure reliable predictions, it is essential that limited sampling methods are validated properly, ideally using a separate group of patients from the one in which the LSS equation or population PK model was derived [52].

This manuscript provides a review of all currently published limited sampling methods for tacrolimus in adult kidney transplant recipients. The predictive performances of each of these methods have been evaluated using an independent cohort of adult kidney transplant recipients. These results have then been used to identify the best method for our patient group, and to examine the general applicability (or otherwise) of these methods.

**Table 1**

Covariates contributing to tacrolimus pharmacokinetic variability

Covariates	Reference
Transplanted organ	[15, 19, 37]
Patient age	[26, 27]
Patient race	[28–30]
Hepatitis C status	[21, 22]
Diabetes status	[36]
Time from transplantation	[24, 25]
Diurnal rhythm	[24, 31]
Food administration	[32, 33]
Corticosteroid dosage	[12, 13, 24]
Co-medication use	[3, 24]
Diarrhoea	[34, 35]
Albumin concentration	[24]
Haematocrit	[24]
Liver dysfunction	[15–18, 20]
Cytochrome P450 isoenzyme and P-glycoprotein genotype and phenotype	[6–11, 14]

## Methods

### Patients

Adults who had undergone kidney transplant surgery at the Princess Alexandra Hospital (Brisbane, Queensland, Australia) were considered for inclusion in this study. Eligibility criteria included an immunosuppressive regimen of

twice daily tacrolimus (Prograf®; Janssen-Cilag, Dublin, Ireland), twice daily mycophenolate mofetil (MMF; Cellcept®; Roche Pharma, Milan, Italy) and once daily prednisolone (Panaftocortelone®; Aspen Pharmacare, St Leonards, New South Wales, Australia). A total of 20 tacrolimus PK profiles were collected from 20 kidney transplant recipients over the period April to June, 2009. The Princess Alexandra Hospital and University of Queensland Ethics Committees approved the study, and all participants provided written informed consent.

### *Blood sampling and analytical method*

Thirteen whole blood samples were collected over a 12 h dosing interval (pre-dose, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 9 and 12 h post-dose) from each subject. Samples were collected into Vacutainer® tubes containing ethylenediaminetetraacetic acid and stored at  $-20^{\circ}\text{C}$  until analysis. There was no restriction on food intake prior to or during blood sampling. Tacrolimus concentrations were determined using a validated high-performance liquid chromatography–tandem mass spectrometry method [55, 56]. This assay is specific for the parent drug tacrolimus, and is linear over the range of  $0.5\text{--}50\text{ ng ml}^{-1}$  ( $r^2 > 0.99$ ). The within-day and between-day imprecision is  $<8\%$ .

### *Limited sampling methods*

A literature search was performed using MEDLINE (1982 to current) and PubMed (1995 to current). Search terms included tacrolimus, therapeutic drug monitoring, limited sampling strategies, multiple linear regression, Bayesian forecasting, population pharmacokinetics, area under the concentration–time curve and kidney transplantation. Relevant primary research papers presenting limited sampling methods for tacrolimus derived in adult kidney transplant recipients were identified and evaluated. Articles were included if they were written in English.

Forty-two multiple linear regression-derived LSS equations were identified [42, 44, 46, 50, 57, 58]. These equations were entered into an Excel spreadsheet. Six population PK models of tacrolimus were identified [54, 59–63]. Covariate information included in the population models was collected from patient medical records. The models were entered into the Bayesian forecasting program TCIWorks, version 1 (The TCIWorks Development Team, Brisbane, Queensland, Australia) [64]. Each study subject was added as a new patient in the system with tacrolimus dosing history entered in chronological order. Additionally, a Web-based consulting service that uses Bayesian analysis to estimate tacrolimus  $\text{AUC}_{0-12}$  from concentration measurements made at 20 ( $\pm 10$ ), 60 ( $\pm 15$ ) and 180 ( $\pm 30$ ) min post-dose was identified [ImmunoSuppressants Bayesian dose Adjustment (ISBA)] [65].

### *Pharmacogenetic analysis*

Cytochrome P450 3A5 (CYP3A5) and multidrug resistant protein-1 (MDR-1) genotype were included as significant

covariates in two of the population PK models [61, 62]. The CYP3A5 and MDR-1 genotyping was performed on blood samples from each study patient. Genomic DNA was extracted from whole blood samples using a QIAamp deoxyribonucleic acid mini kit (Qiagen, Hilden, Germany) and was stored at  $4^{\circ}\text{C}$  until analysis. Real-time PCR was performed with a 7900 Real Time PCR System (Applied Biosystems, Melbourne, Victoria, Australia). The PCR conditions were 10 min at  $95^{\circ}\text{C}$ , then 50 cycles of 15 s at  $92^{\circ}\text{C}$  and 1 min 30 s at  $69^{\circ}\text{C}$ . CYP3A5 6986A>G (rs776746) allelic discrimination was undertaken with a Custom TaqMan® Single Nucleotide Polymorphism (SNP) Genotyping Assay (Applied Biosystems) and VIC and FAM reporters. MDR-1 1236 (rs1128503) and MDR-1 3435 (rs1045642) allelic discrimination was undertaken with TaqMan® Drug Metabolism Genotyping Assays (Applied Biosystems), using assays C\_7586662-10 and C\_7586657-20 for MDR-1 1236 and MDR-1 3435, respectively. MDR-1 2677 (rs2032582) allelic discrimination was undertaken with a custom TaqMan® SNP Genotyping Assay (Applied Biosystems) and VIC and FAM reporters.

### *Predictive methods*

Full tacrolimus  $\text{AUC}_{0-12}$  (AUCf) was estimated from all measured concentration–time points using noncompartmental analysis (trapezoidal rule) and compartmental analysis (two-compartment model with lag time) in WinNonlin® (Pharsight, version 5.2, Pharsight Corporation, North Carolina, USA). The predicted tacrolimus  $\text{AUC}_{0-12}$  (AUCp) was calculated in the following ways.

- 1 Applying relevant concentration measurements within each of the multiple regression LSS equations.
- 2 Applying concentration measurements taken at 0, 1, 2, 4 and 6 h post-dose along with relevant patient covariate values in the Bayesian forecasting program TCIWorks using each of the population PK models.
- 3 Sending concentration measurements taken at 0.25, 1 and 3 h post-dose and requested covariate information to the Web-based consultancy service, ISBA.

The AUCp calculated using each of the limited sampling strategies was compared with the AUCf estimated using noncompartmental analysis. The AUCp calculated using Bayesian forecasting was compared with AUCf estimated using compartmental analysis.

### *Pharmacokinetic and statistical analysis*

Descriptive statistics used were mean  $\pm$  standard deviation (SD) or median with interquartile range (IQR) for continuous variables, and percentages for categorical variables. For univariate comparisons,  $\chi^2$ , Fisher's exact test, Student's unpaired *t*-test and Wilcoxon rank sum test were used, where appropriate.

A Pearson correlation coefficient test was applied to assess the correlation between the following variables: (i)



$C_0$  and AUCf; and (ii) AUCp and AUCf. The AUCp was compared with the AUCf in terms of bias and imprecision according to the guidelines proposed by Sheiner & Beal [66]. Specifically, the four measures used for assessment were as follows.

*Bias:*

- 1 Median prediction error (MPE) = median (AUCp – AUCf).
- 2 Median percentage prediction error (MPPE) = median  $[100\% \times (\text{AUCp} - \text{AUCf})/\text{AUCf}]$ .

*Imprecision:*

- 3 Root median squared prediction error (RMSE) =  $\sqrt{\text{median}(\text{AUC}_p - \text{AUC}_f)^2}$ .
- 4 Median absolute percentage prediction error (MAPE) = median  $[100\% \times |(\text{AUC}_p - \text{AUC}_f)|/\text{AUC}_f]$

Values of MPPE and MAPE of <15% were considered acceptable, as is the norm in clinical studies [52]. The percentage of AUCp estimates within 15% of AUCf was also calculated as a measure of overall predictive ability [67].

Analyses were carried out using the software packages Stata/SE 10.1 (College Station, TX, USA) and Excel 2007 (Microsoft Corporation). Graphs were completed using GraphPad Prism, version 5.0 (GraphPad Software).

## Results

### Limited sampling methods for tacrolimus

Tables 2 and 3 summarize the 42 multiple regression-derived LSSs [42, 44, 46, 50, 57, 58] and the six population PK models [54, 59–63] developed for estimation of tacrolimus exposure in adult kidney transplant recipients. One of the population PK models [54] had no between-subject variability and residual random error estimates and thus could not be evaluated further using TClworks. Information on the population model used by the Web-based consultancy service was not available [65].

### Baseline characteristics

Study participants were divided into early (3–5 days post-transplant surgery) and late cohorts (>3 months post-transplant surgery). Table 4 shows the baseline demographic and clinical characteristics of study participants according to study group. The serum creatinine concentration was significantly higher and serum albumin concentration and haematocrit significantly lower in the early compared with the late group.

Similar values for AUCf were estimated based on non-compartmental and compartmental methods in WinNonlin (mean percentage difference 2.2% and no percentage difference greater than 8.7%). Median (IQR) AUCf (calculated using noncompartmental analysis) was 130.6 (87.7–158.9)  $\mu\text{g h l}^{-1}$  vs. 76.2 (66.5–94.8)  $\mu\text{g h l}^{-1}$  ( $P=0.01$ ) in the

early and late groups, respectively. However, when adjusted for dose, this difference was reversed, with median (IQR) dose-adjusted AUCf being significantly lower in the early post-transplant group compared with the late group [20.2 (10.7–26.5) vs. 41.2 (33.9–56.6)  $\mu\text{g h l mg}^{-1}$  tacrolimus;  $P=0.002$ ] (Figure 1).

### Predictive performance of the different limited sampling methods

*Multiple linear regression equations* The correlation ( $r^2$ ) between  $C_0$  and AUCf (calculated using noncompartmental analysis) for the entire study cohort was 0.53 (Figure 2). The correlation between  $C_0$  and AUCf was poorer in the late compared with the early post-transplant group ( $r^2=0.21$  vs.  $r^2=0.64$ , respectively). The correlation ( $r^2$ ) between  $C_{12}$  and AUCf was higher than the correlation between  $C_0$  and AUCf ( $r^2=0.83$  for the study cohort as a whole,  $r^2=0.87$  in the early group and  $r^2=0.63$  in the late group). Of all time points,  $C_6$  showed the highest correlation with AUCf ( $r^2=0.91$  for the study cohort as a whole,  $r^2=0.90$  in the early group and  $r^2=0.79$  in the late group).

Based on the 42 multiple regression equations, the  $r^2$  between AUCp and AUCf ranged from 0.54 to 0.99. Table 5 summarizes the predictive performance of each of the LSSs. All equations showed a better correlation with AUCf than did  $C_0$ . The MPPE varied from 0.1 to 33.5%, and the MAPE varied from 2.0 to 33.5%. Both MPPE and MAPE were <15% for 29 of the 42 equations (62%). The two equations that incorporated only  $C_0$  measurements displayed the lowest correlations and the greatest bias and imprecision of all of the equations (for equation 18,  $r^2=0.54$ , MPPE 23.3% and MAPE 25.7%; and for equation 39,  $r^2=0.54$ , MPPE 33.5% and MAPE 33.5%).

Equation 28 was superior to all other equations with regard to practicality and performance (sampling confined to the first 4 h post-dose,  $r^2=0.95$ , MPPE –1.1% and MAPE 2.8%). Figure 2 displays the correlation between AUCp and AUCf for each study participant based on this equation. Bias and imprecision are depicted in Figures 3 and 4, respectively, using Bland–Altman plots. As shown in Figure 3, there was no consistent pattern to the direction of bias, with AUCp both over- and underestimating AUCf. However, there was a suggestion of increasing bias at increasing values of AUCf. Figure 4 shows that for equation 28, 18 of 20 AUCp values (90%) fell within 15% of AUCf.

When patients in the early post-transplant group were considered separately, MPPE and MAPE were <15% for 35 (83%) of the 42 equations. When patients in the late group were considered separately, this was the case for 24 of the 42 equations (52%). Equation 28 remained superior regardless of duration post-transplant (early group,  $r^2=0.92$ , MPPE –0.8% and MAPE 2.1%; and late group,  $r^2=0.91$ , MPPE –1.9% and MAPE 3.1%).

The AUCp was also compared with AUCf estimated using compartmental analysis. Generally, bias and

**Table 2** Multiple linear regression methods for estimation of tacrolimus exposure in adult kidney transplant recipients

Eqn no.	Patients (country)	Time post-transplant	Dietary control	Prospective data collection	Assay	Times investigated (h)	Limited sampling equation for estimation of AU <sub>C<sub>0</sub>-12</sub>	Validation method	r <sup>2</sup>	MPE (µg h <sup>-1</sup> )	MPPE (%)	RMSE (µg h <sup>-1</sup> )	MAPE (%)	Reference
1	14* (Spain)	NA	No	No	MEIA	0, 0.5, 1, 2, 4, 6, 8, 12	8.90 + 4.0C <sub>0</sub> + 1.77C <sub>1</sub> + 5.47C <sub>4</sub>	Separate group (n = 13*)	0.97	0.48	0.16	6.3	NA	[50]
2	29 (India)	3–6 months	Yes	Yes	MEIA	0.5, 1, 1.5, 2, 2.5, 4, 6, 8, 12	14.73 + 4.38C <sub>0</sub> + 2.09C <sub>1,5</sub> + 4.06C <sub>4</sub>	Jackknife	0.99	NA	0.35	NA	4.7	[46]
3							19.16 + 6.75C <sub>0</sub> + 3.33C <sub>1,5</sub>		0.95	NA	0.53	NA	6.4	
4							23.90 + 2.74C <sub>0</sub> + 7.88C <sub>4</sub>		0.93	NA	-0.01	NA	6.5	
5	18 (Hong Kong)	1–8 years	Yes	Yes	MEIA	0, 1, 2, 4, 6, 8, 12	1.0 + 0.5C <sub>0</sub> + C <sub>1</sub> + 1.5C <sub>2</sub> + 2C <sub>4</sub> + 2C <sub>6</sub> + 2.9C <sub>8</sub> + 2C <sub>12</sub>	None	1.00	NA	-0.5	NA	0.5	[44]
6							5.4 + 1.1C <sub>0</sub> + C <sub>1</sub> + 1.4C <sub>2</sub> + 2.3C <sub>4</sub> + 2C <sub>6</sub> + 3.3C <sub>8</sub>		0.99	NA	-0.2	NA	0.8	
7							8.3 + 1.2C <sub>0</sub> + 0.9C <sub>1</sub> + 1.6C <sub>2</sub> + 2.7C <sub>4</sub> + 3.7C <sub>6</sub>		0.99	NA	0.5	NA	1.7	
8							10 + 1.4C <sub>0</sub> + 0.8C <sub>1</sub> + 1.6C <sub>2</sub> + 5.5C <sub>4</sub>		0.98	NA	-0.08	NA	2.5	
9							13.3 + 1.2C <sub>0</sub> + 2.4C <sub>2</sub> + 5.6C <sub>4</sub>		0.93	NA	-0.8	NA	3.5	
10							16.2 + 2.4C <sub>2</sub> + 5.9C <sub>4</sub>		0.93	NA	-0.2	NA	3.6	
11							24.5 + 3.8C <sub>0</sub> + 0.9C <sub>1</sub> + 3.3C <sub>2</sub>		0.82	NA	0.5	NA	6.8	
12							44 + 0.8C <sub>1</sub> + 3.5C <sub>2</sub>		0.77	NA	0.6	NA	7.6	
13							26.2 + 3.6C <sub>0</sub> + 4.2C <sub>2</sub>		0.76	NA	0.7	NA	7.7	
14							53.22 + 5.26C <sub>0</sub> + 1.95C <sub>1</sub>		0.57	NA	1.3	NA	9.5	
15	100 (Belgium)	Day 7, 42, 90, 180, 360	Yes	Yes	MEIA	0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12	5.36 + 3.06C <sub>0</sub> + 0.98C <sub>0,5</sub> + 1.61C <sub>2</sub> + 1.13C <sub>3</sub> + 3.96C <sub>4</sub>	None	0.96	NA	-0.57	NA	4.7	[58]
16	50† (Japan)	Day 28	Yes	Yes	MEIA	0, 1, 2, 3, 4, 6, 9, 12	10.11C <sub>0</sub> + 41.41	Separate group (n = 50†)	0.77	-7.4	NA	28.3	NA	[57]
17							6.46C <sub>4</sub> + 59.49		0.62	0	NA	28.3	NA	
18							12.46C <sub>0</sub> + 46.00		0.61	-0.2	NA	27.8	NA	
19							3.73C <sub>3</sub> + 7.44C <sub>9</sub> + 9.96		0.91	-3.8	NA	18.3	NA	
20							5.19C <sub>0</sub> + 7.50C <sub>9</sub> + 21.73		0.82	-5.1	NA	25.2	NA	
21							7.63C <sub>0</sub> + 4.11C <sub>4</sub> + 21.66		0.77	0.6	NA	21.2	NA	
22							2.25C <sub>2</sub> + 5.06C <sub>4</sub> + 47.12		0.68	1.0	NA	26.0	NA	
23							3.45C <sub>3</sub> + 2.75C <sub>6</sub> + 4.75C <sub>9</sub> + 4.75		0.95	-3.3	NA	14.7	NA	
24							2.25C <sub>2</sub> + 1.92C <sub>4</sub> + 7.27C <sub>9</sub> + 6.61		0.93	-3.7	NA	17.8	NA	
25							2.15C <sub>0</sub> + 3.34C <sub>3</sub> + 6.64C <sub>9</sub> + 5.06		0.91	-3.3	NA	17.7	NA	
26							7.04C <sub>0</sub> + 1.71C <sub>2</sub> + 3.23C <sub>4</sub> + 15.19		0.80	1.3	NA	19.9	NA	
27							1.13C <sub>3</sub> + 3.03C <sub>3</sub> + 3.65C <sub>4</sub> + 37.09		0.73	1.5	NA	23.9	NA	
28	15 (Thailand)	>3 months	Yes	Yes	MEIA	0, 1, 2, 4, 6, 8, 12	-5.385 + 3.337C <sub>0</sub> + 0.96C <sub>1</sub> + 1.402C <sub>2</sub> + 6.01C <sub>4</sub>	None	0.98	NA	NA	NA	3.2	[42]
29							-5.496 + 7.189C <sub>0</sub> + 2.357C <sub>1</sub> + 2.131C <sub>2</sub>		0.93	NA	NA	NA	5.9	
30							3.85 + 3.688C <sub>0</sub> + 1.355C <sub>1</sub> + 6.649C <sub>4</sub>		0.97	NA	NA	NA	4.5	
31							-6.103 + 2.383C <sub>0</sub> + 1.911C <sub>2</sub> + 7.582C <sub>4</sub>		0.97	NA	NA	NA	3.9	
32							1.304 + 0.465C <sub>1</sub> + 1.636C <sub>2</sub> + 8.256C <sub>4</sub>		0.96	NA	NA	NA	4.6	
33							9.345 + 8.408C <sub>0</sub> + 3.23C <sub>1</sub>		0.91	NA	NA	NA	7.4	
34							-8.453 + 7.389C <sub>0</sub> + 4.902C <sub>2</sub>		0.85	NA	NA	NA	9.6	
35							8.231 + 2.316C <sub>0</sub> + 9.636C <sub>4</sub>		0.95	NA	NA	NA	4.0	
36							19.648 + 2.456C <sub>1</sub> + 4.049C <sub>2</sub>		0.81	NA	NA	NA	11.0	
37							13.114 + 0.873C <sub>1</sub> + 9.291C <sub>4</sub>		0.95	NA	NA	NA	5.2	
38							-0.192 + 1.888C <sub>2</sub> + 8.783C <sub>4</sub>		0.96	NA	NA	NA	4.8	
39							52.509 + 13.126C <sub>0</sub>		0.61	NA	NA	NA	14.7	
40							62.472 + 4.451C <sub>1</sub>		0.71	NA	NA	NA	13.4	
41							17.295 + 6.995C <sub>2</sub>		0.72	NA	NA	NA	13.2	
42							13.808 + 10.779C <sub>4</sub>		0.94	NA	NA	NA	4.8	

C<sub>0</sub>, tacrolimus concentration at given time; MAPE, mean absolute percentage prediction error; MEIA, microparticle enzyme immunoassay; MPE, mean prediction error; MPPE, mean percentage prediction error; NA, not available; and RMSE, root mean squared prediction error. \*Number of PK profiles (22 subjects in total in study). †Number of PK profiles (50 subjects in total in study).

**Table 3** Population pharmacokinetic models for estimation of tacrolimus exposure in adult kidney transplant recipients

Model	Patients (country)	Time post-transplant	Dietary control	Prospective data collection	Assay	Times investigated (h)	Structural model estimate (%CV)	Statistical model estimate (%CV)	Validation method	Reference
1	70 (Australia)	2–1475 days	No	No	LC-MS/MS	12	$CL/F = \theta_1 + \theta_2/POD + \theta_3/AST \text{ l h}^{-1}$ $V/F = \theta_4 \text{ l}$ $ka = 4.48 \text{ h}^{-1}$ (fixed) $\theta_1 = 23.6$ (12%) $\theta_2 = 31.9$ (50%) $\theta_3 = 76.7$ (48%) $\theta_4 = 1070$ (27%)	IIV $CL/F = 42\%$ (17%) IIV $V/F = 111\%$ (47%) RREa = 3.7 ng ml <sup>-1</sup> (23%)	None	[59]
2	83 (France)	<2 months	No	No	MEIA	12	$CL = \theta_1 \times [1 + POD^{\theta_2}/(POD^{\theta_2} + \theta_3^{\theta_2})]$ $\times \text{PRED l h}^{-1}$ PRED = 1 + $\theta_4$ (if prednisolone dose >25 mg or 1 if not) $V = \theta_5 \text{ l}$ $F = \theta_6\%$ $ka = 4.5 \text{ h}^{-1}$ (fixed) $\theta_1 = 1.81$ (12%) $\theta_2 = 3.81$ (14%) $\theta_3 = 2.54$ (37%) $\theta_4 = 0.575$ (6%) $\theta_5 = 98.4$ (13%) $\theta_6 = 13.7$ (11%)	IIV $CL = 31\%$ (55%) IIV $V = 79\%$ (48%) IIV $F = 32\%$ (56%) RREa = 0.96 ng ml <sup>-1</sup> (58%) RREP = 18.6% (53%)	Bootstrap	[60]
3	31 (Netherlands)	2–52 weeks	No	Yes	MEIA	0, 1, 2, 3, 4, 6, 12	$CL (CYP3A5^{*3/*3}) = \theta_1 \text{ l h}^{-1}$ $CL (CYP3A5^{*1/*1} \text{ or } *1/*3) = \theta_2 \text{ l h}^{-1}$ $V_c = \theta_3 \text{ l}$ $V_p = V_c \text{ L}$ $Q = \theta_4 \text{ l h}^{-1}$ $F = 23\%$ (fixed) $F = \theta_5$ (if prednisone dose >10 mg) $ka = \theta_6 \text{ h}^{-1}$ $\theta_1 = 3.7$ (8%) $\theta_2 = 5.5$ (10%) $\theta_3 = 42$ (10%) $\theta_4 = 10$ (10%) $\theta_5 = 19.5$ (30%) $\theta_6 = 1.6$ (14%)	IIV $CL = 19\%$ (32%) IIV $V_c = 28\%$ (31%) IOV $F = 22\%$ (13%) RREP = 23% (6%)	Bootstrap	[61]

**Table 3**  
Continued

Model	Patients (country)	Time post-transplant	Dietary control	Prospective data collection	Assay	Times investigated (h)	Structural model estimate (%CV)	Statistical model estimate (%CV)	Validation method	Reference
4	19* (Belgium)	Pre-transplant	Yes	Yes	MEIA	0, 1, 2, 4, 8, 12	$CL/F = \theta_1 + CYP3A5 + MDR-1$   $h^{-1}$ CYP3A5 = 34 (19%) (if *1/*1 or *1/*3 or 0 if not) MDR-1 = 10 (21%) (if 1236CC, 2677GG or 3435CC or 0 if not) $V_d/F = \theta_2$   $V_p/F = \theta_3$   $Q/F = \theta_4$   $h^{-1}$ $ka = \theta_5$ $h^{-1}$ $\theta_1 = 22$ (11%) $\theta_2 = 142$ (15%) $\theta_3 = 192$ (17%) $\theta_4 = 43$ (14%) $\theta_5 = 2.18t$ (15%)	IIV $CL/F = 6\%$ (108%) IIV $V_d/F = 33\%$ (45%) IIV $V_p/F = 31\%$ (43%) IOV $CL/F = 40\%$ (23%) RREa = 0.02 ng ml <sup>-1</sup> (12%) RREp = 29% (7%)	Bootstrap, case deletion diagnostics, cross-validation, simulation	[62]
5	32 (France)	Weeks 1 and 2 and months 1, 3 and 6	No	Yes	LC-MS/MS	0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 9	$CL/F = \theta_1/HAEM$   $h^{-1}$ $V_d/F = \theta_2$   $V_p/F = 500$   (fixed) $Q/F = \theta_3$   $h^{-1}$ $ka = \theta_4$ $h^{-1}$ $\theta_1 = 863$ (7%) $\theta_2 = 147$ (16%) $\theta_3 = 60$ (20%) $\theta_4 = 6.5\ddagger$ (6%)	IIV $CL/F = 30\%$ IIV $V_d/F = 26\%$ IIV $Q/F = 63\%$ IIV $ka = 15\% \ddagger$ IOV $CL/F = 71\%$ IOV $Q/F = 27\%$ IOV $ka = 24\%$ RREa = 1.5 ng ml <sup>-1</sup> RREp = 10%	Bootstrap, cross-validation	[63]
6	17 (Netherlands)	2–52 weeks	No	Yes	MEIA	0, 1, 2, 3, 4, 6, 8, 12	$ke = \theta_1$ $h^{-1}$ $V_c = \theta_2$   kg <sup>-1</sup> $K_{12} = \theta_3$ $h^{-1}$ $K_{21} = \theta_4$ $h^{-1}$ $F = 23\%$ (fixed) $ka = \theta_5$ $h^{-1}$ $t_{lag} = \theta_6$ h $\theta_1 = 0.517$ (19%) $\theta_2 = 0.180$ (35%) $\theta_3 = 2.850$ (78%) $\theta_4 = 0.384$ (107%) $\theta_5 = 0.580$ (90%) $\theta_6 = 0.956$ (17%)	Separate group (n = 15)		[54]

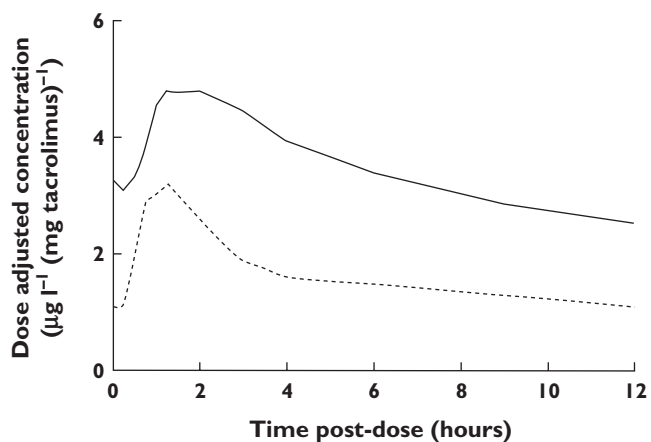
AST, aspartate transaminase; CV, coefficient of variation; CL, clearance,  $CL/F$ , apparent clearance; CYP3A5, cytochrome P450 3A5; F, bioavailability;  $ka$ , absorption rate constant; HAEM, haematocrit; IIV, interindividual variability; IOV, interoccasional variability;  $ke$ , elimination rate constant;  $K_{12}$ , distribution rate constant (central to peripheral compartment);  $K_{21}$ , distribution rate constant (peripheral to central compartment); LC-MS/MS, liquid chromatography–tandem mass spectrometry; MEIA, microparticle enzyme immunoassay; MDR-1, multiple drug resistant protein 1; POD, postoperative day; Q, intracompartamental clearance;  $Q/F$ , apparent intracompartamental clearance; RREa, additive residual random error; RREp, proportional residual random error;  $t_{lag}$ , lag time;  $V_c$ , volume of distribution;  $V_d$ , volume of distribution of central compartment;  $V_p$ , volume of distribution of peripheral compartment;  $V/F$ , apparent volume of distribution; \*renal transplant candidates; †following the morning dose; and ‡transfer rate constant.

**Table 4**

Summary of baseline characteristics of kidney transplant recipients

Characteristic	All subjects	Early post-transplant group*	Late post-transplant group†	P value
Number of patients	20	10	10	1
Tacrolimus dose (mg day <sup>-1</sup> )	7.5 [4, 12.5]	12.5 [11, 15]	4.0 [2.0, 5.5]	0.002
AUCf (µg h l <sup>-1</sup> )	24.1 [18.9, 36.0]	130.6 [87.7, 158.9]	76.2 [66.5, 94.8]	0.01
Dose-adjusted AUCf (mg h l mg <sup>-1</sup> )	30.2 [20.2, 41.2]	20.2 [10.7, 26.5]	41.2 [33.9, 56.6]	0.002
Age (years)	49 ± 11	45 ± 12	53 ± 9	0.1
Male (n (%))	12 (60)	6 (60)	6 (60)	1
Body weight (kg)	80 [62, 98]	91 [74, 104]	74 [56, 91]	0.08
<b>Race</b>				
Caucasian (n (%))	19 (90)	10 (100)	8 (80)	0.14
Asian (n (%))	2 (10)	0 (0)	2 (20)	–
Diabetes (n (%))	2 (10)	0 (0)	2 (20)	0.14
<b>Aetiology of kidney failure</b>				
Glomerulonephritis (n (%))	6 (30)	2 (20)	4 (40)	0.4
Polycystic kidney disease (n (%))	4 (20)	3 (30)	1 (10)	–
Vesicoureteric reflux (n (%))	4 (20)	3 (30)	1 (10)	–
Diabetes (n (%))	1 (5)	0 (0)	1 (10)	–
Other (n (%))	5 (25)	2 (20)	3 (30)	–
<b>Transplant number</b>				
1 (n (%))	17 (85)	9 (90)	8 (80)	0.5
2 (n (%))	3 (15)	1 (10)	2 (20)	–
<b>Transplant type</b>				
Living donor (n (%))	7 (35)	4 (40)	3 (30)	0.6
Deceased donor (n (%))	13 (65)	6 (60)	7 (70)	–
Duration since transplant (days)	44.5 [4, 569]	4 [4, 4]	569 [193, 1941]	0.001
Serum creatinine (µmol l <sup>-1</sup> )	140 [103, 220]	196 [146, 280]	103 [101, 134]	0.006
Serum albumin (g l <sup>-1</sup> )	32 [28, 38]	29 [26, 31]	38 [36, 40]	0.0008
Haematocrit	0.33 [0.26, 0.36]	0.27 [0.25, 0.29]	0.36 [0.35, 0.39]	0.002
Serum bilirubin (µmol l <sup>-1</sup> )	13 [10, 14]	12 [9, 14]	13 [10, 15]	0.4

Values expressed are medians [interquartile range], except mean ± SD for age and median (range) for MMF dose. AUCf, full MPA AUC<sub>0-12</sub> calculated using noncompartmental analysis (trapezoidal rule). \*Days 3–5 post-transplantation. †>3 months post-transplantation.



**Figure 1**

Dose-adjusted tacrolimus concentration vs. time post-dose for the early (3–5 days post-transplant) and late groups (>3 months post-transplant). Solid line is the late post-transplant group and dotted line is early post-transplant group

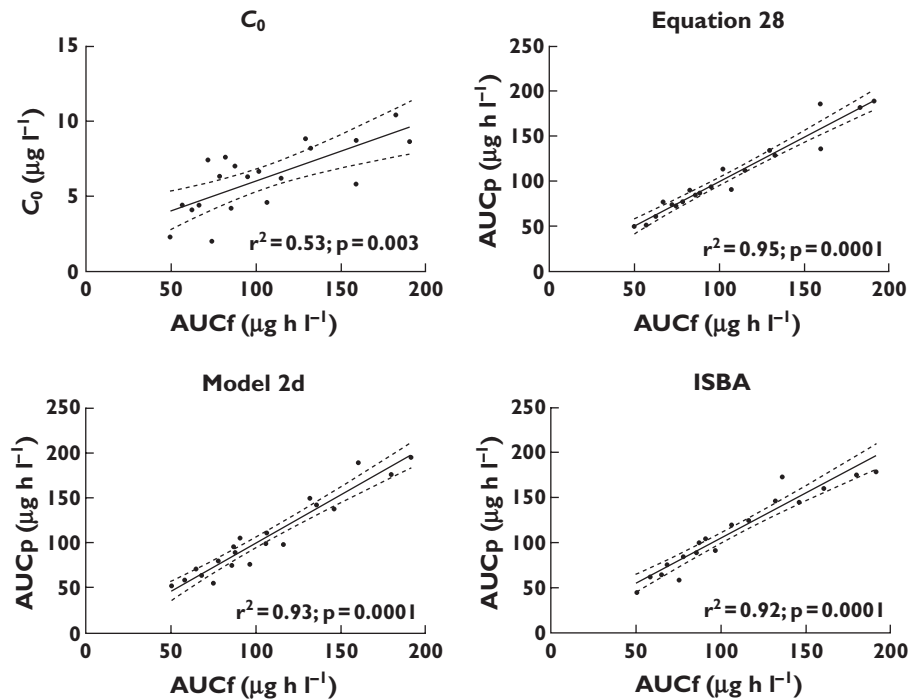
imprecision estimates were slightly inferior (data not shown). However, equation 28 remained superior regardless of metric used, and 26 of the 29 equations that had previously been identified as yielding clinically acceptable

bias and imprecision estimates continued to do so (all except equations 12, 20 and 37).

*MAP Bayesian estimators* Table 6 summarizes the predictive performance of each of population PK models in TCI-Works when varying concentration time points from 0 to 6 h post-dose were applied. It also shows the predictive performance of the Web-based consultancy service when concentration time points at 0.25, 1 and 3 h post-dose were used. Regardless of the model used, Bayesian AUC estimates derived from only C<sub>0</sub> values showed poor correlation with AUCf (calculated using compartmental analysis; r<sup>2</sup> = 0.27–0.54) and unacceptable imprecision (MAPE 17.5–31.6%). In most cases, correlation, bias and imprecision estimates progressively improved with inclusion of a greater number of concentration time points. Generally, population models required at least one concentration time point greater than 2 h post-dose to predict tacrolimus AUCf with acceptable bias and imprecision.

Model 2, using time points at 0, 1, 2 and 4 h post-dose (so-called model 2d) [60], showed slightly superior bias and imprecision estimates compared with all other models (sampling confined to 4 h post-dose, r<sup>2</sup> = 0.93, MPPE 1.6% and MAPE 7.4%). Figure 2 displays the correlation between AUC<sub>p</sub> and AUC<sub>f</sub> for each study participant based on model





**Figure 2**

Correlation between AUCf and  $C_0$ , equation 28, model 2d and ISBA estimates. Continuous lines represent the linear regression lines. Dotted lines represent the 95% confidence intervals for each linear regression line

2d. Bias and imprecision are depicted in Figures 3 and 4, respectively. As shown in Figure 3, there was no consistent pattern to the direction of bias, with AUCp both over- and underestimating AUCf. Again, there was a suggestion of increasing bias at increasing values of AUCf. The dotted line in Figure 4 demonstrates that 15 of 20 AUCp values (75%) fell within 15% of AUCf.

Utilizing three concentration time points over the first 3 h post-dose (0.25, 1 and 3 h post-dose), the Web-based consultancy service also showed clinically acceptable predictive power ( $r^2 = 0.92$ , MPPE 6.6% and MAPE 9.6%; Figures 3 and 4).

Very similar results were obtained when AUCp was compared with AUCf estimated using noncompartmental analysis (data not shown).

## Discussion

This study evaluated the performance of published limited sampling methods for tacrolimus using an independent cohort of 20 adult kidney transplant recipients co-treated with mycophenolate mofetil and prednisolone. Poor correlation between  $C_0$  and AUCf was demonstrated, particularly in those further from transplantation. Alternatively, the majority of the multiple regression-derived LSSs showed acceptable predictive power, regardless of post-transplant duration. This included several LSSs based on

time points 2 h or less post-dose. When population PK models were applied in a Bayesian forecasting program, at least one concentration time point greater than 2 h post-dose appeared necessary to predict tacrolimus AUC<sub>0-12</sub> with acceptable levels of bias and imprecision.

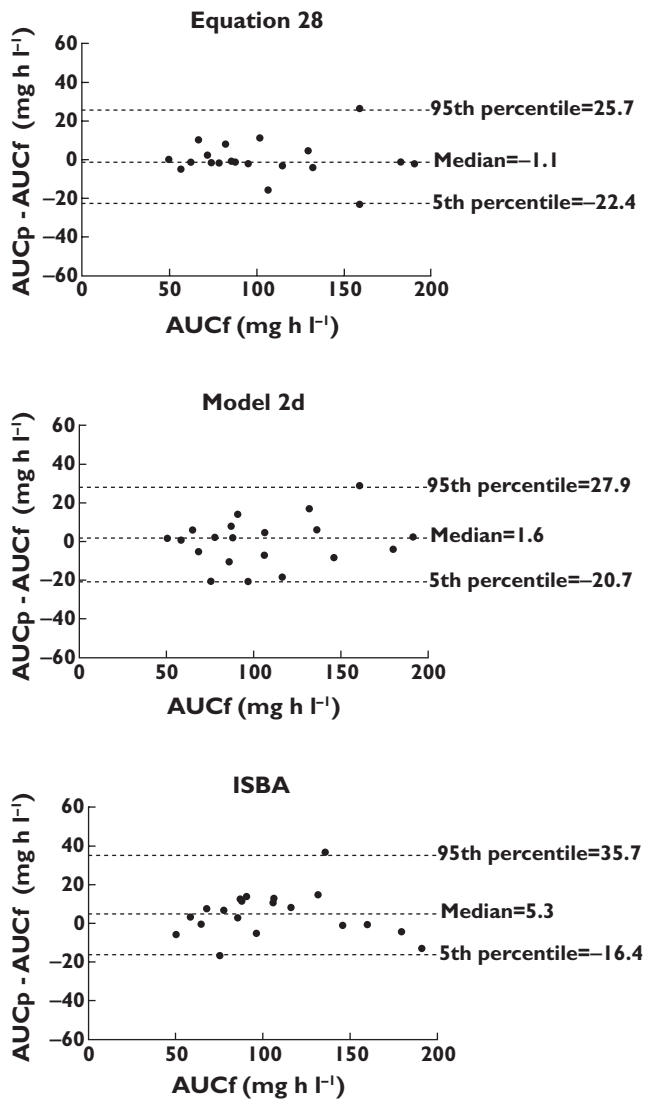
This study provides a summary of all currently published limited sampling methods for tacrolimus in adult kidney transplant recipients. The majority of LSSs and population PK models developed to date have been based on small patient numbers, with 42% involving  $\leq 20$  and 75% involving  $\leq 50$  participants. Most (83%) were derived from tacrolimus concentrations measured by microparticle enzyme immunoassay (MEIA) rather than liquid chromatography–tandem mass spectrometry technology. Ethnicities of participants varied, as did the time post-transplant when sampling occurred. While most (75%) used prospectively collected data, only a minority (25%) were externally validated using a separate group. When reported for the LSS studies, bias and imprecision estimates were generally within clinically acceptable limits. For the PK models, proportional residual random error was as high as 29%.

Measurement of pre-dose ( $C_0$ ) tacrolimus concentrations is currently routine clinical practice. However, consistent with the majority of previous studies, we saw only moderate correlation between  $C_0$  and AUCf ( $r^2 = 0.53$ ), with the wide range of the 95% confidence interval suggesting suboptimal imprecision (depicted in Figure 2). Similarly, we

**Table 5** Predictive performance of multiple linear regression methods for prediction of tacrolimus exposure in adult kidney transplant recipients

Eqn no.	Limited sampling equation for estimation of AUC <sub>0-12</sub>	Times used(h)	r <sup>2</sup>	MPE(μg h <sup>-1</sup> )	MPPE(%)	RMSE(μg h <sup>-1</sup> )	MAPE(%)	% AUCp within 15% AUCf	Reference
1*	8.90 + 4.0C <sub>0</sub> + 1.77C <sub>1</sub> + 5.47C <sub>4</sub>	0, 1, 4	0.95	7.6	9.3	8.1	7.3	85	[50]
2*	14.73 + 4.38C <sub>0</sub> + 2.09C <sub>1.5</sub> + 4.06C <sub>4</sub>	0, 1.5, 4	0.92	6.9	7.7	10.4	12.2	60	[46]
3*	19.16 + 6.75C <sub>0</sub> + 3.33C <sub>1.5</sub>		0.86	2.4	2.5	12.2	13.2	60	
4	23.90 + 2.74C <sub>0</sub> + 7.88C <sub>4</sub>		0.80	17.9	20.6	20.9	23.2	30	
5*	1.0 + 0.5C <sub>0</sub> + C <sub>1</sub> + 1.5C <sub>2</sub> + 2C <sub>4</sub> + 2C <sub>6</sub> + 2.9C <sub>8</sub> + 2C <sub>12</sub>	0, 1, 2, 4, 6, 8, 12	0.99	1.0	1.6	2.0	2.0	95	[44]
6*	5.4 + 1.1C <sub>0</sub> + C <sub>1</sub> + 1.4C <sub>2</sub> + 2.3C <sub>4</sub> + 2C <sub>6</sub> + 3.3C <sub>8</sub>		0.99	1.0	0.9	2.4	2.1	90	
7*	8.3 + 1.2C <sub>0</sub> + 0.9C <sub>1</sub> + 1.6C <sub>2</sub> + 2.7C <sub>4</sub> + 3.7C <sub>6</sub>		0.99	-0.7	1.1	3.2	3.2	100	
8*	10 + 1.4C <sub>0</sub> + 0.8C <sub>1</sub> + 1.6C <sub>2</sub> + 5.5C <sub>4</sub>		0.95	-0.7	-0.7	5.7	6.2	85	
9*	13.3 + 1.2C <sub>0</sub> + 2.4C <sub>2</sub> + 5.6C <sub>4</sub>		0.90	1.3	1.6	7.5	9.1	80	
10*	16.2 + 2.4C <sub>2</sub> + 5.9C <sub>4</sub>		0.88	-0.8	-1.0	8.7	9.6	80	
11*	24.5 + 3.8C <sub>0</sub> + 0.9C <sub>1</sub> + 3.3C <sub>2</sub>		0.89	1.8	1.9	11.8	12.8	60	
12*	44 + 0.8C <sub>1</sub> + 3.5C <sub>2</sub>		0.86	-1.1	-1.4	13.8	14.5	50	
13*	26.2 + 3.6C <sub>0</sub> + 4.2C <sub>2</sub>		0.89	1.1	0.9	9.4	8.4	65	
14	53.22 + 5.26C <sub>0</sub> + 1.95C <sub>1</sub>		0.80	15.0	13.0	19.4	20.0	45	
15*	5.36 + 3.06C <sub>0</sub> + 0.98C <sub>0.5</sub> + 1.61C <sub>2</sub> + 1.13C <sub>3</sub> + 3.96C <sub>4</sub>	0, 0.5, 2, 3, 4	0.97	2.0	3.2	7.1	5.0	85	[58]
16	10.11C <sub>0</sub> + 41.41	0, 2, 3, 4, 9	0.90	5.0	5.6	18.6	20.7	40	[57]
17	6.46C <sub>4</sub> + 59.49		0.77	24.1	25.0	24.8	25.0	30	
18	12.46C <sub>0</sub> + 46.00		0.54	25.7	23.3	28.1	25.7	30	
19*	3.73C <sub>3</sub> + 7.44C <sub>9</sub> + 9.96		0.91	-1.2	-1.1	7.7	9.3	75	
20*	5.19C <sub>0</sub> + 7.50C <sub>9</sub> + 21.73		0.82	-3.2	-2.7	11.7	13.9	60	
21	7.63C <sub>0</sub> + 4.11C <sub>4</sub> + 21.66		0.77	8.6	10.2	15.4	18.1	35	
22	2.25C <sub>2</sub> + 5.06C <sub>4</sub> + 47.12		0.89	22.2	23.7	22.2	23.7	30	
23*	3.45C <sub>3</sub> + 2.75C <sub>6</sub> + 4.75C <sub>9</sub> + 4.75		0.94	-5.0	-5.1	6.8	7.4	75	
24*	2.25C <sub>2</sub> + 1.92C <sub>4</sub> + 7.27C <sub>9</sub> + 6.61		0.94	-2.3	0.1	3.7	3.9	90	
25*	2.15C <sub>0</sub> + 3.34C <sub>3</sub> + 6.64C <sub>9</sub> + 5.06		0.91	-0.6	-1.0	6.2	7.5	80	
26*	7.04C <sub>0</sub> + 1.71C <sub>2</sub> + 3.23C <sub>4</sub> + 15.19		0.86	13.2	10.9	15.3	15.0	50	
27	1.13C <sub>2</sub> + 3.03C <sub>3</sub> + 3.65C <sub>4</sub> + 37.09		0.88	16.4	19.5	19.2	19.5	45	
28†	-5.385 + 3.337C <sub>0</sub> + 0.96C <sub>1</sub> + 1.402C <sub>2</sub> + 6.01C <sub>4</sub>	0, 1, 2, 4	0.95	-1.1	-1.1	2.8	2.8	90	[42]
29*	-5.496 + 7.189C <sub>0</sub> + 2.357C <sub>1</sub> + 2.131C <sub>2</sub>		0.87	-3.5	-3.4	13.6	11.6	65	
30*	3.85 + 3.688C <sub>0</sub> + 1.355C <sub>1</sub> + 6.649C <sub>4</sub>		0.94	5.7	7.2	6.7	8.1	75	
31*	-6.103 + 2.383C <sub>0</sub> + 1.911C <sub>2</sub> + 7.582C <sub>4</sub>		0.88	0.3	0.3	8.3	8.8	65	
32*	1.304 + 0.465C <sub>1</sub> + 1.636C <sub>2</sub> + 8.256C <sub>4</sub>		0.96	1.9	2.2	6.9	7.7	70	
33	9.345 + 8.408C <sub>0</sub> + 3.23C <sub>1</sub>		0.80	6.5	6.1	14.5	17.9	40	
34*	-8.453 + 7.389C <sub>0</sub> + 4.902C <sub>2</sub>		0.85	0.6	0.6	8.7	7.8	75	
35	8.231 + 2.316C <sub>0</sub> + 9.636C <sub>4</sub>		0.80	11.6	14.9	15.1	17.5	40	
36*	19.648 + 2.456C <sub>1</sub> + 4.049C <sub>2</sub>		0.83	4.2	3.8	10.5	12.8	65	
37*	13.114 + 0.873C <sub>1</sub> + 9.291C <sub>4</sub>		0.88	8.6	9.2	10.3	13.1	50	
38*	-0.192 + 1.888C <sub>2</sub> + 8.783C <sub>4</sub>		0.86	0.9	1.5	10.9	9.3	65	
39	52.509 + 13.126C <sub>0</sub>		0.54	35.6	33.5	35.2	33.5	25	
40	62.472 + 4.451C <sub>1</sub>		0.66	27.2	22.3	29.7	24.0	25	
41*	17.295 + 6.995C <sub>2</sub>		0.83	4.1	4.5	11.1	11.3	60	
42	13.808 + 10.779C <sub>4</sub>		0.77	13.1	12.2	17.3	18.9	45	

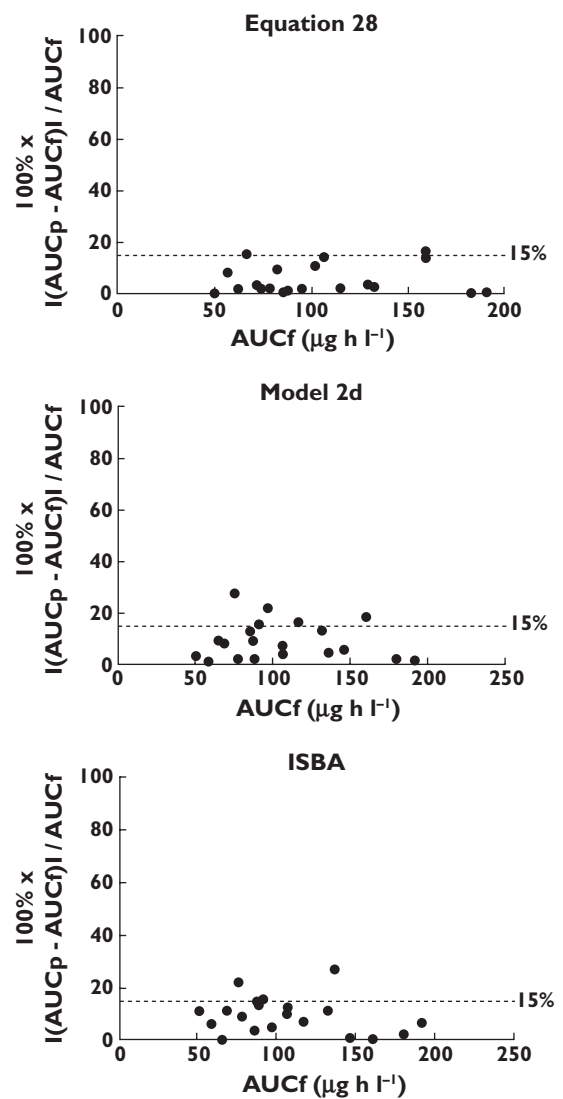
C<sub>0</sub>, tacrolimus concentration at given time; MAPE, median absolute percentage prediction error; MPE, median prediction error; MPPE, median percentage prediction error; RMSE, root median squared prediction error. \*Acceptable bias and imprecision. †Best performance of all equations with regard to practicality and performance.


**Figure 3**

Bland–Altman plots comparing the difference between AUCf and AUCp and the average of AUCf and AUCp, when AUCp was estimated from equation 28 or model 2d and using ISBA. Dotted lines represent median differences and the 5th and 95th percentiles

found that limited sampling methods that relied solely on  $C_0$  values showed poor ability to predict AUCf (Tables 5 and 6). For unclear reasons, but also consistent with previous studies [5, 38], the relationship between  $C_0$  and AUCf was particularly weak during the late post-transplant phase ( $r^2 = 0.64$  in the early group vs. 0.21 in the late group). The correlation between  $C_{12}$  and AUCf was substantially higher than the correlation between  $C_0$  and AUCf ( $r^2 = 0.83$  vs. 0.53). Possibly, failure by patients to self-administer their evening dose of medication at the specified time on the night prior to blood sampling may have been responsible, an occurrence that would be even more likely outside of the trial setting.

The majority (69%) of multiple regression-derived LSSs showed acceptable predictive power for AUCf (bias and


**Figure 4**

Scatter plots of the absolute percentage prediction error vs. AUCf, when AUCp was estimated from equation 28 or model 2d and using ISBA. Accuracy within 15% of AUCf is represented by the dotted lines

imprecision <15% for both parameters), regardless of duration post-transplantation. This was particularly the case for equation 28 ( $AUC_{0-12} = -5.385 + 3.337C_0 + 0.96C_1 + 1.402C_2 + 6.01C_4$ ), which not only demonstrated the highest predictive power in our cohort as whole ( $r^2 = 0.95$ , MPPE  $-1.1\%$  and MAPE 2.8%), but also maintained superior predictive power when applied separately to early and late groups. This equation was derived in a study involving 15 Thai kidney transplant recipients, all of whom were >3 months post-transplant [42]. Tacrolimus was administered in the fasting state, and concentrations were measured with MEIA (known to overestimate tacrolimus concentrations by up to 30% due to interference by metabolites [23]). Given the markedly different study conditions and demographic of the derivation population compared with our

**Table 6**

Predictive performance of population models for prediction of tacrolimus exposure in adult kidney transplant recipients

Model	Limited sampling equations	Times used (h)	r <sup>2</sup>	MPE (µg h l <sup>-1</sup> )	MPPE (%)	RMSE (µg h l <sup>-1</sup> )	MAPE (%)	% AUCp within 15% AUCf	Reference
1a	CL/F = 23.6 + 31.9/POD + 76.7/AST l h <sup>-1</sup>	0	0.34	-5.9	-4.9	22.3	26.4	30	[59]
1b	V/F = 1070 l	0, 1	0.65	2.9	5.0	27.0	22.9	30	
1c	ka = 4.48 h <sup>-1</sup> (fixed)	0, 1, 2	0.71	5.2	5.0	14.6	19.2	45	
1d	IIV CL/F = 42%	0, 1, 2, 4	0.75	1.9	1.3	12.5	12.5	55	
1e*	IIV V/F = 111%	0, 1, 2, 4, 6	0.83	3.2	2.3	9.2	8.6	70	
1f*	RREa = 3.7 ng ml <sup>-1</sup>	0, 1, 4	0.74	0.1	0	12.9	13.0	60	
1g		0, 4	0.68	-7.4	-5.2	16.2	15.9	45	
2a	CL = 1.81 × [1 + POD <sup>2.54</sup> / (POD <sup>2.54</sup> + 3.81 <sup>2.54</sup> )] × PRED l h <sup>-1</sup>	0	0.27	8.2	9.3	20.1	17.5	40	[60]
2b	PRED = 1 + 0.575 (if prednisolone dose >25 mg or 1 if not)	0, 1	0.75	0.7	1.2	30.0	21.8	25	
2c	V = 98.4 l	0, 1, 2	0.83	3.9	4.7	13.7	15.4	50	
2dt	F = 13.7%	0, 1, 2, 4	0.93	1.6	1.6	6.6	7.4	75	
2e*	ka = 4.5 h <sup>-1</sup> (fixed)	0, 1, 2, 4, 6	0.95	-2.1	-1.4	4.9	4.6	90	
2f*	IIV CL = 31%	0, 1, 4	0.91	-0.1	-0.1	8.1	9.2	70	
2g*	IIV V = 79%	0, 4	0.88	-3.3	-4.1	9.9	9.7	75	
	IIV F = 32% RREa = 0.96 ng ml <sup>-1</sup> RREp = 18.6%								
3a	CL (CYP3A5*3/*3) = 3.7 l h <sup>-1</sup>	0	0.52	30.7	25.7	43.0	31.6	20	[61]
3b	CL (CYP3A5*1/*3) = 5.5 l h <sup>-1</sup>	0, 1	0.64	11.0	14.4	21.1	20.8	40	
3c	V <sub>c</sub> = 42 l	0, 1, 2	0.73	8.2	10.0	18.3	16.4	50	
3d	V <sub>p</sub> = 42 l	0, 1, 2, 4	0.86	6.4	6.5	14.9	12.7	55	
3e*	Q = 10 l h <sup>-1</sup>	0, 1, 2, 4, 6	0.89	4.7	6.7	12.1	9.7	60	
3f*	F = 23% (fixed)	0, 1, 4	0.82	6.7	9.2	17.7	13.6	55	
3g	F = 19.5% (if prednisone dose >10 mg)	0, 4	0.72	18.3	14.8	28.3	23.8	40	
	ka = 1.6 h <sup>-1</sup> IIV CL = 19% IIV V <sub>c</sub> = 28% RREp = 23%								
4a	CL/F = 22 + 34 (if CYP3A5*1/*1 or *1/*3) + 10 (if MDR-1 1236CC, 2677GG or 3435CC) l h <sup>-1</sup>	0	0.54	20.9	24.2	31.9	28.1	35	[62]
4b		0, 1	0.69	-1.2	-0.5	29.1	25.1	40	
4c	V <sub>c</sub> /F = 142 l	0, 1, 2	0.82	-2.4	-2.4	14.2	14.1	50	
4d*	V <sub>p</sub> /F = 192 l	0, 1, 2, 4	0.93	-8.6	-7.2	9.4	9.4	65	
4e*	Q/F = 43 l h <sup>-1</sup>	0, 1, 2, 4, 6	0.94	-4.7	-5.0	5.4	6.5	65	
4f*	ka = 2.18 h <sup>-1</sup>	0, 1, 4	0.83	-4.6	-4.6	13.0	14.1	55	
4g	IIV CL/F = 46%	0, 4	0.82	9.8	8.0	14.8	15.8	50	
	IIV V <sub>c</sub> = 33% IIV V <sub>p</sub> = 31% RREa = 0.02 ng ml <sup>-1</sup> RREp = 29%								
5a	CL/F = 863/HAEM	0	0.42	18.9	17.0	29.4	22.5	30	[63]
5b	V <sub>c</sub> /F = 147 l	0, 1	0.70	-6.3	-6.7	15.8	13.8	50	
5c	V <sub>p</sub> /F = 500 l (fixed)	0, 1, 2	0.76	-2.4	-2.7	13.5	13.5	50	
5d*	Q/F = 60 l h <sup>-1</sup>	0, 1, 2, 4	0.87	-1.1	-1.3	9.5	9.8	80	
5e*	ka = 6.5 h <sup>-1</sup>	0, 1, 2, 4, 6	0.91	-0.2	-0.2	8.1	7.7	80	
5f*	IIV CL/F = 30%	0, 1, 4	0.86	-0.7	-0.3	10.1	8.1	65	
5g	IIV V <sub>c</sub> /F = 26%	0, 4	0.69	20.1	17.3	26.1	22.9	25	
	IIV Q/F = 63% IIV ka = 15% RREa = 1.5 ng ml <sup>-1</sup> RREp = 10%								
ISBA Web-based service*	Population model not published Information on covariates (postoperative day, diabetes status, assay used) is supplied by the user	0.25, 1, 3	0.92	5.3	6.6	8.0	9.6	85	[54]

AST, aspartate transaminase; CV, coefficient of variation; CL, clearance, CL/F, apparent clearance; CYP3A5, cytochrome P450 3A5; F, bioavailability; ka, absorption rate constant; HAEM, haematocrit; IIV, interindividual variability; IOV, interoccasional variability; MAPE, median absolute percentage prediction error; MDR-1, multiple drug resistant protein 1; MPE, median prediction error; MPPE, median percentage prediction error; POD, postoperative day; Q, intracompartamental clearance; Q/F, apparent intracompartamental clearance; RMSE, root median squared prediction error; RREa, additive residual random error; RREp, proportional residual random error; V, volume of distribution; V<sub>c</sub>, volume of distribution of central compartment; V<sub>p</sub>, volume of distribution of peripheral compartment; V/F, apparent volume of distribution; IIV + IOV. \*Acceptable bias and imprecision. †Best performance of all equations with regard to practicality and performance.

population, the superior performance of this equation was surprising. However, by showing that applicability cannot always be predicted, it highlights the importance of validating any LSS prior to applying it to an alternative population. Of note, five of eight equations based on time points 2 h or less post-dose showed bias and imprecision estimates of <15%.

Alternatively, in our cohort, on first application of the population PK models developed to date in a Bayesian forecasting program, at least one concentration time point greater than 2 h post-dose appeared necessary to predict tacrolimus  $AUC_{0-12}$  with acceptable bias and imprecision. The predictive power of the models progressively increased with the inclusion of a greater number of concentration time points. This was expected, as it allows for greater reliance on measured data rather than on the predictive power of the underlying model. Model 2 [60], which was developed from the largest number of patients ( $n = 83$ ; Table 2) and considered postoperative day and prednisolone dosage as covariate parameters, was marginally superior to all other models. However, even when concentration time points greater than 2 h post-dose were employed, its predictive ability was inferior to the performance of the highest performing multiple regression-derived LSS.

These data suggest some limitations with the population models developed to date. All models were derived from small, relatively homogeneous populations, lessening the likelihood of applicability to alternative groups. Additionally, all were associated with reasonably large residual random variability (greater than 20% in most cases). Furthermore, there was inconsistent consideration of the influence of relevant covariates on tacrolimus pharmacokinetics. Staatz *et al.* [5] included postoperative day and aspartate aminotransferase (AST), while Antignac *et al.* [60] included postoperative day and prednisolone dose. Neither considered the influence of genotype, despite its well-documented contribution to variable tacrolimus exposure. Alternatively, Press *et al.* [61], Musuamba *et al.* [62] and Benkali *et al.* [63] considered genotype [variably examining the influence of polymorphisms in CYP 3A4 and 3A5, P-glycoprotein (ABCB1/MDR1), and the pregnane X receptor (PXR) genes], but failed to consider the influence of days of therapy. The Web-based consultancy service requested provision of only postoperative day, assay used for tacrolimus measurement and diabetic status, while the study of Scholten *et al.* [38] considered only the impact of patient weight. Haematocrit and time of drug administration (morning vs. evening) were also found to be significant covariates in some studies [46, 47], but were not considered in others. Additional concerns with the studies of Staatz *et al.* [5] and Antignac *et al.* [60] included use of only  $C_0$  values to derive population PK parameters and the retrospective nature of data collection.

It is important to note in this study that LSS and Bayesian forecasting methods were tested in a controlled

setting, where strict adherence to sampling times was possible. Compared with Bayesian analysis, multiple regression-derived LSSs are dependent on reasonably exact timing of concentration measurements. Accurate timing may be more difficult to achieve in 'real-world' practice, thereby potentially affecting the clinical utility of this method. As well as allowing greater flexibility of timing of samples, another advantage of Bayesian predictions is that the population models on which they are based can be continually improved as more patient-specific data become available. As the ability of population models to reflect drug pharmacokinetics improves, the ability of Bayesian estimators to predict  $AUC_{0-12}$  reliably improves simultaneously. Thus, despite the weaknesses apparent in the population models published to date, the abovementioned theoretical advantages of Bayesian analysis mean that, in the future, this methodology may prove to be the most desirable to derive limited sampling methods for use in clinical practice. In this regard, the clinically acceptable AUC estimates returned by the Web-based consultancy service are encouraging. Use of such a service removes the requirement for specialist software and user expertise, making this methodology more accessible to the clinician.

Another interesting finding from our study was higher dose-adjusted tacrolimus AUCf in those >3 months post-transplant compared with those in their first post-transplant week (Figure 1). A similar increase in dose-adjusted exposure over time was seen in the study of Scholten *et al.* [38]. In our cohort, this may be the consequence of the significantly lower serum albumin and haematocrit concentrations observed in the early post-transplant group (Table 2). Given that tacrolimus binds extensively to albumin and haemoglobin [68], a decrease in albumin and haematocrit should be associated with an increase in tacrolimus free fraction [5]. This in turn should lead to an increase in apparent oral total clearance and a decrease in total tacrolimus whole blood concentrations. Alternatively, given that CYP3A enzymes involved in tacrolimus metabolism are induced by corticosteroids [69], steroid tapering over time may be contributory. Another possible explanation may be poor gut motility, impairing absorption in the early post-transplant group. Regardless of mechanism, the increase in dose-adjusted AUCf over time is of particular interest when viewed in conjunction with our finding of poorer correlation of  $C_0$  with AUCf in the later post-transplant period. Together, these findings suggest a risk of misinterpretation of chronic drug exposure if  $C_0$  values alone are used for tacrolimus therapeutic drug monitoring.

The primary limitation of our study relates to the relatively small sample size, which exposes our data to potential ascertainment bias. However, our study population was similar in overall demographic to our larger transplant population, and our results are concordant with those of previous studies. A further limitation is that where concentration time points specified by models did not correspond



with our sampling time points, linear extrapolation from measured concentrations was required (multiple regression-derived LSS equations 5 and 6). Given the potential error inherent in this process, our bias and imprecision estimates may not truly demonstrate the predictive power of these particular equations. Additionally, although we calculated AUC<sub>0-12</sub> using compartmental and noncompartmental analyses, values obtained are still estimates, and thus may not be truly reflective.

Despite these limitations, our study clearly shows that limited sampling methods have superior ability to predict tacrolimus exposure compared with C<sub>0</sub> monitoring. Given that collection of multiple samples is likely to incur significant costs and prove inconvenient and time consuming for patients and medical personnel, particularly in the outpatient setting, it is likely that these limited sampling methods may be of particular use on an infrequent basis in the later post-transplant period when the relationship between C<sub>0</sub> and AUC<sub>0-12</sub> appears to be especially poor, or in patients having a particularly complicated post-transplant course. Future research should be aimed at improving existing population models so as to improve the predictive power of Bayesian methodology. Additionally, it is important to note that this study addresses only those methods available for tacrolimus therapeutic drug monitoring. It provides no data showing clinical relevance of any methodology. Prospective randomized controlled trials are required to establish a target range for AUC<sub>0-12</sub> and to confirm that the improved AUC predictions afforded by limited sampling methods translate into improved clinical outcomes.

## Competing Interests

SC has been on Advisory Boards for Janssen-Cilag. DJ has received speakers' grants and honoraria from Janssen-Cilag. The other authors have no competing interests to declare.

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