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ARTICLE

Fasting Status and Circadian Variation Must be Considered When Performing AUC-based Therapeutic Drug Monitoring of Tacrolimus in Renal Transplant Recipients

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Therapeutic drug monitoring (TDM) is mandatory for the immunosuppressive drug tacrolimus (Tac). For clinical applicability, TDM is performed using morning trough concentrations. With recent developments making tacrolimus concentration determination possible in capillary microsamples and Bayesian estimator predicted area under the concentration curve (AUC), AUC-guided TDM may now be clinically applicable. Tac circadian variation has, however, been reported, with lower systemic exposure following the evening dose. The aim of the present study was to investigate tacrolimus pharmacokinetic (PK) after morning and evening administrations of twice-daily tacrolimus in a real-life setting without restrictions regarding food and concomitant drug timing. Two 12 hour tacrolimus investigations were performed; after the morning dose and the following evening dose, respectively, in 31 renal transplant recipients early after transplantation both in a fasting-state and under real-life nonfasting conditions (14 patients repeated the investigation). We observed circadian variation under fasting-conditions: 45% higher peak-concentration and 20% higher AUC following the morning dose. In the real-life nonfasting setting, the PK-profiles were flat but comparable after the morning and evening doses, showing slower absorption rate and lower AUC compared with the fasting-state. Limited sampling strategies using concentrations at 0, 1, and 3 hours predicted AUC after fasting morning administration, and samples obtained at 1, 3, and 6 hours predicted AUC for the other conditions (evening and real-life nonfasting). In conclusion, circadian variation of tacrolimus is present when performed in patients who are in the fasting-state, whereas flatter PK-profiles and no circadian variation was present in a real-life, nonfasting setting.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Circadian variation of tacrolimus (Tac) is controversial. Most Tac population pharmacokinetic (PK) models are based on fasting-day data.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ It investigated circadian variation in Tac PK and the effect on Tac PK-profiles when administered in a real-life setting with regard to food and concomitant drug timing.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ In a real-life nonfasting setting, the PK-profiles were flat without circadian variation. The study supports circadian variation of Tac under fasting conditions. Data on the real-world behavior of the patients are needed for a population PK model to predict area under the concentration curve (AUC) during both conditions.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ Proposed Tac AUC-target levels need to be redefined due to circadian variation and flat real-life nonfasting PK-profiles. The association between high peak concentrations and side effects of Tac may be overestimated given the flat real-life nonfasting PK-profiles. The effect of real-life dosing of Tac may very well be present for other drugs and should be investigated for drugs where TDM is indicated.

Following organ transplantation, there is a need for life-long immunosuppressive therapy. For the last 10–15 years, the calcineurin inhibitor tacrolimus (Tac) has been the cornerstone in most transplant centers.[1] The narrow therapeutic index and large pharmacokinetic (PK) interindividual and intra-individual variability makes therapeutic drug monitoring (TDM) of Tac mandatory,[2] and is normally performed using morning trough concentrations.

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When Tac was introduced in transplant protocols, importance of avoiding acute rejections led to TDM targeting high Tac trough concentrations. High concentrations induce nephrotoxicity and development of other side effects, like hypertension, post-transplant diabetes mellitus, neurotoxicity, and cancer.[3,4] In combination with mycophenolate mofetil (MMF) and modern induction therapy, the recommended Tac trough concentration target has gradually been reduced.[5,6] There is still room for improving long-term outcomes following renal transplantation,[7,8] and improved tailoring of the Tac dosing may be an important contributor.[9] The area under the concentration vs. time curve (AUC), reflecting total systemic Tac exposure, should theoretically be a more relevant measure for both efficacy and side effects compared with trough concentrations.[10] A recent consensus report also recommended AUC thresholds and advocates the need for prospective AUC-dosed studies.[10] By utilizing limited sampling strategies (LSS), preferably by capillary microsampling, in combination with population PK model-derived Bayesian estimators have made AUC-targeted dosing of Tac applicable in clinical practice.[11,12] However, data used to develop most Tac population PK models are based on data from clinical trials.[13,14] Such data are generally obtained in selected patients under highly controlled conditions (i.e., fasting, without concomitant drugs at time of Tac dose administration); hence, these results may not reflect a real-life situation of individual transplant recipients. In addition, the majority of AUC data are obtained during the day (i.e., following the morning dose of Tac). Because Tac has shown circadian variation, with higher drug exposure after the morning dose,[15–18] using models that assume a similar PK-profile following the morning dose and evening dose will introduce biased Tac exposure 0–24-hour AUC ($AUC_{0–24}$) predictions. In addition, Tac PK is also affected by food consumption.[19] If there is a correlation between systemic Tac exposure and long-term outcomes, models reflecting the real-life scenario over the entire dosing interval may prove advantageous.

The primary aim of this study was to investigate Tac PK after the morning and evening administration of twice-daily Tac in a real-life setting with regard to food and concomitant drug timing. Second, we aimed to determine the predictive performance of Tac AUC predictions using LSS and Bayesian estimators from a nonparametric population PK model.

METHODS

Study design

A prospective, open, nonrandomized PK study was performed at the National Transplant Center in Norway, Oslo University Hospital – Rikshospitalet, from December 2015 to May 2017. Renal transplant recipients older than 18 years using twice-daily Tac (Prograf; Astellas Pharma Ltd., Chertsey, UK) without concomitant drugs known to interact with Tac PK were included.

The study was conducted in accordance with ethical principles in the Declaration of Helsinki, guidelines for Good Clinical Practice, and was approved by the Norwegian Medicine Agency (EudraCT number: 2015-004734-10) and the local ethic committee (reference number 2015/2098). All patients received verbal and written information and signed an informed consent before entering the study.

Immunosuppressive treatment

Maintenance therapy consisted of a combination of Tac, MMF, and steroids. Tac was initiated on the day of transplantation, given a starting dose of 0.04 mg/kg for immunological standard-risk patients and adjusted to a trough (C_0) target range of 3–7 μg/L. For immunological high-risk patients (presence of donor specific antibodies at time of engraftment), Tac starting dose was 0.05 mg/kg and dose adjusted to a C_0 target of 8–12 μg/L. MMF was given at a fixed dose of 750 mg twice-daily from the day of transplantation and dose adjustments were only performed in case of side effects. Prednisolone was administered according to a fixed tapering schedule starting at 20 mg/day (80 mg/day in high-risk patients) the day after transplantation and tapered to a maintenance dose of 10 mg/day by weeks 4–8. All patients received induction therapy with basiliximab 20 mg on day 0 and day 4 after transplantation, and intravenous methylprednisolone 250 mg (standard-risk) or 500 mg (high-risk) on day 0. High-risk patients also received intravenous humane immune globulins 0.4 g/kg daily on days 0 and 4 and rituximab 375 mg/m^2 on day 0.

Tacrolimus analysis

Tac whole-blood samples were collected using vacutainers with spray-coated potassium EDTA acid (4 mL Vacuette K_2EDTA; Greiner Bio-One, Monroe, NC). The analysis was performed using liquid chromatography tandem mass spectrometry, as previously reported.[20] Lower limit of quantification was 0.6 μg/L, with imprecision coefficients of 9.0% at 2.3 μg/L and 6.0% at 7.0 μg/L.

Pharmacokinetic investigation

In the early post-transplant phase (2–8 weeks after transplantation), two 12-hour PK investigations were performed in succession (following morning and evening doses). In almost half of the participants, the PK investigations were repeated within 1 month (**Figure 1**).

Blood samples were collected predose (0 hour) and 11 times postdose; approximately after 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 12 hours. Exact sampling times in h:min were recorded. At the time of transplantation, patients were instructed to take their Tac doses at 9 AM and 9 PM, and this was also applied in the study. For inclusion, the Tac dosage had to be unchanged for at least 5 days prior to the PK investigation.

Patients were investigated either after administering their immunosuppressive medications as in their everyday life (i.e., both with regard to food consumption) in association to the Tac dose and concomitant drug administration (i.e., "real-life" nonfasting dose administration), or they were restricted to fast 2 hours before and after the Tac dose administration (i.e., no food, drinks, caffeine, or tobacco); concomitant drugs were administered simultaneously with Tac also on these occasions (i.e., "fasting" dose administration).

With this study design, 12-hour Tac PK were investigated following 4 different dosing scenarios: (1) fasting morning dose, (2) fasting evening dose, (3) real-life nonfasting morning dose, and (4) real-life nonfasting evening dose.

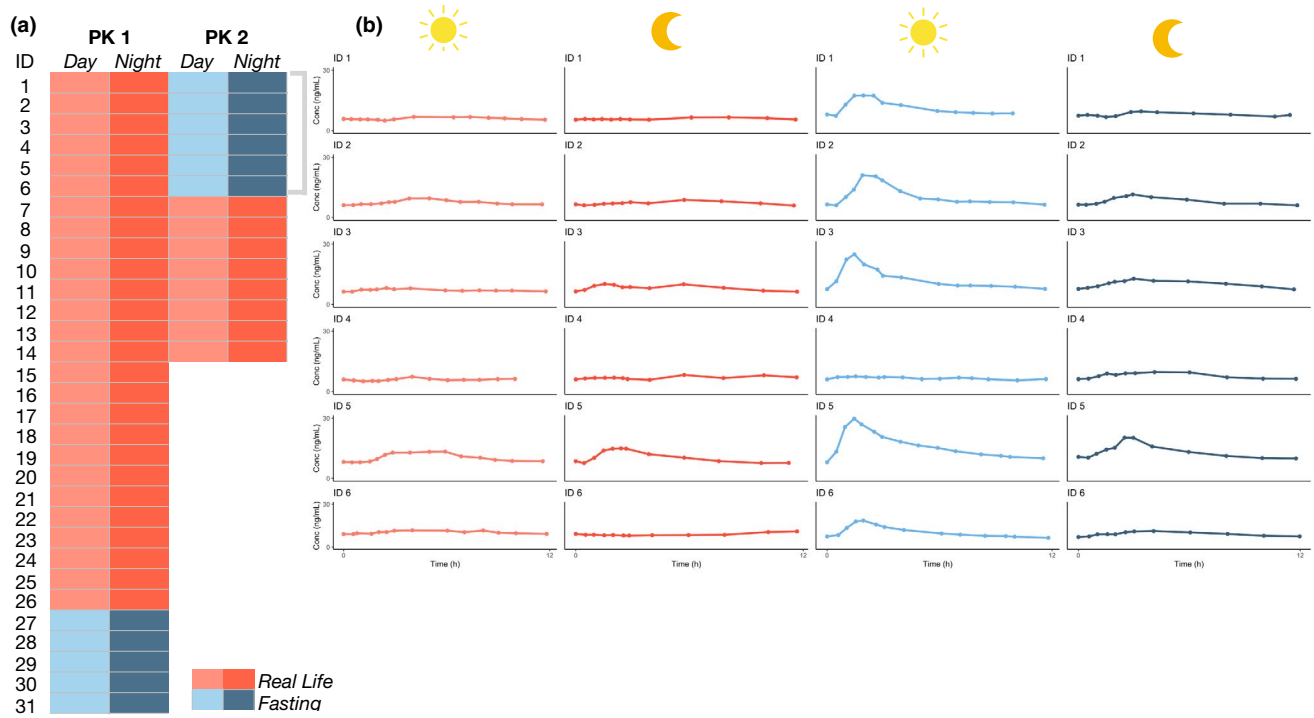

Figure 1 Overview of the pharmacokinetic (PK) investigations. (**a**) A table representing the PK investigations after the morning and the evening doses performed in a real-life nonfasting setting and under fasting conditions. The various colors represent the four different dose scenarios. The headings "Day" represents the morning dose and "Night" the evening dose. All 31 patients performed the PK investigation number 1 (PK1), and 14 patients performed the second investigation (PK2). (**b**) Six patients performed the PK investigations both in a real-life nonfasting setting and under fasting conditions, giving 12 hour PK-profiles with paired data from the 4 different dose scenarios. The various colors represent the four different dose scenarios, whereas the "sun" and the "moon" symbols represent the morning and the evening doses, respectively.

Pharmacokinetic calculations

The trapezoidal method was used to calculate AUC for the dose intervals after the morning dose ($AUC_{0–12}$) and the evening dose ($AUC_{12–24}$). In each dose interval, the maximum concentration (C_{max}) was determined as the highest observed concentration. The actual observed time of C_{max} (T_{max}) in relation to the respective dose administration was also determined. Three different trough concentrations were assessed on the investigation days: prior to the morning dose (C_0), prior to the evening dose (C_{12}), and 12 hours after the evening dose (C_{24}).

AUC determined by limited sampling strategies

Different LSS were used to predict individual Tac AUC using a previously developed and validated nonparametric population PK model as Bayesian estimator.[21,22] The model was adapted to also handle the flatter real-life nonfasting and evening-time PK profiles obtained in the present study (see **Supplementary Material**). The *makeAUC* function in the Pmetrics package for R (linear model) was used to calculate model-derived $AUC_{0–tau}$ values over respective dose interval.[23] The predictive performance for AUC determination when using the Bayesian estimator derived from the adapted model, used in combination with different LSS, was evaluated in a validation dataset, not previously used for developing the adapted model, by comparing the different LSS-derived AUCs with respective trapezoidal determined AUCs.

The LSS tested included the validated sampling times of 0, 1, and 3 hours, as previously published[12] and single trough concentrations (C_0 for $AUC_{0–12}$ and C_{12} for $AUC_{12–24}$). In addition, the multiple model optimal sample time function (MMopt) in Pmetrics,[23] weighted for AUC, was used to determine the best LSS using three optimal sampling times for the real-life nonfasting and evening PK-profiles. The MMopt function in the Pmetrics package for R was used to determine the sampling times that minimize the risk of misrepresenting the patients as the wrong set of support points in the model (i.e., estimating the wrong set of individual PK parameters).[24]

Statistical analyses

Population characteristics are summarized as median (range). For comparison between the paired PK variable following the morning and evening doses of Tac, the Wilcoxon signed rank test was used. The different trough concentrations were compared using nonparametric Friedman test, and correlation between AUC and trough concentrations were calculated using Spearman's rank correlation coefficient. A two-tailed P value < 0.05 was considered significant.

Agreement between the respective LSS derived AUCs and the trapezoidal determined reference AUC were assessed by C-statistics, with concordance correlation coefficient (CCC), total deviation index (TDI), and coverage probability (CP),

as previously described.[25] CCC is a correlation coefficient measuring the agreement between two measurements; the values can range from 0 to 1, where 1 reflects perfect correlation and 0 no correlation. TDI is a measure of the proportion of data within a pre-set boundary for an allowed difference between the reference and the estimations. CP can range from 0 to 1, and is an estimate of whether a given TDI is less than a prespecified fixed percentage. Predefined accepted agreement levels were determined to be: CCC $\geq$ 0.9, TDI $\leq$ 15%, and CP $\geq$ 0.85.[12]

RESULTS

Patients

Thirty-one stable renal transplant recipients (74% men) were prospectively enrolled in the study between 13 and 54 days post-transplant. Demographic data and patient characteristics are presented in **Table 1**, and were considered representative of our kidney transplant population. All included patients, except one, received concomitant MMF. Four (13%) were immunological high-risk patients and three patients (10%) were CYP3A5 expressers (all *CYP3A5*1/*3*). No overall difference in Tac PK was observed between CYP3A5 expressers and nonexpressers.

All patients performed 2 successive 12-hour PK investigations (morning and evening doses), and 14 patients (45%) also repeated these PK investigations within 7–28 days (median 14 days; **Figure 1**). A total of ninety 12-hour PK profiles: 45 from the morning dose and 45 from the successive evening dose were obtained in the present study. In 11 of these morning-evening dose investigations (i.e., twenty-two 12-hour PK investigations), Tac was administered in fasting conditions, as defined in Methods. In the other 34 morning and evening dose investigations (sixty-eight 12-hour PK investigations), Tac was administered as in a real-life setting (**Figure 1**). Patients were told to do "as normal." Patient-reported time of food consumption for breakfast was < 0.5 hours before/after the morning dose, dinner 3–4.5 hours before the evening dose, and supper < 0.5 hours before/after the evening dose.

Table 1 Patient characteristics and demographic data (*n* = 31)

	Number (%)	Median (range)
Male	23 (74)	
Living donor	15 (48)	
First transplant	28 (90)	
Pre-emptive transplantation	13 (42)	
Standard immunological risk	27 (87)	
CYP3A5 genotype		
**1/*1*	0 (0)	
**1/*3*	3 (10)	
**3/*3*	26 (84)	
Unknown	2 (6)	
Age, years		62 (22–78)
Height, cm		175 (159–192)
Weight, kg		79 (52–103)
Donor age, years		55 (6–73)
Time since transplantation to PK1, days		22 (13–54)
P-creatinine, μmol/L		122 (70–192)
Hematocrit, %		36 (29–44)
Tacrolimus dose, mg/day		5 (3–14)
Prednisolone dose, mg/day		15 (7.5–20)
Mycophenolate mofetil dose, mg/day		1,500 (720–1,500)

CYP3A5, cytochrome P450 3A5; PK1, first pharmacokinetic investigation.

Chronopharmacokinetics

Fasting dose administration. In fasting conditions, Tac PK displayed circadian variation (**Table 2**) with slower absorption and reduced exposure following the evening dose (**Figure 2**): AUC and C_{max} (median [range]) were significantly higher following the morning dose ($AUC_{0–12}$: 127 [77–200] μg h/L, C_{max}: 20.6 [7.4–31.8] μg/L) compared with the evening dose ($AUC_{12–24}$: 102 [84–155] μg h/L, C_{max}: 11.5 [9.4–20.3] μg/L), $P < 0.006$. Additionally, T_{max} was significantly shorter after the morning dose (1.5 [1.3–2.0] hours vs. 3.9 [2.0–10.1] hours), $P = 0.003$. However, there were no significant differences between the three respective trough levels: C_0 (7.5 [5.4–9.2] μg/L), C_{12} (7.1 [6.0–10.7] μg/L), or C_{24} (7.2 [6.0–9.9] μg/L), $P = 0.761$. The correlations among $AUC_{0–12}$, $AUC_{12–24}$, or $AUC_{0–24}$ with C_0, C_{12}, or C_{24} were only moderate and not statistically significant (**Table 3**).

Real-life nonfasting dose administration. Administering Tac in a real-life nonfasting setting showed slow absorption PK profiles without indication of circadian variation on PK

Table 2 Chronopharmacokinetics of tacrolimus under fasting and real-life nonfasting dose administration

	Fasting (*n* = 11) Median (range)	Real-life (*n* = 34) Median (range)
Tacrolimus total daily dose, mg	6 (3–11)	5 (3–14)
AUC, μg h/L		
Morning dose	127 (77–200)	82 (55–128)
Evening dose	102 (84–155)	80 (53–129)
Comparison	***P* = 0.006**	*P* = 0.083
C_{max}, μg/l		
Morning dose	20.6 (7.4–31.8)	8.9 (5.3–18.4)
Evening dose	11.5 (9.4–20.3)	8.4 (5.8–15.2)
Comparison	***P* = 0.008**	*P* = 0.334
T_{max}, hours		
Morning dose	1.5 (1.3–2.0)	4.0 (0.7–9.2)
Evening dose	3.9 (2.0–10.1)	4.1 (1.0–11.7)
Comparison	***P* = 0.003**	*P* = 0.077
C_{12}, μg/l		
Morning dose	6.6 (5.4–10.7)	5.9 (3.4–9.5)
Evening dose	7.2 (4.9–9.9)	5.6 (4.0–11.1)
Comparison	*P* = 0.286	*P* = 0.912

Data shown as AUC (calculated using the trapezoidal method), the observed C_{max} and T_{max}, and the measured concentration 12 hours after the dose (C_{12}).
Comparison between the morning dose and evening dose calculated using nonparametric Wilcoxon signed rank test.
Bold type indicates significant difference between the morning and evening doses.
AUC, area under the concentration vs. time curve; C_{max}, maximum concentration; T_{max}, time to reach maximum concentration; C_{12}, the concentration 12 hours after dose administration.

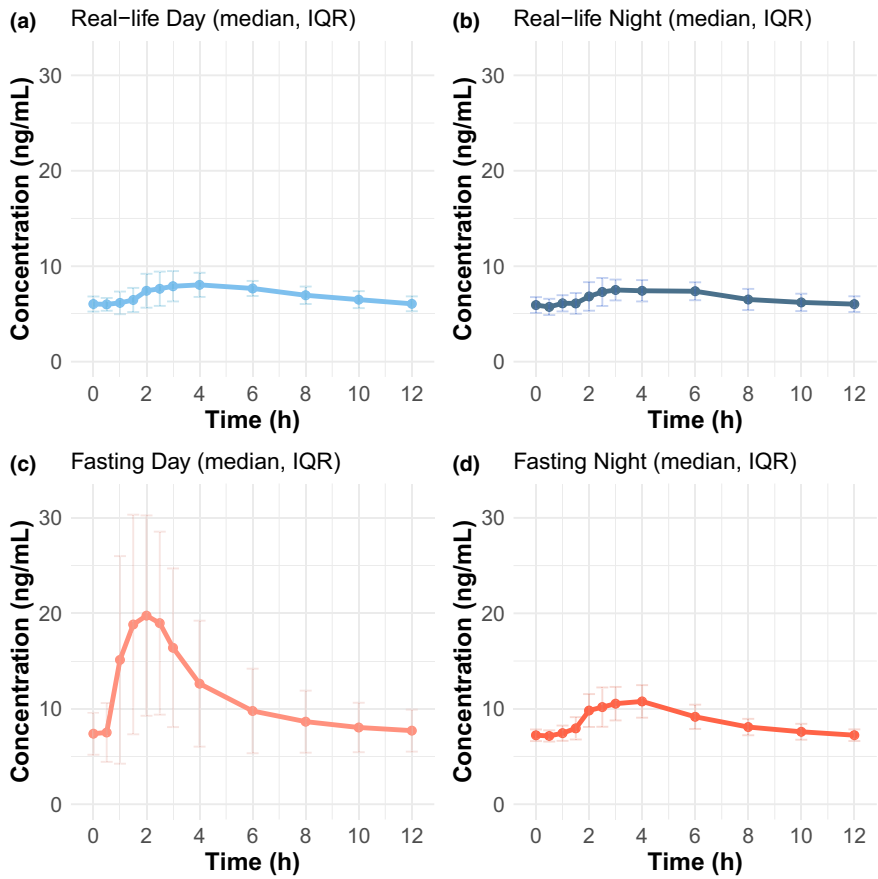

Figure 2 Median curves for the four different dose scenarios. Individual time-corrected concentrations were used to make median curves with related interquartile range (IQR) for the four different dose scenarios: (**a**) real-life nonfasting morning dose (n = 34 in the 12-hour PK-profiles), (**b**) real-life nonfasting evening dose (n = 34 in the 12-hour PK-profiles), (**c**) fasting morning dose (n = 11 in the 12-hour PK-profiles), and (**d**) fasting evening-dose (n = 11 in the 12-hour PK-profiles.

parameters (**Figure 2**). There were no differences in AUC, C_{max}, or T_{max} between morning and evening doses (**Table 2**). In addition, trough levels (median [range]) did not vary during the 24-hour dosing interval: C_0 (5.9 [3.5–9.2] µg/L), C_{12} (5.9 [3.4–9.5] µg/L), or C_{24} (5.5 [4.0–11.1] µg/L), P = 0.262. The correlations among $AUC_{0–12}$, $AUC_{12–24}$, or $AUC_{0–24}$ with any of the trough values C_0, C_{12}, or C_{24} were strong. The highest correlation coefficient was found for $AUC_{0–24}$ and C_{24} (**Table 3**, Spearman's rho 0.866, $P < 0.001$).

In 6 of the 14 patients repeating the PK investigations, both fasting and real-life nonfasting dose conditions were investigated. The paired PK-profiles from all four 12-hour dose intervals showed high variation (**Figure 1**). The population PK

Table 3 Correlations between AUC and trough concentrations under fasting and real-life nonfasting dose administration

	$AUC_{0–12}$		$AUC_{12–24}$		$AUC_{0–24}$	
	Fasting (n = 11)	**Real-life (n = 34)**	**Fasting (n = 11)**	**Real-life (n = 34)**	**Fasting (n = 11)**	**Real-life (n = 34)**
C_0	0.345 P = 0.298	0.820 **$P < 0.001$**	0.282 P = 0.401	0.801 **$P < 0.001$**	0.309 P = 0.355	0.857 **$P < 0.001$**
C_{12}	0.527 P = 0.096	0.807 **$P < 0.001$**	0.509 P = 0.110	0.818 **$P < 0.001$**	0.518 P = 0.102	0.859 **$P < 0.001$**
C_{24}	0.573 P = 0.066	0.799 **$P < 0.001$**	0.464 P = 0.151	0.838 **$P < 0.001$**	0.509 P = 0.110	0.866 **$P < 0.001$**

Reported Spearman's rank correlation coefficient.
AUC calculated using the trapezoidal method.
Bold type indicates significant correlation.
AUC, area under the concentration vs. time curve; $AUC_{0–12}$, area under the concentration vs. time curve after the morning dose; $AUC_{12–24}$, area under the concentration vs. time curve after the evening dose; $AUC_{0–24}$, total daily area under the concentration vs. time curve; C_0, trough concentration right before the morning dose; C_{12}, trough concentration right before the evening dose; C_{24}, trough concentration 12 hours after the evening dose.

Table 4 Population PK model derived parameter values for the four different dose scenarios

	Fasting morning dose[a] (*n* = 11)		Fasting evening dose[b] (*n* = 8)		Real-life morning dose[b] (*n* = 22)		Real-life evening dose[b] (*n* = 22)	
Parameters	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**
Absorption rate constant, Ka hours	1.50	0.33	0.79	1.35	0.70	0.84	0.21	1.43
Apparent clearance, L/h	12.9	11.2	22.0	5.8	18.3	16.1	25.7	11.5
Apparent intercompartment clearance, L/h	44.1	56.6	85.6	41.6	95.4	94.2	17.3	123.8
Apparent central volume of distribution, L	107	31	269	208	339	217	305	439
Apparent peripheral volume of distribution, L	866	1094	13457	9290	21626	16936	29956	16717
Lag time week 2–4 post-transplant, hours	0.52	0.40	2.02	0.47	1.86	2.46	1.27	1.23
Lag time after first month post-transplant, hours	0.58	0.26	2.32	2.45	1.28	1.89	1.52	2.61

IQR, interquartile range; PK, pharmacokinetic.
[a]Used the previous developed population PK model. [b]Used the adapted version of the previous developed population PK model (derived from the model-adaption dataset; see **Supplementary Digital Content, Methods page 1–2**).

parameters of the model are presented in **Table 4**. The absorption rate was higher and the apparent clearance lower for the fasting morning dose when compared with the fasting evening dose and the real-life nonfasting morning and evening doses.

Limited sampling strategy determined AUC

Fasting dose administration. The previously validated LSS of Tac with samples obtained at 0, 1, and 3 hours postdose predicted $AUC_{0–12}$ with high accuracy and precision (**Table 5**). CCC was 0.922 (95% confidence interval (CI): 0.800–1.0), reflecting high precision and accuracy for the LSS-predicted AUCs. TDI was 13.4 (95% CI: 6.5–20.3), which means that 85% of the predicted AUCs showed an error ranging from –13.4% to +13.4% compared with reference (trapez) AUC. CP was 0.854 (95% CI: 0.607–1.0), which indicates that < 15% of the predicted AUCs had an error greater than ±15%. Using MMopt sampling times (1, 3, and 6 hours postdose) for the slow-absorption profiles (fasting evening dose) resulted in predicted $AUC_{12–24}$ of accepted agreement (**Table 5**) in the validation dataset (*n* = 3). A single trough concentration did not predict neither $AUC_{0–12}$ nor $AUC_{12–24}$ within the acceptance limit.

Real-life nonfasting dose administration. The LSS with samples obtained at 0, 1, and 3 hours postdose or a single trough concentration did not show acceptable agreement for real-life nonfasting AUC predictions (**Table 5**). Using the MMopt determined sampling times (1, 3, and 6 hours postdose) for predictions of both $AUC_{0–12}$ and $AUC_{12–24}$ showed overall better agreement with trapezoidal $AUC_{0–12}$ and $AUC_{12–24}$ compared with LSS 0, 1, and 3 hours for fasting conditions: CCC was 0.946 (95% CI: 0.897–0.995),

Table 5 Agreement between population PK estimated AUC, applying different number of samples, compared with reference AUC

Sampling times	CCC (95% CI)	TDI (95% CI)	CP (95% CI)
$AUC_{0–12}$ – fasting morning dose (*n* = 11)[a]			
Full-profiled, 12 samples	**0.991** (0.975, 1.0)	**4.6** (2.5, 6.7)	**1.0** (0.965 1.0)
3-sample LSS, 0, 1, and 3 hours	**0.922** (0.800, 1.0)	**13.4** (6.5, 20.3)	**0.854** (0.607, 1.0)
Trough only	0.482 (0.023, 0.914)	52.5 (18.8, 86.3)	0.331 (0.217, 0.445)
$AUC_{12–24}$ – fasting evening dose (*n* = 3)[b]			
Full-profiled, 12 samples	**0.988** (0.735, 1.0)	**3.2** (0, 15.0)	NA
3-sample LSS, 0, 1, and 3 hours	**0.938** (0.196, 1.0)	**7.7** (0, 30.4)	NA
Trough only	0.874 (0.165, 1.0)	12.5 (0, 42.0)	NA
3-sample LSS,[c] 1, 3, and 6 hours	**0.944** (0.340, 1.0)	**7.2** (0, 23.4)	NA
$AUC_{0–12}$ and $AUC_{12–24}$ – real-life morning dose and evening dose (*n* = 24)[b]			
Full-profiled, 12 samples	**0.974** (0.951, 0.997)	**7.7** (5.2, 9.9)	**0.994** (0.954, 1.0)
3-sample LSS, 0, 1, and 3 hours	0.788 (0.621, 0.955)	25.3 (17.2, 33.4)	0.608 (0.284, 0.455)
Trough only	0.424 (0.236, 0.612)	81.5 (54.0, 108.9)	0.227 (0.160, 0.294)
3-sample LSS,[c] 1, 3, and 6 hours	**0.946** 0.897, 0.995)	**11.2** (7.9, 14.5)	**0.934** (0.823, 1.0)

Reference AUC calculated using the trapezoidal method.
Bold type indicates better agreement than the prespecified boundaries: CCC ≥ 0.9, TDI ≤ 15, and CP ≥ 0.85.
AUC, area under the concentration vs. time curve; $AUC_{0–12}$, area under the concentration vs. time curve after the morning dose; $AUC_{12–24}$, area under the concentration vs. time curve after the evening dose; CCC, concordance correlation coefficient; CI, confidence interval; CP, coverage probability index; LSS, limited sampling strategy; NA, not available (too few samples – see **Supplementary Digital Content 1**); TDI, total deviation index.
[a]AUC calculated using the previous developed population PK model. [b]AUC calculated in the validation dataset using the adapted version of the previous developed population PK model. [c]LSS using sampling times closest to the multiple model optimal sampling times determined by the MMopt-function in Pmetrics.

and TDI and CP were 11.2 (95% CI: 7.9–14.5) and 0.934 (95% CI: 0.823–1.0), respectively.

DISCUSSION

In a real-life nonfasting setting, Tac does not show the well-known PK profile with a C_{max} about 20 μg/L after about 1–2 hours.[26] Instead, the PK profiles are flat, with a very slow absorption rate. C_{max} was less than half, and the systemic exposure about two-thirds of that obtained following morning dose administered under fasting conditions in the present study. It is indeed important to point out that this comparison was performed against fasting morning doses because Tac showed circadian variation when administered in a fasting state (but not in the real-life nonfasting setting); with slower absorption and flatter PK-profiles after the evening dose. When fasting, the AUC was on average 20% and C_{max} 45% higher after the morning dose compared with the evening dose. The circadian variation that was consistently observed after fasting Tac dose administration did, however, not influence the various trough levels investigated (C_0, C_{12}, and C_{24}). In the literature, there are some conflicting reports with respect to circadian variation of Tac exposure,[15,17,27,28] but there is a tendency in support of circadian variability under fasting conditions. After the evening dose and under real-life nonfasting dose administration, the absorption rate constant was lower, reflecting a slower absorption when compared with the fasting morning dose. In addition, apparent clearance was higher when compared with the fasting morning dose, most likely reflecting a decreased oral bioavailability rather than higher clearance. With adaptions of the parameter boundaries for absorption constant and lag time of a previous developed population PK model, AUC determinations were possible both for the fasting and the real-life nonfasting setting, but other optimal sampling time strategies were required.

Our data raise several important questions regarding current and future Tac TDM recommendations and evaluations. Some studies have demonstrated a satisfying correlation between Tac trough concentrations and AUC.[29,30] However, this has not been reproduced in other studies, and as in agreement with our fasting-day data, the general view is that the correlation between trough and AUC is relatively poor.[31–33] Although AUC is regarded the optimal measurement of drug exposure, for practical reasons, morning trough concentrations are today widely used in the routine for Tac dose individualization. According to the present results, it may, however, be that the correlation is greater in the real-life nonfasting setting, considering the flat curves and the strong correlation between C_{24} and $AUC_{0–24}$ (r = 0.866). Hence, one may argue that there is not so much to gain by doing AUC-monitoring, as trough in this setting better reflects the systemic exposure of Tac. It should also be kept in mind that the actual AUC in the fasting and nonfasting conditions are very different. If performing AUC-monitoring, data reflecting the real-life situation are needed to develop more clinically appropriate population PK models for dose individualization, because most PK models presented in the literature will not perform well on real-life data.

There is a lack of studies addressing the optimal total daily Tac exposure ($AUC_{0–24}$).[34] The proposed $AUC_{0–24}$ target ranges have to be redefined because fasting day and night AUCs are not similar, and the fasting-day AUC cannot just be doubled. For the last decades, we have been following the low-dose Symphony protocol from the time of transplantation.[5] With this approach, the 24-hour Tac AUC is in the lowest range of the suggested target,[10,35] mainly as a result of either the circadian variation when fasting or dose administration performed relatively close to food consumption. As almost all available PK studies and population PK models are based on fasting-day data, further research involving prospective studies investigating Tac $AUC_{0–24}$ in patients at different immunological risk and time after transplantation, performed during nonregulated conditions, where patients eat and take their medications as in their everyday routine, is strongly warranted. With the use of capillary microsampling and patients performing blood sampling at home, it might be possible to perform such clinical trials, within reasonable cost and effort boundaries for both patients and investigators.[12]

An important drawback of the clinical implementation of AUC-guided dosing of Tac is that blood samples are not convenient to obtain following the evening dose, and accurate predictions of the full 24-hour Tac exposure is thus not feasible. Based on the current data, we evaluated potentially clinical applicable sampling strategies for predictions of AUC following the evening dose ($AUC_{12–24}$; data not shown). Samples closest to the MMopt sampling times (1, 3, and 6 hours) were tested, but samples during sleep were avoided (between 11 PM and 7 AM). In this regard, the best strategy with the highest agreement in C-statistics was to use samples 1, 2, and 10 hours after the evening dose (e.g., at 10 PM and 11 PM and again at 7 AM the next morning when utilizing a 9 AM to 9 PM dosing scheme (CCC was 0.895 (95% CI: 0.795–0.995), TDI 16.6% (95% CI: 12.0–21.1), and CP 0.816 (95% CI: 0.620–1.0)).

Once-daily Tac is suggested to increase adherence.[36,37] An additional hypothesized clinical benefit of using once-daily Tac formulations has been to avoid the high peak concentrations and the large peak-to-trough variation, which is present with the twice-daily formulation (when administered in a fasting state).[38–40] Most of the patients at our transplant center take their Tac dose without respecting the ±2-hour fasting rule, and as clearly shown in the present study, the high peaks following administration of the twice-daily Tac formulation will then be avoided. This raises the question of the actual need and benefit of giving a prolonged-release formulation, as a close to similar PK-profile can be achieved by administering the twice-daily formulation closer to food consumption.

The main strength of the present study is the rich sampling obtained following both the morning dose and the evening dose of Tac. In total, 1,187 Tac samples have been investigated in the present study, on average, 26 per 24-hour PK investigation. This ensures detailed individual description of Tac PK during the full 24-hour interval. Second, the study was performed in a real-life setting: patients took their medications as in their everyday routine. This study obviously also has some limitations. First,

this study is performed in the early post-transplant phase, and because Tac PK change during the first 6–12 months after transplantation,[15,35,41] the results from the present study should be extrapolated with care to the long-term follow-up situation. Second, even though the validation metrics of the adapted population PK model were convincing, relatively few PK-profiles of the different dose scenarios were included in the development and validation datasets (see **Supplementary Material**), so the results have to be interpreted with caution. The numbers of patients with complete dual data, performing both fasting and real-life nonfasting investigations, are very low (n = 6). Finally, only the immediate-release formulation of Tac was investigated. It will be important to also investigate if these effects are present with prolonged-release formulations.

These findings raise several questions pertaining to the optimal monitoring of Tac in a standard clinical setting. If the exposure of Tac following an evening dose is less influenced by intake of food, such restrictions are unnecessary and can be omitted when advising patients on their drug habits.

In summary, our results demonstrated that dosing Tac in real-life, without respecting the ±2-hour fasting rule, showed rather flat PK-profiles and no circadian variation. Dosing Tac under fasting conditions in the morning produced the well-known Tac PK-profile, with a sharp peak after ~ 1–2 hours. Circadian variation was present with fasting administration and the profiles after the evening dose were flat and quite similar to the real-life nonfasting profiles. Following real-life nonfasting dose administration, the correlation between trough (C_{24}) and total daily exposure ($AUC_{0–24}$) was high. LSS in combination with population PK model-derived Bayesian estimators was able to accurately predict AUC for both fasting and real-life nonfasting dose administration, but different optimal sampling times for predictions of AUC were required. Data on the real-world behavior of the patients are needed for a population PK model to predict AUC during both dose scenarios. Whether this will improve long-term outcome needs to be verified in a large prospective clinical trial.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

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