AUC-guided dosing of tacrolimus prevents progressive systemic overexposure in renal transplant recipients

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Background. Tacrolimus has a narrow therapeutic window, and bioavailability is known to vary considerably between renal transplant recipients. Most centers still rely on measurement of trough levels, but there are conflicting reports on the correlation between tacrolimus trough levels and systemic exposure, as measured by the area-under-the-concentration-over-time curve ($AUC_{(0-12h)}$).

Methods. We developed and validated a two-compartmental population-based pharmacokinetic model with Bayesian estimation of tacrolimus systemic exposure. Subsequently, we used this model to apply prospectively AUC-guided dosing of tacrolimus in 15 consecutive renal transplant recipients. The main objective was to study intrapatient variability in the course of time.

Results. Bayesian forecasting with a two-point sampling strategy, a trough level, and a second sample obtained between two and four hours post-dose significantly improved the squared correlation with the $AUC_{(0-12h)}$ ($r^2 = 0.94$). Compared with trough level monitoring only, this approach reduced the 95%-prediction interval by 50%. The Bayesian approach proved to be feasible in clinical practice, and provided accurate information about systemic tacrolimus exposure in individual patients. In the AUC-guided dosing cohort the apparent clearance of tacrolimus decreased gradually over time, which was not reflected in corresponding trough levels.

Conclusion. This simple, flexible method provides the opportunity to tailor immunosuppression, and should help minimize tacrolimus-related toxicity, such as nephrotoxicity and post-transplant diabetes mellitus.

The currently available calcineurin inhibitors cyclosporine microemulsion and tacrolimus both have a narrow therapeutic window, which makes regular drug monitoring necessary. Most transplantation centers adjust the dose of these drugs to reach or maintain a defined trough level range. Especially for cyclosporine, several studies have already documented that estimates of systemic drug exposure, and the absorption profile in particular, represented by the area-under-the-concentration over time curve (AUC), correlate better with clinical events (acute rejections, nephrotoxicity) as compared with trough levels [1–3]. Prospective studies in de novo renal transplant recipients have shown that targeting certain predefined cyclosporine $AUC_{(0-4h)}$ or C2 levels was associated with a very low incidence of acute rejection episodes [4, 5].

For tacrolimus, most centers still rely on trough level monitoring, but there are conflicting data about the correlation with systemic exposure. Some studies reported a reasonable squared correlation coefficient between trough levels and tacrolimus $AUC_{(0-12h)}$ (r^2 0.60 to 0.85) [6–8], others found poor correlations ($r^2 < 0.50$) [9, 10]. The observed differences may originate from at least three factors, including differences in sample sizes, type of correlation tests used, and time interval after transplantation, at which the studies were done.

In general, a considerable variation in $AUC_{(0-12h)}$ can be expected in relation to a single trough level, which of course, is augmented by the range of trough levels that is thought to be acceptable in clinical practice (e.g., tacrolimus trough levels between 10–20 ng/mL early post-transplant and 5–10 ng/mL in stable transplant recipients). Similar to cyclosporine, patients with low systemic tacrolimus exposure in relationship to tacrolimus trough levels could be expected to have an increased risk of developing acute or chronic rejection, whereas patients with a high AUC/C_0 ratio are likely to be overdosed.

Prevention of tacrolimus overexposure by AUC monitoring may be relevant to reduce nephrotoxicity,

hypertension, and hypercholesterolemia, but the impact of controlled systemic exposure on these side effects has not been studied. The increased risk for tacrolimus-treated patients to develop post-transplant diabetes mellitus [11] and polyoma-virus–associated nephropathy [12], in comparison with cyclosporine treated patients, may theoretically be reduced by AUC monitoring. Before the impact of tacrolimus drug exposure can be studied over longer periods of time, a simple and flexible strategy is needed to estimate systemic drug exposure, since "full" 12-hour AUC sampling is not a realistic option in daily practice. The major disadvantage of limiting sampling models with a mathematically derived equation [9] is the imperative of accurate timing of the blood samples. When a sample is taken 15 minutes too late, the mathematic equation is no longer valid [13].

Bayesian forecasting is a therapeutic drug monitoring tool that uses pharmacokinetic parameter estimates (such as mean population drug clearance and volume of distribution) along with expected associated variability and information about the patient (e.g., body weight, renal function) to predict drug concentrations achieved with specific doses [14]. Pharmacokinetic parameters for each patient become individualized, and the influence of the population parameters decreases [15]. Optimally, these techniques also inform the clinician of the next appropriate dose to maintain or reach the desired drug concentration. The number of blood collections needed, and the time to reach the required drug concentrations can be reduced [15–17]. We previously described a population-based, two-compartmental computer model for cyclosporine [18] that uses Bayesian forecasting combined with a limited sampling strategy, and now used the same program to analyze pharmacokinetic data obtained in tacrolimus-treated renal transplant recipients. After building and validating a new model for tacrolimus, we prospectively applied AUC-guided dosing to a cohort of de novo patients during the first postoperative year. The main objective was to study intraindividual pharmacokinetic changes in this for systemic exposure standardized cohort, in order to be able to design an optimal pharmacokinetic monitoring strategy for tacrolimus-treated renal transplant recipients.

METHODS

Population-based model

Using the kin pop module of the pharmacokinetic software package MW/Pharm version 3.33 (Mediware, Groningen, the Netherlands) [16], a population two-compartment model with a lag-time and first-order absorption pharmacokinetics was calculated using the tacrolimus dose and the blood concentration values of 20 tacrolimus curves (blood concentration at t = 0, 1, 2, 3, 4, 6, 8, 12 hours) obtained from 17 renal trans-

Table 1. Tacrolimus pharmacokinetic parameters derived from the model building set of 20 curves, obtained in 17 renal transplant recipients

| Parameter		Mean (SD)
t_{lag} *hours*	Lag time	0.956 (0.161)
F	Oral bioavailability	0.23 (fixed)
Ka h^{-1}	Absorption rate constant	0.580 (0.524)
V_1 *L/kg*	Apparent volume of distribution of central compartment	0.180 (0.063)
K_{elm} h^{-1}	Elimination rate constant	0.517 (0.096)
K_{12} h^{-1}	Distribution rate constant (central to peripheral compartment)	2.850 (2.219)
K_{21} h^{-1}	Distribution rate constant (peripheral to central compartment)	0.384 (0.410)

Table 2. Characteristics of renal transplant recipients whose tacrolimus curves (N = 64) were used for validation of the model, and of patients treated with AUC-guided dosing

	Validation set (N = 26) Mean (range)	AUC-dosing set (N = 15) Mean (range)
Age *years*	46.9 (20–65)	45.9 (33–65)
% Male	65	80
Renal disease		
Hereditary	27%	33%
Glomerulonephritis	31%	27%
Hypertension, nephrosclerosis	19%	20%
Other or unknown	23%	20%
Race		
Caucasian	80%	87%
African	8%	13%
Oriental	12%	0%
Body weight *kg*	78.5 (53–114)	81.5 (70–108)
Procedure		
Cadaveric, heart-beating	39%	47%
Cadaveric, non–heart-beating	15%	13%
Living related donation	31%	27%
Living unrelated donation	15%	13%
Delayed graft function (need for dialysis)	NA	20%
Cockroft clearance *mL/min*	60.4 (12–96)	65.6 (16–95) at 1 year

plant recipients (6 females, 11 males; mean age 45.4 years, mean body weight 73.1 kg; 30% living donation), and taken at different time points post-transplantation (11 curves between 2 and 6 weeks, 9 curves between 6 and 52 weeks post-transplantation). Whole blood concentrations (ng/mL) were determined by micro particle enzyme immunoassay (MEIA; Abbott Laboratories, Abbott Park, IL, USA). The MW/Pharm program uses an iterative two-stage Bayesian procedure to calculate the mean and standard deviations of the relevant pharmacokinetic parameters, as shown in Table 1.

Validation of the model and different limited sampling strategies

The population-based model was validated in another cohort of 26 renal transplant recipients. The characteristics of this validation group are summarized in Table 2. The calculated mean population pharmacokinetic parameters were individualized for each of 64 curves
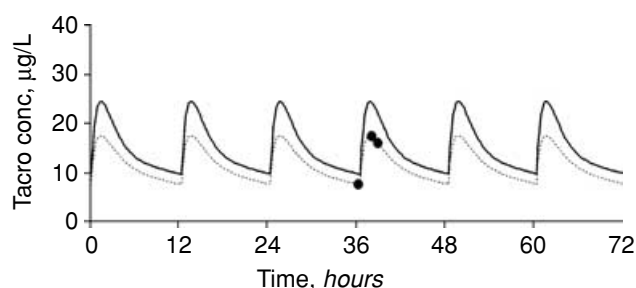
Fig. 1. Tacrolimus blood concentration time curve according to the population-based model (continuous line), the measured tacrolimus blood concentrations at t = 0h, 2h, 3h in a patient (●), and the tacrolimus blood concentration time curve according to the model after fitting the population parameters to the measured concentrations (dotted line), after which the AUC_{0-12h} is calculated by the model.

(blood concentration data points at t = 0, 1, 2, 3, 4, 6, 8 hours), based on the tacrolimus dose and different sampling methods, using the maximum a posteriori (MAP) Bayesian fitting method. Twenty-two of the curves were obtained in the early postoperative phase (i.e., within two weeks after transplantation), 42 curves were obtained between six weeks and 52 weeks post-transplantation. For each of the combination of time points, individualized pharmacokinetic parameters were calculated with the model, from which the AUC_{0-12h} was derived (Fig. 1). For comparison, the AUC_{0-12h} of the 64 curves was also calculated using the equation based strategy as described by Wong et al [9]: $AUC_{0-12h} = 16.2 + 2.4 \times C2h + 5.9 \times C4h$. The "standard" or reference AUC_{0-12h} of the 64 curves was calculated from all tacrolimus blood concentrations using the trapezoidal method (Kin fit module; MW/Pharm).

Statistics

The AUCs calculated by the different methods were compared to the standard AUC by Pearson's correlation coefficient. C0h (trough levels), C1h, C2h, C3h were also correlated to the standard AUC by Pearson's correlation coefficient. Predictive performance was investigated by calculating the prediction precision and bias according to Sheiner and Beal [19].

AUC-guided dosing

This study was approved by the Medical Ethic Committee of the Leiden University Medical Center. Fifteen consecutive de novo renal transplant recipients were included, and their characteristics are summarized in Table 2. These patients were prospectively treated according to the following AUC-guided dosing protocol: tacrolimus (starting dose 0.1 mg/kg) was given in a twice-daily schedule starting three hours before surgery. In the first week, target trough levels were 12.5 ng/mL (range 10–15 ng/mL). Tacrolimus "full" AUC_{0-12h} was deter-

mined at weeks 2, 6, 12, 26, and 52 using the pharmacokinetic model. Limited sampling estimates of AUC_{0-12h} were obtained at weeks 4, 8, 10, 17, 21, and 39. After each AUC-assessment dose adjustments were made to reach the predefined target AUC_{0-12h}: 210 ng.h/mL within the first six weeks (corresponding with a trough level of 12.5 ng/mL, derived from the model using mean population-based PK parameters), and 125 ng.h/mL thereafter (corresponding with a trough level of 7.5 ng/mL). Because according to the model there was a linear correlation between dose and AUC, dose adjustments were made by the model according to the formula $D_{new} = D_{current} \times AUC_{target}/AUC_{current}$. Concomitant immunosuppressive medication consisted of prednisolone (100 mg day 1–3, 50 mg day 4, 20 mg day 5–14, 15 mg day 15–21, 10 mg after day 22), mycophenolate mofetil, 500 mg b.i.d., and basiliximab prophylaxis, 20 mg on days 0 and 4. Drugs that are known to alter concentrations of tacrolimus were prohibited.

Statistics

Mean pharmacokinetic variables (±SD) resulting from this strategy [C_{trough} (AUC/dose), Tmax, Cmax] were calculated from data obtained at time points of full 0- to 12-hour AUC sampling (t = 2, 6, 12, 26, and 52-week post-transplantation) and analyzed by repeated measurements analysis of variance (ANOVA) (SPSS, version 11.0, Chicago, IL, USA).

RESULTS

AUC monitoring

The relationship between tacrolimus trough levels and standard $AUC_{(0-12h)}$, as calculated by trapezoidal rule, is plotted in Figure 2A. We found a squared correlation coefficient of 0.79, which was comparable with previous studies [6–8]. As a single sample strategy, the three-hour postdose tacrolimus level had an improved correlation ($r^2 = 0.88$) with the standard AUC, but still a wide range of the 95% prediction interval, as is illustrated in Figure 2B, indicating that the precision is not optimal. The correlation between the Bayesian estimates of $AUC_{(0-12h)}$ using the tacrolimus concentrations at 0 and 3 hours and the standard $AUC_{(0-12h)}$ was significantly better ($r^2 = 0.96$, Fig. 2C). This strategy resulted in a markedly improved precision for an individual measurement. The squared correlation coefficients, bias, and precision of all the strategies tested to estimate systemic exposure compared with the standard $AUC_{(0-12h)}$ as determined by trapezoidal rule are summarized in Table 3. All two-point strategies including a trough level with either a two-, three-, or four-hour sample had a strong correlation with the standard AUC ($r^2 = 0.94, 0.96$, and 0.95, respectively). Introduction of more samples further
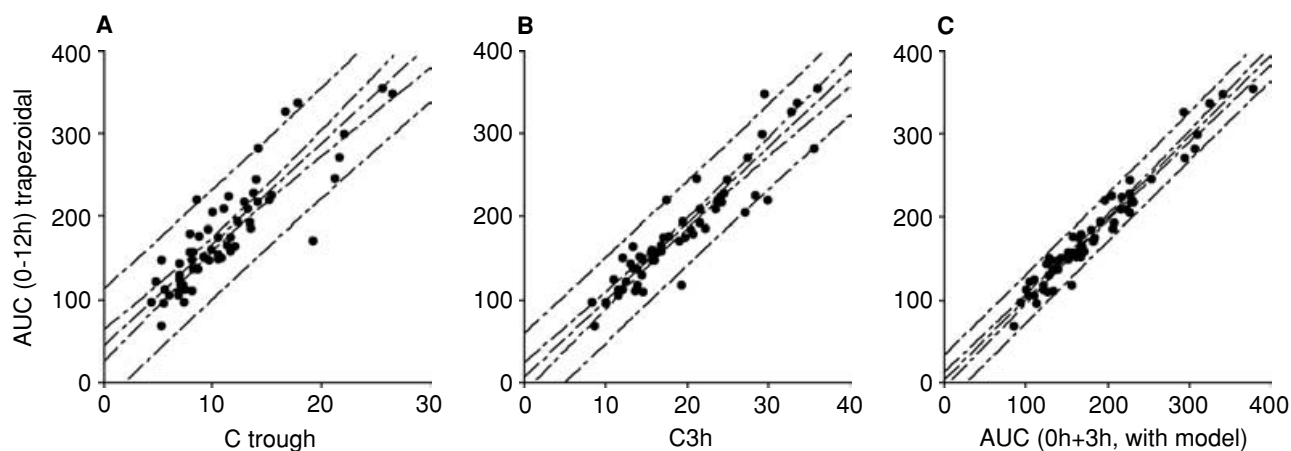
Fig. 2. Relationship between either (*A*) predose level (C$_{trough}$, ng/mL), (*B*) a single sample at 3 hours postdose (C3h, ng/mL), or (*C*) Bayesian estimates of AUC$_{0-12h}$ using blood concentrations at 0 and 3 hours postdose (AUC 0h +3h, ng.h/mL) and the AUC$_{0-12h}$ calculated by trapezoidal rule. The inner lines (– · –) demonstrate the 95% confidence interval, the outer lines the 95% prediction interval.

improved the estimation of systemic exposure. The performance of the limited sampling model, as described by Wong, was comparable ($r^2 = 0.92$), but the imperative of exact timing to draw samples at two and four hours postdose make this approach inflexible and less attractive for daily practice. When only the curves obtained two weeks after transplantation were evaluated, the correlation of trough levels with the standard AUC was even worse ($r^2 = 0.67$). In contrast, the correlations of Bayesian estimates derived from all limited sampling procedures were hardly affected (Table 3). This underlines not only the large interpatient variability especially in the early posttransplant period, but also the robustness of the model.
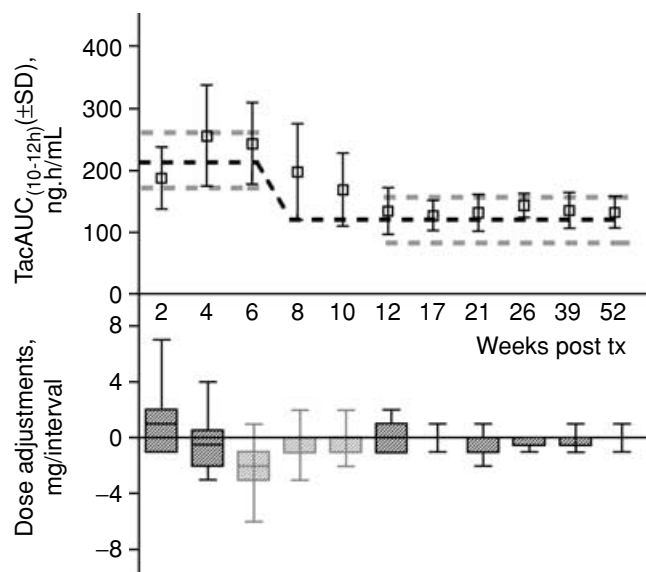
AUC-guided dosing

A total of 15 consecutive de novo renal transplant recipients received tacrolimus-based immunosuppression, and were prospectively dosed using the described model and predefined AUC targets. Cumulative incidence of biopsy proven acute rejection at one year was 6.6% (1 of 15 patients). The only acute rejection episode we encountered occurred at day four post-transplantation in a living, unrelated donation procedure, and could not be attributed to a low tacrolimus AUC. Patient and graft survival at one year both were 100%. At one year post-transplantation, mean GFR, as calculated by the Cockroft formula, was 66 mL/min (SD ± 21), mean total cholesterol 5.3 mmol/L (SD ± 1.3, 32% of patients used statins), mean systolic blood pressure 139 mm Hg (SD ± 16), mean diastolic blood pressure 82 mm Hg (SD ± 9), mean number of antihypertensive drugs per patient 1.6 (SD ± 1.0). In Figure 3, for every time point (weeks) at which tacrolimus AUC was estimated, both the mean actual AUCs (± SD) and dose corrections (mg/12 hrs) needed to reach the predefined AUC targets (dotted

Table 3. Pearson correlation coefficients (r^2), mean prediction error (MPE), and mean absolute prediction error (MAPE) of different strategies to estimate systemic exposure compared with AUC$_{0-12h}$ determined by trapezoidal rule, based on 64 curves of 26 renal transplant recipients treated with tacrolimus

Sampling strategy	r^2 (All curves)	MPE (%)	MAPE (%)	r^2 <2 wks post Tx (22 curves)	r^2 >2 wks post Tx (42 curves)
0h	0.79	−3.0	13.4	0.67	0.78
1h	0.48	−7.6	21.2	0.77	0.36
2h	0.77	−3.4	13.3	0.86	0.74
3h	0.88	−1.3	9.5	0.82	0.91
0, 2h[a]	0.94	1.4	7.6	0.93	0.95
0, 3h[a]	0.96	0.3	7.1	0.97	0.96
0, 4h[a]	0.95	−1.3	6.7	0.94	0.96
0, 2, 3h[a]	0.96	0.8	6.4	0.96	0.97
0, 1, 3h[a]	0.97	3.7	6.5	0.97	0.98
0, 2, 4h[a]	0.97	−1.1	5.2	0.96	0.98
0, 1, 2, 3h[a]	0.97	2.7	6.0	0.97	0.98
0, 1, 2, 3, 4h[a]	0.98	1.4	4.8	0.98	0.99
0, 1, 2, 3, 4, 6, 8, 12h[a]	0.99	−0.4	2.2	0.99	0.99
16.2+2.4*C2h +5.9*C4h	0.92	−6.5	8.2	0.96	0.92

[a]Bayesian estimation.

lines) are plotted. It is important to stress that no dose corrections were made in between these time points. There were no episodes of suspected nephrotoxicity, resulting in dose reductions. Despite the corrections made at week two and week four ("early phase"), the standard deviation of tacrolimus AUCs at week four and week six was considerable. These data indicated that intraindividual changes in pharmacokinetic parameters are still occurring in this early phase after renal transplantation. Between week six and 12 by protocol the target AUC was stepwise reduced to 125 ng.h/mL. The AUCs obtained at 12 weeks and later were defined as the "steady phase,"

Fig. 3. AUC-guided tacrolimus dosing in de novo kidney transplant recipients. Upper: Mean tacrolimus AUCs (± SD) in ng.h/mL and target AUC (dotted line) ± 20% (gray dotted lines). Lower: Dose corrections (± SD and range) in mg, calculated by the model, to reach these targets. Beyond 6 weeks (according to the protocol), the target AUC was lowered, which was implemented gradually between 6 and 10 weeks post-transplantation for safety reasons. For this reason, the dose corrections at these time points are shown in gray.

and showed stabilization of the intrapatient variability. This was also reflected in a decrease of dose corrections in the "steady phase" needed to maintain the AUCs within the target range.

Further analysis of the tacrolimus curves, obtained at week two and week six, indicated an increase in the AUC that was predominantly determined by the absorption phase of the curve (Fig. 4A and B). Not only in the "early phase," but also between weeks 12 and 52, there was a rise in the tacrolimus dose corrected concentration curves (Fig. 4C and D). The corresponding pharmacokinetic variables are summarized in Table 4, showing a steady and significant increase over time of (AUC/dose), while this was not reflected in a change of (C_{trough}/dose) in the "stable phase" ($P = 0.01$, repeated measurements ANOVA analysis, linear test of within subjects contrast).

DISCUSSION

There is increasing evidence that dosing of cyclosporine microemulsion, guided by estimation of the absorption profile of the drug, is superior as compared with trough level monitoring. Adequate systemic exposure was associated with a reduced incidence of acute rejection episodes, cyclosporine-related nephrotoxicity, and cardiovascular risk factors, including hypertension and hypercholesterolemia. A critical appraisal of tacrolimus monitoring by trough levels only has thus far not received much attention, partly because earlier reports suggested that tacrolimus trough levels correlated better with systemic exposure [8]. Our data indicate that also in case of maintenance therapy with tacrolimus, a trough level is not a reliable tool to estimate systemic exposure in renal transplant recipients. Until now, no conclusive prospective trials [10, 20] have been published that evaluated the correlation between tacrolimus exposure and rejections or side effects. Kuypers et al [21] reported a tendency for a lower incidence of acute rejection in relationship with simultaneous, adequate AUCs for both tacrolimus (>150 ng.h/mL) and mycophenolate mofetil (>45 ng.h/mL) at day seven compared with patients who did not reach both targets. Although no differences in AUC were found in patients with versus patients without hypertension, hyperlipidemia, and nephrotoxicity, they did report a significant higher AUC in patients with infectious complications compared to patients without infections.

Our population-based computer model with Bayesian fitting improved significantly the estimation precision to predict the AUC, requiring a trough level in combination with only one additional, but timed, sample. For illustration: a trough level of 7.5 ng/mL corresponded with a mean $AUC_{(0-12h)}$ of 135 ng.h/mL, with a 95% prediction interval of 69 to 202 ng/mL. This interval could be reduced to 108 to 166 ng/mL when a two-point (0 and 3 hours) strategy was followed. By analogy, the 95% prediction interval of a trough level of 12.5 ng/mL could be improved from an AUC range from 162 to 294 to a range of 198 to 256 ng.h/mL.

The major advantage of this model is that it can handle sampling at any time point between two and four hours postdose without influencing its predictive performance. This approach is simple and feasible in the outpatient clinic setting. In contrast, limited sampling strategies based on a mathematical formula become useless when the obligatory predefined time points are not met. Also, monitoring guided by a single C2h or C3h level is very much dependent on accurate timing of the intake of the drug and the drawing of the samples [13].

We tested our model-based approach prospectively in a cohort of renal transplant recipients at various time points in the first postoperative year. Early after transplantation, there was a change in the absorption phase in at least 50% of the patients we studied. The performance of the model combined with two-point sampling, however, was not influenced by these time-dependent changes in individual pharmacokinetics. In contrast, the worst correlation of trough levels with the trapezoidal AUCs was found in the early period after transplantation ($r^2 = 0.67$). Changes in absorption or elimination of tacrolimus, resulting in suboptimal systemic exposure, are not reliably identified by trough level monitoring. This is illustrated by the fact that in our data (validation set and AUC guided dosing set) only a limited fraction of curves with a trapezoidal AUC
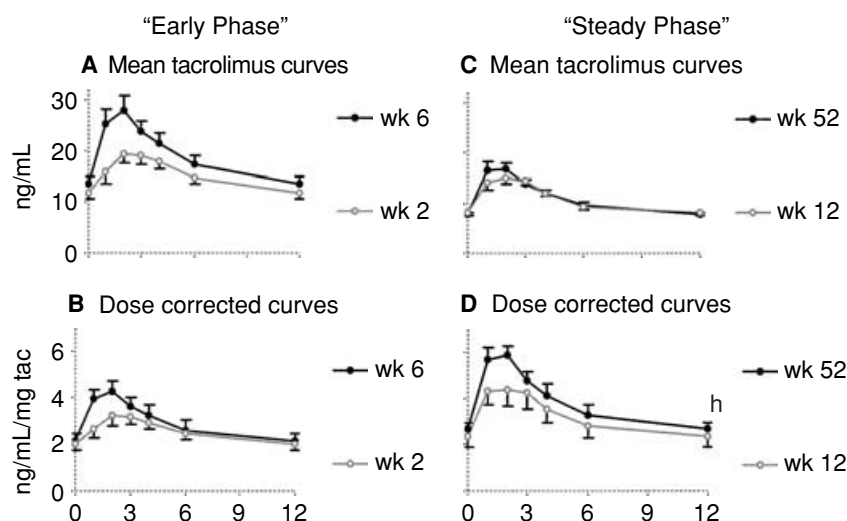
Fig. 4. Tacrolimus concentrations (mean ± SE) (*A*, *C*) and dose-corrected tacrolimus curves (*B*, *D*) in the early phase post-transplant (target AUC: 210 ng.h/mL) (A, B), and in the "steady" phase (target AUC: 125 ng.h/mL) (C, D) in de novo kidney transplant recipients.

Table 4. Mean values of pharmacokinetic variables with time

Weeks post Tx	AUC (0-12h) ng.h/mL (±SD)	C_{trough} ng/mL (±SD)	Tmax minutes post dose	Cmax ng/mL	Dose tacro mg/12h	Body weight kg	Dose/weight mg/kg	C_{trough}/dose conc./mg	AUC/dose AUC/mg
(Early phase)									
2 weeks	181 (± 50)	11.9 (± 4.7)	130	23.1	6.0	77.5	0.077	2.1	30[a]
6 weeks	224 (± 67)	13.6 (± 5.5)	121	29.5	6.5	74.8	0.087	2.5	34[a]
(Steady phase)									
12 weeks	129 (± 35)	8.0 (± 2.4)	108	17.5	3.4	74.5	0.046	2.8	38[a]
26 weeks	139 (± 20)	8.1 (± 2.1)	92	19.1	3.4	76.0	0.044	2.8	42[a]
52 weeks	130 (± 23)	7.7 (± 1.6)	78	18.5	2.9	76.8	0.038	2.9	45[a]

[a]Significant, $P < 0.01$ by repeated measures ANOVA analysis.

deviating more than 20% from the target AUCs would have been detected using trough levels. Using Bayesian estimates with sampling at zero and three hours post-dose, this proportion is considerably higher (Table 5). To narrow the range of accepted trough levels would only result in a limited improvement of the proportion and, of course, impair the test specificity. For example, in the stable period, the sensitivity and specificity of a trough level >9 ng/mL to predict an AUC >150 ng.h/mL were 0.65, respectively, 0.88 [for comparison: trough level >10 ng/mL: sensitivity 0.50, specificity 0.90; AUC(0h3h) >150 ng.h/mL: sensitivity 0.95, specificity 0.98].

In the first months after transplantation we observed a considerable standard deviation of the actual reached AUCs despite dose adjustments. This indicated that early after transplantation an AUC measurement has a limited predictive value for drug exposure in the following weeks, which still makes, in our opinion, frequent monitoring necessary. After this period only minor dose corrections were needed to maintain patients within the defined AUC target range (100–150 ng.h/mL). The difficulty to reach the target AUC in the first weeks post-transplantation reflects the changes in intra-individual PK parameters in this time period. Repeated measurements every three or four days could help to signalize these changes earlier,

whereas the simplicity of the model facilitates these kinds of strategies. Also, the system could theoretically be improved by putting the time-related PK changes into the model. However, the disadvantage of this kind of strategies would be that the simplicity of the model is affected.

In this intensively monitored group of patients with controlled systemic exposure, the most striking change in pharmacokinetic parameters was the consistent increase of dose corrected AUC [(AUC/dose)] of tacrolimus with time. Since AUC/dose is equal to F/CL, this is a reflection of a decrease in apparent clearance, which can be a result from either an increasing bioavailability, by improving of absorption, or a decrease in the actual elimination clearance of tacrolimus over time. Especially early post-transplantation, the change of the absorption profiles, as shown in Figure 3, suggests that changing absorption kinetics in individual patients may be the principal cause for this phenomena. Presystemic metabolism of tacrolimus by the gastrointestinal cytochrome P450 3A (CYP3A) isoenzymes and removal by P-glycoprotein transport is extensive, and likely to contribute significantly to large variability in the rate and extent of drug absorption [22, 23]. CYP3A4 expression is highly variable between individuals, with up to 30-fold differences in small intestine expression [24]. Expression of

Table 5. Relatioship of trough levels and of Bayesian of AUC_{0-12h} using blood concentrations at 0 and 3 hours postdose (AUC 0h3h, ng.h/mL) with trapezoidal AUCs and their performance as a test to detect "abnormal" exposure of tacrolimus

Early period (N = 65 curves)

	AUC trapezoidal ng.h/mL				AUC trapezoidal ng.h/mL		
	<170	170–250	>250		<170	170–250	>250
Ctrough ng/mL				AUC(0h.3h) ng/mL			
<10	14	8	0	<170	22	2	0
10–15	7	15	4	170–250	1	26	0
>15	2	5	10	>250	0	0	14
Test performance		Sens	Spec	Test performance		Sens	Spec
High Ctrough > overexposure		0.71	0.86	High AUC(0h3h) > overexposure		1.00	1.00
Low Ctrough > underexposure		0.61	0.81	Low AUC(0h3h) > underexposure		0.96	0.95

Stable period (n = 72 curves)

	AUC trapezoidal ng.h/mL				AUC trapezoidal ng.h/mL		
	<100	100–150	>150		<100	100–150	>150
Ctrough ng/mL				AUC(0h3h) ng/mL			
<5	1	2	0	<100	6	1	0
5–10	7	37	10	100–150	2	42	1
>10	0	5	10	>150	0	1	19
Test performance		Sens	Spec	Test performance		Sens	Spec
High Ctrough > overexposure		0.50	0.90	High AUC(0h3h) > overexposure		0.95	0.98
Low Ctrough > underexposure		0.13	0.97	Low AUC(0h3h) > underexposure		0.75	0.98

Sens, sensitivity, spec, specificity. Overexposure was defined as a (trapezoidal) AUC_{0-12h}, more than 20% above the target AUC (>250 ng.h/mL, early period; >150 ng.h/mL, stable period), underexposure as an AUC_{0-12h}, more than 20% under target (<170 ng.h/mL, early period; <100 ng.h/mL, stable period).

CYP3A4 in the gut mucosa varies along the intestinal tract, the upper small intestine being the major site for CYP3A4-mediated first-pass metabolism in humans [25, 26]. P-glycoprotein lowers intracellular concentrations of tacrolimus by pumping absorbed drug back into the intestinal lumen. P-glycoprotein may regulate access of tacrolimus to CYP3A enzymes, preventing these enzymes from being overwhelmed by high drug concentrations [24]. Tacrolimus is repeatedly transported out of the intestinal mucosal cells, and then passively reabsorbed. At least theoretically, this continuous repeated exposure could lead to more efficient metabolism [27]. P-glycoprotein shows significant interindividual variability, with two- to eight-fold variation found in small intestine biopsies from kidney transplant recipients and healthy volunteers [28]. P-glycoprotein mRNA levels increase longitudinally along the intestine [29]. In addition, factors such as the poor aqueous solubility of tacrolimus and alterations in gut motility may cause intraindividual variability in tacrolimus exposure to CYP450 and P-glycoprotein systems and, hence, random intraindividual variability in tacrolimus bioavailibility.

Using trough levels as monitoring tool, this effect would not have been appreciated since the corresponding trough levels remained consistently within the generally accepted target range of 5 to 10 ng/mL. These data suggest that there may be a "silent" and progressive increase in the systemic exposure in tacrolimus-treated patients, despite stable trough levels. It is important to note that all patients in the present study received a standard dose of 10 mg prednisolone beyond day 22 after transplantation. The observed pharmacokinetic changes can, therefore, not be attributed to a change in induction of CYP3A as a result of steroid tapering [30, 31].

CONCLUSION

We present a simple and reliable model-based approach, which, with only one additive sample, significantly improved estimation of tacrolimus exposure. A trough level in combination with a second sample, obtained somewhere between two and four hours postdose, is sufficient to accurately and reliably estimate tacrolimus AUC, even in the early post-transplant period. Especially in the first months ("early phase") posttransplantation, this method can serve as a tool to prevent under- or overexposure compared to trough level monitoring, but changes in intraindividual pharmacokinetics with time make it necessary to repeat AUC estimations frequently in this phase. After the first three months, the intraindividual changes in AUC are minimal, and in stable outpatients, AUC estimates can be done every

three months to prevent systemic overexposure with time. The applicability of AUC-guided tacrolimus dose adjustments can be expanded to overt tacrolimus-associated complications, such as post-transplant diabetes mellitus, polyomavirus-associated nephropathy, or unpredictable changes in tacrolimus exposure in relation to diarrhea [32]. A prospective study in which AUC-guided dosing is directly compared with trough level monitoring is the only way to determine the impact on the prevention of acute rejection episodes, nephrotoxicity, and other known side effects. Once the strategy, to control exposure over time, is optimized, different target levels of AUC could be tested in relationship to clinical outcome.

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