Research Article

Two-hour post-dose cyclosporine monitoring does not fit all in kidney transplantation

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Background: In transplant patients given cyclosporine (CsA), 2-hour post-dose sampling (C_2) has been proposed as the most accurate single-sample surrogate marker for the area under the time-concentration profile (AUC). Further optimization of this CsA monitoring is however, required. Objectives: The study, designed in 58 stable adult kidney transplant recipients more than 1 year post-surgery, was aimed at defining the single-point sampling strategy that best predicts CsA AUC_{n-12}, evaluating the precision of these strategies in AUC</sub> prediction according to different CsA absorption profiles, and establishing the predictivity of CsA pharmacokinetic parameters on graft outcome. **Results:** Regression analysis showed that C_2 values (r = 0.902) best correlated with AUC₀₋₁₂, whereas a lower correlation coefficient was found for C₀ (r = 0.769). However, a large error in AUC prediction was documented even with the C₂ equation model, with 33% of the estimations being unacceptable. Although C₂ is considered a useful surrogate of CsA C_{max}, only 22% of our estimations had the maximum CsA concentration at this time point, whilst 74% CsA peaked within 1 h. Of note, in 'low' absorbers, C₀ but not C₂ identified the most accurate surrogate marker for AUC₀₋₁₂. In a retrospective study, multivariate logistic regression analysis of the interaction between pharmacokinetic parameters and graft function outcome showed that C₀ but not C₂ significantly predicted an increase in serum creatinine greater than 20% and/or a decline in glomerular filtration rate of greater than 20% at the last available follow-up as compared with baseline evaluation. Similar predictive value of C_0 but not C_2 was obtained considering dialysis as the end point. **Conclusions:** In adults, stable kidney transplant recipients, a 2 h post-dosing CsA blood level is not a universal, accurate predictor of drug exposure and graft function outcome.

Cyclosporine (CsA) – a key immunosuppressant in organ transplantation - is characterized by a narrow therapeutic index and variable absorption and hence close monitoring of the drug is required to optimize dosing [1]. The utility of CsA trough level (C_0) measurement as a surrogate for clinical effects was initially proposed based upon reports of a correlation between low C₀ values and an increased incidence of acute rejection episodes, as well as high concentrations of nephro- or hepatotoxicity [2]. Nevertheless, in routine clinical practice this approach was proven as not always confident due to the fact that significant groups of patient's experienced acute rejection or CsA nephrotoxicity despite trough CsA levels within the suggested therapeutic range [1]. The best predictor of CsA exposure is the area under the time-concentration curve (AUC), calculated from the complete pharmacokinetic profile that for drugs administrered twice a day, must be measured from 0 to 12 h after CsA administration [1,3,4]. This approach is, however, expensive, time-consuming and increases the discomfort of the patient as it requires multiple sampling analyses.

As an alternative, abbreviated AUC profiles with different sampling protocols have been proposed but are still seldom a feasible option in routine out-patient clinical monitoring [5,6].

In the last few years, evidence has accumulated that blood sampling at 2 h post-dosing (C_2) provides a good estimate of drug exposure, expressed as AUC_{0-4} , in the early post-transplant period [7]. This could reflect the fact that within the CsA pharmacokinetic profile achieved with the microemulsion formulation, the C2 level is, in most cases, close to or at the peak CsA blood concentration (Cmax), which in turn is effective in predicting acute rejection [8-10]. Despite this, C₂ measurement is now replacing C_0 as an index of a patient's exposure to CsA in clinical transplantation - the accuracy and precision of this strategy is as of yet ill-defined. This mainly applies to patients late after transplant when graft function is stabilized and low maintenance immunosuppression is administered. Moreover, the impact of the individual CsA absorption profile on the ability of C_2 to be an appropriate surrogate marker of AUC has not been examined thus far.

The present study was designed in stable kidney transplant recipients, more than 1 year post-surgery in order to:

- Define the single-point sampling strategy that best predicts CsA AUC₀₋₁₂
- Evaluate the precision of the single-point strategy in AUC prediction, according to different CsA absorption profiles
- Assess the usefulness and feasibility of abbreviated AUC equations in selected cases
- Compare the predictive value of C_0 and C_2 monitoring on long-term graft function outcome

Materials & methods Patients

A total of 58 patients among those undergoing kidney transplantation at the Kidney Transplant Center of the Ospedali Riuniti Bergamo between November 1989 and January 1997 underwent the pharmacokinetic study. They were selected according to an agreement to:

- Undergo a 'per protocol' complete 12 h pharmacokinetic profile evaluation
- Be at least 1 year after kidney transplant
- Have stable graft function in the previous 4 months (defined by less than 15% differences in serum creatinine values during monthly evaluations)

Patients entered the study independently of whether they had previously delayed graft function and/or acute graft rejection. They were 11 female and 47 males between the ages of 18 and 64 years, with a cadaver donor kidney transplant who underwent pharmacokinetic studies after a median of 1513 days post-surgery (range: 370 to 2796 days), and regularly followed at the Unit of Nephrology of the Department of Medicine and Transplantation, Ospedali Riuniti di Bergamo -"Mario Negri" Institute for Pharmacological Research, Bergamo, Italy. At the time of the pharmacokinetic study, the patients were on triple (CsA Neoral, azathioprine and corticosteroids, n = 49; CsA Neoral, mycophenolate mofetil, and corticosteroids, n = 4) or dual (CsA Neoral, corticosteroid, n = 4; CsA Neoral, azathioprine, n = 1 immunosuppressive therapy. All patients were on the new microemulsion CsA formulation from at least 1 year. None of the 58 patients changed their therapy throughout the study period. Traditional C0-based monitoring was adopted and targets of blood levels used were those suggested by the current literature [11,12].

The study protocol was described in detail to the patients before admission and written informed consent to perform the study was obtained in each instance.

Aims

The primary aim of the study was to establish the precision of C₀ and C₂ single-point monitoring in predicting the full CsA AUC_{0-12} – the gold standard pharmacokinetic parameter to reliably monitor daily exposure of patients to drugs administered twice a day - according to individual CsA absorption profiles in adult kidney transplant recipients on stable graft function and longterm maintenance immunosuppressive therapy. As secondary aims, the ability of C₀ and C₂ pharmacokinetic parameters to predict subsequent renal graft function outcome was explored. These clinical outcomes included changes in serum creatinine concentration and glomerular filtration rate (GFR), as well as graft loss. In particular, we considered as a poor outcome an increase in serum creatinine of greater than or equal to 20% and/or a decline in GFR (measured by the Nankivell equation [13]) of greater than or equal to 20% over values at the time of pharmacokinetic evaluation (baseline), and/or return to dialysis up to the last available visit. Thus, the median follow-up for clinical outcome analyses was 1967 days post-pharmacokinetic evaluation.

Study design

All patients were fasted overnight, had a light lunch 4 h after CsA dosage and had free access to drinking water. The pharmacokinetics were based on an analysis of blood samples collected from the antecubital vein at $0 (C_0)$ and 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 h after drug administration. Blood samples were analyzed by high-performance liquid chromatography (HPLC) as previously described [14]. The CsA blood concentration-time profile was recorded for all patients, together with the time to reach the maximum concentration (T_{max}) . The area under the blood concentration curve from time equal to 0 to the last sampling point (12 h) (AUC_{0-12}) was calculated by the trapezoidal rule. CsA concentrations are expressed as ng/ml and CsA AUC $_{0-12}$ as ng*h/ml.

To examine the impact of different CsA absorption profiles on the accuracy of the singlepoint sampling strategy to predict AUC_{0-12} , patients were defined as 'slow' or 'rapid' absorbers, according to the maximum CsA concentration achieved within or after 1 h postdosing. In fact, it is now well known that there are a proportion of patients who show delayed absorption of CsA, documented by a shift towards the right in the CsA T_{max} [15]. In addition, the degree of drug absorption may be characterized not only by the T_{max}, but also by the ratio between the maximum to the minimum (or basal) concentration observed after CsA administration [16,17]. To this, we also evaluated the interaction between the absorption profile and AUC prediction considering patients as 'low', 'intermediate' or 'high' CsA absorbers. Patients exhibiting C_{max}/C_0 values more than 1 standard deviation (SD) below or above the mean value were defined as 'low' or 'high' absorbers respectively, and those within 1 SD of the mean were considered as 'intermediate' absorbers [16,17].

From the pharmacokinetic profiles, abbreviated AUC for CsA were also estimated using four different equations. In particular, the equation by Gaspari and colleagues [18] uses a three-point strategy (sampling at 0, 1, 3 h post CsA dosing), (AUC₀₋₁₂= $5.189 \times C_0 + 1.267 \times C_1 + 4.150 \times C_3 + 135.079$) and that by Keown and colleagues (AUC₀₋₁₂ = $12.34 \times C_0 + 2.48 \times C_2 + 441.42$) considers only C₀ and C₂ sampling points [17]. The equation by Cantarovich and colleagues which calculates first AUC₀₋₄ using the trapezoidal rule

Table 1. Baseline characteristics at the time of pharmacokinetic study.				
Gender (F/M)	11/47			
Age (years)	44 (18–64)			
Body weight (Kg)	69 (42–117)			
Serum creatinine (mg/dl)	1.42 (1.00–4.70)			
GFR (ml/min)	60 (16.9–88.1)			
CsA dose (mg/kg/day)	2.85 (1.43–5.15)			
C ₀ (ng/ml)	100 (41–190)			
C ₂ (ng/ml)	573 (190–1277)			
AUC ₀₋₁₂ (ng/ml)	2942 (1405–5073)			
Time from Tx to PK study (days)	1513 (370–2796)			
DGF (n)	5			
Acute graft rejection (n) [§]	36			

Values are median (range).

CsA: Cyclosporine; DGF: Delayed graft function; GFR: Glomerular filtration rate; PK: Pharmacokinetics. [§] Number of patients with at least one rejection episode (all within the first 6 months posttransplantation, thus before the pharmacokinetic study). (sampling at 0,1,2 and 4 h post-CsA administration), was also considered [7]. C₀ was then used for the estimation of 12 h CsA blood level based on the assumption of a steady state condition. Finally, C₁₂ levels were used to calculate AUC₀₋₁₂, assuming the area between 4 and 12 h as a trapezoid. As an alternative strategy with the four sampling model equation, we also plotted measured AUC₀₋₄ against the experimental AUC₀₋₁₂ and obtained an equation (AUC₀₋₁₂ = 1.36 AUC₀₋₄ + 312.39) used to predict the full AUC.

In addition, on the morning of the pharmacokinetic study, blood was drawn to estimate graft function as serum creatinine concentration and GFR by the Nankivell equation [13]. These values were considered as the baseline of graft function. Thereafter, patients were followed as out-patients to monitor the outcome of graft function and ultimately graft loss up to the last available follow-up (range: 225 to 2782 days; median 1967 days to baseline visit). During this period, none of the patients modified the regimen or dosage of their immunosuppression. The efficacy of the pharmacokinetic parameters, particularly C_0 , C_2 and AUC₀₋₁₂, to predict graft function outcome (as change in serum creatinine concentration and in GFR over baseline, or return to dialysis) was assessed. Moreover, to eliminate the effect on kidney graft outcome of factors/events occurring before the pharmacokinetic study, the age at transplantation, gender, occurrence of delayed graft function and acute rejection episodes, serum creatinine concentration and proteinuria 1 year postsurgery, serum creatinine at the time of the pharmacokinetic evaluation, and CsA dosage were considered in the multivariate analysis.

Statistical analyses

Linear regression analysis between blood CsA concentrations ranging from 0 to 12 h post-dosing or different AUCs predicted with limited sampling points versus the full experimental CsA AUC_{0-12} were performed. Agreement between the predicted and measured AUC was estimated using regression analysis and the percentage error in AUC prediction was calculated as:

$$\frac{\text{predictedAUC} - \text{measuredAUC}}{\text{measuredAUC}} 100$$

We considered an acceptable error within a range of $\pm 15\%$ [5].

The potential bias associated with the AUC prediction was measured as the sum of the



squared differences between the predicted and the measured $AUC_{0-12:}$

$$\Sigma$$
(AUC_{predicted} - AUC_{measured}) ^{\wedge^2}

With this approach, the potential influence of positive and negative bias is avoided [19]. The smaller the sum of squared differences the better the fit of the line to the data.

The ability of pharmacokinetic parameters to predict graft function outcome was assessed by means of multivariate logistic regression analysis. The PROC LOGISTIC of the SAS System Software (version 8) was used for this evaluation. The model included age at transplantation, gender, occurrence of delayed graft function or acute rejection, serum creatinine and proteinuria 1 year post-surgery and at the time of the pharmacokinetic studies, occurrence of acute rejection before and CsA dose at the time of kinetic study, and the pharmacokinetic parameters of interest. Statistical significance was set at p < 0.05.

Results

Baseline characteristics

At the time of the pharmacokinetic study, median body weight was 69 kg and all patients had stable graft function for more than 4 months (Table 1). Baseline characteristics, including serum creatinine concentration (median 1.42 mg/dl) and estimated GFR by means of the Nankivell equation (median 60 ml/min) are provided in Table 1. All patients were on antihypertensive treatment and none assumed prokinetic drugs or statins.

Prediction of CsA exposure by single points

As demonstrated in Table 2, the regression analysis between individual point CsA blood concentrations and AUC_{0-12} documented the best correlation with C_7 values (r = 0.916). The derived equation gave a mean error in AUC prediction of 1.6 ± 13.3% with 79.6% of the predicted AUCs within the acceptable threshold for error of -15–15%.

When only sampling points early post-CsA dosing near the peak drug concentration were considered, the best correlation was found with C_2 values (r = 0.902), whereas the correlation for C_0 was weaker (r = 0.769) (Table 2). The C_2 derived AUC₀₋₁₂ showed a good correlation with measured AUC₀₋₁₂ with a mean error in AUC₀₋ 12 prediction of 2.2 ± 15.8%. However, the associated error in AUC₀₋₁₂ prediction was acceptable for 67.3% of the estimations but yielded values ranging from -34.7 to 60.3% in the remaining (Table 2) (Figure 1). The error in AUC_{0-} 12 prediction using a C₀-based equation was on average 4.2 ± 21.6%, acceptable for even lower percentage of estimations (57.1%) and very largely distributed in the remaining (from -8.5% to 75.4%) (Table 2) (Figure 1).

Time-point of	Correlation	Regression equation	Error range (%)	% of samples
Sampling (II)	coefficient (I)			15% error
0	0.769	$AUC = 20.21C_{o} + 978.06$	-38.5 to 75.4	57.17%
0.5	0.383	$AUC = 1.58C_{0.5} + 2570.6$	-50.9 to 123.3	31.6%
1	0.751	$AUC = 2.01C_1 + 1447.5$	-53.1 to 71.7	44.9%
2	0.902	$AUC = 4.16C_2 + 628.40$	-34.7 to 60.3	67.3%
3	0.885	AUC = 6.70C ₃ + 720.97	-25.7 to 65.4	68.4%
4	0.840	$AUC = 7.47C_4 + 1082.1$	26.3 to 60.2	67.3%
5	0.769	AUC = 20.21C ₅ + 978.06	-38.5 to 75.4	57.1%
6	0.916	$AUC = 14.76C_6 + 511.24$	-29.0 to 45.7	73.5%
7	0.916	$AUC = 17.67C_7 + 537.49$	-28.2 to 53.3	79.6%
8	0.911	AUC = 19.93C ₈ + 473.91	-26.1 to 47.5	72.4%
9	0.905	AUC = 23.88C ₉ + 352.42	-29.8 to 43.3	77.6%
10	0.903	AUC = 23.81C ₁₀ + 508.97	-29.8 to 60.3	74.5%
11	0.886	AUC = 27.31C ₁₁ + 427.87	-29.5 to 39.6	73.5%
12	0.872	AUC = 27.40C ₁₂ + 535.57	-34.2 to 46.6	73.5%
T _{max} §	0.839	$AUC = 2.49C_{max} + 918.3$	-41.2 to 52.4	64.3%

Table 2. Comparison of measured AUC₀₋₁₂ versus predicted AUC₀₋₁₂ values obtained by using single sampling points.

§ 1.26 ± 0.54 h.

Impact of CsA absorption on AUC prediction C_2 is considered a useful surrogate of CsA C_{max} , at least early post-transplantation [8]. However, only 22% of our estimations had the maximum CsA concentration at this time point, while for 74%, CsA peaked within 1 h. The mean peak time was 1.26 ± 0.54 h. Given the variability in T_{max} which reflects individual CsA absorption profiles, we investigated

whether this difference would alter the efficacy of C_2 measurements to predict AUC₀₋₁₂ better than C_0 .

As shown in Table 3, in 'slow' absorbers, no significant difference between C₂ and C₀ levels in predicting CsA AUC₀₋₁₂ was found. This was not the case for 'rapid' absorbers where the time point concentration associated with the best correlation with AUC₀₋₁₂ was C₂ (Table 3).

Table 3. Correlation coefficient (r) between predicted AUC using near-the-peak time-points and measured AUC₀₋₁₂.

		T _{max}				
Time-point of sampling (h)	Overall (n = 58)	Slow absorbance (n = 15)	Rapid absorbance (n = 43)	Low absorbance (n = 4)	Intermediate absorbance (n = 45)	High absorbance (n = 9)
0	0.769	0.813	0.756	0.982	0.819	0.921
0.5	0.383	0.809	0.404	0.872	0.421	0.185
1	0.751	0.789	0.891	0.642	0.765	0.872
2	0.902	0.825	0.950	0.937	0.906	0.908
3	0.885	0.947	0.939	0.984	0.889	0.875
4	0.840	0.822	0.945	0.974	0.847	0.961

To define the pattern of absorption, the patients are divided in: slow ($T_{max} > 1$ h), rapid ($T_{max} < 1$ h) absorbers according to the T_{max} . As an alternative definition, patients are also grouped as: low, intermediate and high absorbers according to the ratio between maximum and minimum CsA concentration. Low absorbers exhibited C_{max}/C_0 values more than 1 SD below the mean value, and high absorbers exhibited values greater than one SD above the mean value.

limited sampling strategies.					
Time point of sampling (h)	Correlation coefficient (r)	Error range(%)	% of samples within -15 to 15% error	∑(pred-meas) ^2 ^{§§§}	
0, 1, 3	0.984	-11.1 to 22.0	96.9	$4.6 \times 10_{6}$	
0, 2	0.922	-31.5 to 50.0	72.4	1.7 × 10 ₇	
0, 1, 2, 4 [§]	0.986	-7.8 to 34.3	59.2	2.8 × 10 ₇	
0, 1, 2, 4 ^{§§}	0.965	-29.6 to 19.1	94.9	7.5 × 10 ₆	

 S^{c} C_{0} was used for the estimation of 12 h levels, based on an assumption of a steady state condition. C_{12} levels were then used to calculate AUC₀₋₁₂ h, assuming the area between 4 and 12 h as a trapezoid.

 $5^{5:}$ AUC₀₋₄ calculated using the trapezoidal rule. AUC₀₋₁₂ predicted with the equation derived from the regression analysis between measured AUC₀₋₄ and AUC₀₋₁₂: Y = 1.36 × AUC₀₋₄ + 312.39

§§§: Meas: Measured; Pred: Predicted.

A lower correlation coefficient between C_2 and AUC₀₋₁₂ than with C_0 values was found in the small cohorts of 'low' and 'high' absorbers (Table 3). At variance, in the large cohort of 'intermediate' absorbers, C_2 value was the best predictor of CsA AUC₀₋₁₂ (r = 0.906 for C_2 and r = 0.819 for C_0).

AUC₀₋₁₂ prediction by limited sampling points

We compared four limited sampling strategies among those proposed thus far [5] and determined which one would best predict AUC₀₋₁₂ after CsA administration when applied to our 58 pharmacokinetic profiles. First, a model equation using a three-point strategy (0, 1, 3 h) we previously described was explored [18]. A very high correlation (r = 0.984) between predicted and measured AUC_{0-12} was found (Table 4). With this threepoint model, the associated error in AUC prediction, which ranged from -11.1 to 22%, was acceptable for 96.5% of the estimations. Using the two-point equation (0, 2 h) proposed by Keown [17] the correlation coefficient was still high (r = 0.922), but data were more scattered as documented by a larger interval in the error range (Tble 4). Two alternative approaches use four-point model equations (0, 1, 2, 4 h) [7]. As shown in Table 4, the correlations with the experimental AUC_{0-12} were high but the error range in AUC prediction was relatively large for both predictions. To compare more closely the four equation models, the sums of the individual squared differences between predicted and measured AUC were calculated. According to this analysis, the lowest associated error in AUC prediction was found with the three-point model equation (Table 4).

Relationship between CsA pharmacokinetics and graft function outcome

Table 5 reports mean values of pharmacokinetic parameters measured at the time of the CsA kinetic study according to whether patients had more or less than 20% renal function deterioration (as compared with graft function at time of pharmacokinetics) or return to dialysis at the last available follow-up – median (1967 days after pharmacokinetic evaluation). During this period, none of the patients experienced events or illnesses associated with acute renal function deterioration, including acute rejection episodes, and CsA dose remained unchanged. No renal biopsy to prove chronic allograft nephropathy or CsA toxicity was performed.

Table 5. Association between cyclosporine pharmacokinetics and clinical outcome in 58 kidney transplant recipients on maintenance immunosuppression.

	Δ S. Creat. and/or Δ GFR		Dialysis		Combined [§]	
	<20% (n = 27)	≥20% (n = 31)	No (n = 48)	Yes (n = 10)	No (n = 26)	Yes (n = 32)
C _o	93 ± 30	110 ± 38	99 ± 34	116 ± 41	92 ± 31	110 ± 37
C ₂	568 ± 186	618 ± 230	591 ± 224	615 ± 136	568 ± 190	616 ± 227
AUC ₀₋₁₂	2756 ± 659	3289 ± 1031	2985 ± 935	3311 ± 766	2745 ± 669	3282 ± 1015

[§]Combined graft function outcome: increased S. creatinine >20%, glomerular filtration rate decline >20%, dialysis.

A total of 31 patients experienced poor graft function outcome as shown by an increase in serum creatinine of greater than or equal to 20% and/or a decline in GFR of greater than or equal to 20% over values at the time of pharmacokinetic evaluation. Of 58 patients, ten lost the graft and returned on dialysis replacement therapy. Multivariate logistic regression analysis of the interaction between individual pharmacokinetic parameters and graft function outcome is shown in Table 6. C_0 but not C_2 or AUC₀₋₁₂ significantly predicted an increase in serum creatinine of greater than or equal to 20% and/or a decline in GFR of greater than or equal to 20% at the last available follow-up. A similar predictive value was obtained for dialysis end point. When the three graft function end points were combined, predictivity was achieved with C₀ levels and AUC₀₋₁₂, but not with C₂ values.

At baseline, 12 of the 58 patients had severe graft impairment, a condition that might have biased the results. To take into account this potential confounding factor, an additional multivariate analysis - restricted to the subgroup of 46 patients with normal renal function or moderate renal dysfunction (serum creatinine < 2 mg/dL) – was performed. Also using this approach, C₀, but not C₂, was a significant predictor of the combined graft function outcome with higher CsA blood levels in patients who experience graft deterioration compared with those with stable renal function (CsA C_0 124 ± 37 versus 92 ± 33, p = 0.03; CsA C_2 633 ± 244 versus 583 ± 201 , p = 0.82). To examine the impact of different antirejection regimens, a multivariate analysis was also performed considering only the 49 patients on triple immunosuppressive therapy with CsA neoral, steroids and azathioprine. The relationship of pharmacokinetic parameters to graft function outcomes was similar to that reported in the overall patient population (data not shown). The remaining nine

Table 6. Multivariate logistic regression analysis of the abilityof baseline pharmacokinetic parameters to predict graftfunction outcome.

Δ S. Creat and/or Δ GFR	Dialysis	Combined§
0.017	0.03	0.009
0.86	0.60	0.46
0.08	0.64	0.04
	Δ S. Creat and/or Δ GFR 0.017 0.86 0.08	ΔS. Creat and/or ΔGFR Dialysis 0.017 0.03 0.86 0.60 0.08 0.64

§ Combined graft function outcome: increased S.creatinine ≥20%, glomerular filtration rate decline ≥20%, dialysis. All are p-values of multivariate analysis.

patients belonging to other different immunosuppressive regimens represent a group too small in number for sufficient analysis.

Discussion

The results of the present study show that in kidney transplant patients on maintenance CsA Neoral-based immunosuppression, CsA blood concentration at 2 h post-dosing (C₂) best correlated with the measured full AUC₀₋₁₂, when clinically applicable, near-the-peak, single sampling points were considered. A lower correlation coefficient was found for the C₀ single point.

These findings confirm previous analyses showing that 2 h post-dose sampling point (C_2) correlated most closely with AUC₀₋₄ in both the early [20,21] and later post-transplant periods [7,21–23]. Results from adult renal [24], liver [8,9] and cardiac patients [7,10] as well as pediatric renal [23] and liver transplant recipients [22], have demonstrated a correlation coefficient (r) of greater than 0.89 for C_2 and AUC_{0-4} . These studies also found a relatively poor correlation between C₀ and AUC₀₋₄ (r<0.70). C₂ was identified as the single sample that provides the most accurate surrogate marker for AUC_{0-4} . Other groups have also found correlations between C₂ and AUC_{0-8} [25]. However, these studies do not allow the elucidation of a definitive conclusion on the predictive value of C2, since both AUC0-4 and AUC₀₋₈ do not reflect the full daily exposure of patients to CsA.

The novelty of our study rests on the fact that for the first time, the value of the single C_2 sampling point in predicting CsA daily exposure was tested against the complete measured AUC₀₋₁₂ parameter instead of the abbreviated AUC. Moreover, while this pharmacokinetic relation was thus far assessed in adult kidney transplant recipients within the first 3 months post-surgery [21] or in pediatrics with stable renal transplant [23], here we extended the observation of C_2 monitoring to the most common situation of adult patients with stable and long lasting (>1 year) kidney graft.

In actual fact, the correlation depends on the range of values considered for this analysis and data with quite a high correlation may be in poor agreement [26]. This was the case of the C_2 equation model we used to predict AUC, that although better than the C_0 strategy, gave a large limit of agreement – from -34 to 60% – with an unacceptable over or underestimation of the actual CsA AUC in more than 30% of estimations. Thus, given the wide error in prediction in

a substantial group of estimations, the C_2 singlesample predictor strategy cannot be viewed as a universal, reliable approach for CsA monitoring – at least in adult kidney transplant recipients.

Not all kidney transplant recipients behave in the same manner as for absorption, distribution, metabolism and elimination of CsA [27], despite the fact that these pharmacokinetic challenges have been improved with the microemulsion formulation Neoral [8,28]. Although it has been suggested that the C2 time point incorporates a measure of CsA absorption, distribution and possibly elimination [15], individual variation of the absorption state could have accounted for the large error in AUC prediction we found using the C2 sampling model. Interindividual variation in the absorption of CsA segregates three distinct populations of patients defined as 'low', 'intermediate' and 'high' absorbers [16,17]. Here we documented for the first time that C₂ sampling exhibited a different pattern of prediction of CsA exposure based on the patient's absorption profile. Indeed, a lower correlation coefficient was observed between C_2 -predicted and measured AUC₀₋₁₂, than with Co-predicted AUC values in 'low' and 'high' absorbers when absorption profiles were established considering mean values of C_{max}/C_0 and one SD.

A low absorber of CsA may be a 'true' low absorber, or because the patient experiences markedly delayed absorption, he or she may be a 'slow' absorber in that they experience an extended time to peak CsA concentration [15,29]. Even considering this latter possibility, no difference between C_2 and C_0 levels in predicting AUC was found in the subgroup of 'slow' absorbers. These findings indicate that C_2 is not the best time–point predictor of AUC in the kidney transplant population of 'low' and 'slow' absorbers. Conversely, in the 'true low' absorbers, C_0 identifies the most accurate surrogate marker for AUC₀₋₁₂.

For the 'slow' absorbers, in which neither C_2 nor C_0 sampling offers the best AUC predictive model, the possibility would be the use of abbreviated kinetic profiles with few sampling points [5]. In the present study, we confirmed that in stable renal transplant recipients a limited strategy of three-point sampling taken early after CsA dosing (0, 1, 3 h), allowed an excellent and reliable prediction of the actual AUC. This was better than other proposed abbreviated sampling strategies which require blood collection at 0 and 2 h [17] or 0, 1, 2 and 4 h post-CsA dosing [7].

The pharmacokinetic rationale for using C₂ instead of conventional C₀ concentrations to monitor patients receiving CsA Neoral has been borne out by clinical trials that demonstrate a reduced incidence and severity of acute rejection in de novo renal and liver transplant patients and improvements in safety profile in both renal and hepatic maintenance patients [30-35]. Moreover, in de novo renal transplant recipients, C2 was consistently the best surrogate marker for C_{max} (the peak concentration of CsA) throughout the first 3 months post-transplant [21]. C_{max} is known to coincide with the peak pharmacodynamic effect of CsA Neoral [36-38], such that C2 is not only considered a sensitive measure of CsA absorption, but may be also an accurate index of immunosuppression. In our long-term kidney transplant recipients on maintenance CsA-based immunosuppressive regimen however, C_{max} coincided with C₂ in only 22% of the pharmacokinetic estimations. Although this fact does not negate that C2 might be the best surrogate marker of the absorption phase, it poses some reasonable concerns. Indeed, these findings lessen the value of C_2 monitoring as an index of CsA absorption and probably as a surrogate marker of the pharmacodynamic effect of CsA, at least in stable renal transplant patients long enough post surgery.

Chronic allograft nephropathy is the major cause of progressive renal failure in renal transplant recipients to which chronic CsA administration plays a relevant role [39]. CsA nephrotoxicity may account for the paradox of modest improvement in long-term outcome being much less than predicted by reduction or abolition of early acute rejection [40]. A kidney transplant salvaged from immunological injury by CsA therapy may then be subsequently damaged and lost by chronic nephrotoxicity caused by the same agent. In the long term, the predictable impact of reducing CsA dose and thus drug exposure, after months or years post transplant, is a slower rate of renal function deterioration and eventual graft loss in respect to the standard CsA regimen [39,41-43]. However, this requires appropriate CsA drug monitoring. Despite CsA C₂ blood measurement being claimed to be as effective a marker of chronic allograft dysfunction [44], available data are very scanty to be conclusive. Our retrospective analysis on maintenance renal transplant recipients extends and challenges these findings. Patients with declining renal function or progression to end-stage renal disease had higher C₀, C₂ and AUC₀₋₁₂ compared with those

Highlights

- Cyclosporine (CsA) is a narrow therapeutic agent that requires close monitoring.
- Different pharmacokinetic approaches have been proposed to guide CsA dosing.
- Data regarding the best strategy to monitor CsA exposure in patients with stable graft function is lacking.
- In kidney transplant patients on maintenance CsA Neoral-based immunosuppression, CsA concentration at 7 h post-dosing showed the best correlation with daily drug exposure.
- When only single near-the-peak points were considered, CsA C₂ levels provided the higher degree of correlation with CsA area under the curve (AUC)₀₋₁₂ but lack in precision, with an unacceptable associated error in AUC predictor for 35% of estimations.
- In kidney transplant recipients with an atypical absorption profile, C₂ is not the best time-point predictor of CsA AUC.
- In long-term kidney transplant recipients on maintenance CsA, C₂ coincided with maximum concentration only in 22% of patients.
- CsA trough levels, but not C₂, were significant predictors of graft dysfunction, graft loss and long-term CsA nephrotoxicity, expressed by an increase in serum creatinine or a decrease in glomerular filtration rate.

possessing stable renal function. Of note, by multivariate analyses, C_0 but not C_2 was a significant predictor of graft dysfunction and graft loss. Indeed, after considering factors that could also have influenced clinical outcome and ultimately contributed to graft loss, C_0 was still an independent predictor of the clinical events. Similar findings were obtained even after excluding from the analysis patients with more severe graft function impairment at baseline that could have been a confounding factor. This observation is clinically relevant since patients with normal of near normal renal function at baseline are presumably those who will receive the longest benefit from optimizing therapeutic drug monitoring.

Our results are at variance with those recently published by Di Paolo and colleagues [45]. They prospectively monitored a large, heterogeneous cohort of kidney transplant recipients after switching from a C_0 - to a C_2 -based CsA doseadjustment protocol. After 3 years of follow-up, they found a better graft function in patients with mean C_2 levels greater than 661ng/ml compared with those with lower CsA C_2 concentrations. However, patients with atypical

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absorption profiles were not considered. In the present study patients were monitored for a longer follow-up, exposed to lower CsA C_2 concentrations and treated with different concomitant immunosuppressive regimens, making difficult a formal comparison between the two studies.

Our study has certainly some shortcomings. This is a retrospective analysis of a small cohort of stable kidney transplant recipients monitored using CsA C_0 levels. Thus, we can not exclude that the use of other monitoring strategies, such as C_2 values, might lead to different conclusion. Prospective, multicenter, clinical trials with full CsA pharmacokinetic profiles are needed to definitely establish the best strategy to monitor CsA exposure in all transplant recipients, including those characterized by peculiar drug absorption profiles.

Conclusions

In stable kidney transplant recipients on a longterm CsA Neoral-based regimen, C_2 is not necessarily a superior surrogate marker of the daily exposure to the drug compared with C_0 , at least in patients monitored with the C_0 sampling strategy.

We would suggest that stable kidney transplant recipients should undergo at least one complete pharmacokinetic profile in order to be classified according to his/her CsA absorption pattern. Although the number of patients with atypical CsA absorption profiles is limited, usually not exceeding 25 to 30% of all the treated subjects, they may still deserve attention. In the 'low' absorber patients, C₀ monitoring is a very reliable alternative to C₂, whereas for 'slow' absorbers, a limited strategy would be advisable. As additional clinical data from C₂ studies in renal transplantation become available, the potential benefit of C₂ over C₀ monitoring will be better defined.

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