Cyclosporin exposure correlates with 1 year graft function and histological damage in renal transplanted patients

Salvatore di Paolo1, Annalisa Teutonico1, Giovanni Stallone1, Barbara Infante1, Antonio Schena1, Giuseppe Grandaliano1, Michele Battaglia2, Pasquale Ditonno2 and Paolo F. Schena1

1Department of Emergency and Organ Transplantation, Division of Nephrology and 2Department of Emergency and Organ Transplantation, Division of Urology, University of Bari, Bari, Italy

Abstract

**Background.** Cyclosporin (CsA) level obtained 2 h after the morning dose (C2) has been shown to accurately predict total CsA exposure and acute rejection (AR) risk, whereas conventional trough levels (C0) do not. The impact of C2 monitoring on long-term kidney graft function, independent from AR risk, is still unclear, however, and it was assessed in the present study.

**Methods.** We enrolled 39 CsA-treated renal transplant recipients and used 1 year graft function and histological structure as surrogate markers of graft outcome. CsA dose was adjusted according to C2 levels.

**Results.** In the first 7 days after grafting, 40–51% of patients failed to reach target C2 levels; nevertheless, at 1 year the incidence of AR was only 2.5% and graft and patient survival was 100%. The decrease of serum creatinine (12–6 months) was associated with significantly higher C2 levels over time (P = 0.0003) and lower intrapatient variability of CsA relative absorption (CV) (P = 0.0006). One year graft biopsy showed chronic tubulointerstitial lesions in 54.5% of patients. Both C2 mean levels and the percentage CV independently predicted the severity of chronic histological lesions (R = 0.69, P < 0.0001).

**Conclusions.** Higher C2 levels, within the proposed target range values, seem to be associated with better renal function and structure.

**Keywords:** C2 monitoring; chronic allograft nephropathy; cyclosporin; graft function; kidney transplantation

Introduction

Cyclosporin (CsA) has a narrow therapeutic window and dosing is, therefore, dependent on accurate and reliable drug monitoring. The widespread adoption of Neoral®, the microemulsion formulation of CsA, has significantly improved clinical outcomes, due to its greater and more consistent absorption of CsA, improved dose-linearity and reduced inter- and intrapatient variability [1].

Research in renal transplant patients has shown that the area under the time-concentration curve (AUC), based on 12 h pharmacokinetics, strictly reflects drug exposure and is a sensitive predictor for acute rejection (AR) and graft survival at 1 year [2]. Unfortunately, this approach is too inconvenient, costly and difficult to perform on a routine basis.

The measurement of the absorption and exposure of CsA during the first 4 h of the dosing interval (AUC0–4) correlates very closely with full 12-h AUC [1], is a more practical monitoring tool and is very effective in differentiating between patients with normal and impaired absorption of CsA [1]. This 4 h period is associated with a large amount of intrapatient and interpatient variability and is an indicator of the patient’s ability to absorb CsA from the intestine [3]. Furthermore, blood concentrations during the early post-dose period correlate well with the pharmacodynamic effects of calcineurin inhibition and inhibition of interleukin-2 production [4,5]. Most importantly, it has been demonstrated that the CsA level obtained 2 h after the morning dose (C2) correlates most closely with AUC0–4 and can predict the risk of AR, thereby offering a rapid, practical and effective strategy for routine CsA microemulsion monitoring in both the early and later stages post-transplantation [3,6–10]. In contrast, conventional trough level measurements of CsA (C0) correlate poorly with AUC0–4 and do not accurately predict total CsA exposure and rejection risk [2,6]. Therefore, adjustment of Neoral dose based on C2 level has been proposed as...
a more effective monitoring strategy than conventional 
$C_0$, to optimize the use of the calcineurin antagonist.

The impact of $C_2$ monitoring on long-term kidney 
graft function, independent from AR risk, is still 
undefined, however. Former studies were aimed to 
identify the optimal cut-off value to be targeted during 
the early post-operative period to achieve a low rate of 
AR at 3 months post-graft, whereas the most appro-
priate $C_2$ target remains to be defined to maximize 
Neoral’s immnosuppressive effect and to minimize its 
toxicity in the longer term after renal transplantation.

In-centre $C_0$ monitoring of de novo kidney allograft 
recipients receiving basiliximab, Neoral, mycopheno-
late mofetil and low-dose steroids has previously 
resulted in an extremely low rate of AR. Thus, the 
primary objective of this study was to evaluate the 
clinical impact of $C_2$ monitoring on kidney graft out-
come, taking 12 month graft function and histological 
structure as surrogate markers of late graft outcome 
[11,12], and to explore the possible correlation of renal 
graft function with $C_2$ levels.

**Subjects and methods**

**Patients**

Patients who were ≥18 years of age, who were recipients of 
cadaveric or living-related kidney transplants, who received 
Neoral on a b.i.d. regimen and who were able to give 
informed consent were qualified for inclusion in the study. 
Patients who had a known liver disease, gastrointestinal dis-
 ease or other disorders that may have altered the absorption 
or metabolism of CsA; those who were multiorgan 
recipients or were previously transplanted with any organ; 
those with PRA >80% at any time-point; and recipients 
of dual transplant or marginal kidneys or an HLA-identical 
organ were excluded from the study. The quality of the 
donor kidney was evaluated by histological examination of 
pre-transplant biopsy: a histological score of four or more 
qualified the organ as marginal [13].

Thus, in the period from May 2001 through April 2002, 39 
kidney transplant recipients were enrolled at our transplant 
centre (55.7% of the total number of transplants performed in 
that period). Both donors and recipients were Caucasians. 
Their demographics and clinical characteristics are reported in 
Table 1. The minimum follow-up was 12 months. In all 
patients, both $C_0$ and $C_2$ levels were measured, but CsA dose 
was adjusted exclusively according to $C_2$ monitoring. Strict 
collaboration with inpatient and outpatient nursing staff 
greatly facilitated timely blood sampling.

Whole blood samples were collected at days 3, 5, 7, 10 and 14 
after the introduction of CsA, then weekly up to the end of 
the third month, thrice a month in the following 3 months and 
twice from month 7 through month 12, for a total of at least 
36 measures for each patient. All measures of CsA blood 
levels were performed ≥48 h after the last change of dose.

**Immunosuppressive regimen**

All patients received immunoprophylaxis with basiliximab 
20 mg intravenous (i.v.) on the day of transplant and 20 mg 
i.v. on day 4 and maintenance triple-drug immunosup-
pression consisting of Neoral, mycophenolate mofetil and 
prednisone.

Treatment with Neoral was commenced at a dose of 
12–14 mg/kg given orally in two divided doses, starting 48 h 
after transplantation. Target therapeutic ranges for $C_2$ were 
1600–2000 ng/ml during the first 4 weeks, 1400–1600 in the 
second month, 1200–1400 in the third month, 800–1200 
during months 4–6, declining to 600–1000 ng/ml during 
months 7–12 after engraftment [14].

CsA-related nephrotoxicity was defined as 30% increase in 
serum creatinine (sCr) that was not attributed to any other 
identifiable cause and that improved with decrease in the 
Neoral dose.

Mycophenolate mofetil was given at 1 g per os. twice daily, 
starting from the second day after transplantation and was 
adjusted according to white blood cell counts or other 
relevant parameters.

All patients were treated with corticosteroids (500 mg 
methylprednisolone intraoperatively, then 200 mg prednisone 
daily, tapered to 25 mg by day 8 and to 5 mg by month 3).

**Histological examination**

All grafted kidneys underwent surgical biopsy, performed 
immediately after graft reperfusion (0 h biopsy). Methods of 
processing and scoring criteria have been described 
elsewhere [13].

The diagnosis of AR was always confirmed by percuta-
aneous core needle biopsy, classified according to the Banff 
criteria [15]. Delayed graft function (DGF) was diagnosed 
if sCr increased or remained unchanged immediately after 
surgery during three consecutive days. All patients with DGF 
derwent routine graft biopsy after the first post-transplant 
week (and at weekly intervals thereafter): if acute rejection 
was diagnosed, the graft was categorized as primary function. 
In fact, no such case could be observed in the population 
studied.

All recruited patients were asked to undergo protocol 
biopsy of the kidney graft at ~1 year after transplant. The 
histopathologist was blinded to any clinical features of the 
patients and to $C_2$ and sCr levels. Chronic lesions were scored 
according to Banff criteria [15], after subtracting for the 
lesions already present at baseline biopsy, to calculate the
C₂ levels and chronic renal graft dysfunction

Statistical methods

The results of the quantitative variables are expressed as means ± SD and those of the qualitative variables as proportions.

Intratentive variability of CsA relative absorption [measured as dose- and weight (mg/kg)-corrected C₂ (DWC.C₂)] [8] was expressed as the coefficient of variation (CV) (months 2–12): %CV = SD of mean DWC.C₂/mean DWC.C₂. We used a conventional receiver operating characteristic (ROC) curve to analyse %CV values in order to determine the cut-off point that yielded the highest combined sensitivity and specificity with respect to distinguishing patients with an increase of sCr over time from those with a decrease (i.e. an amelioration of renal graft function at the end of follow-up).

For each patient, the mean C₂ represented the mean of C₂ levels measured in each month (1–12) at the scheduled visits (see above). For the first month, only the measures recorded at days 7, 14, 21 and 28 were considered. For those levels not obtained within the expected time window (120 ± 15 min), values were discarded and, on almost every occasion, patients were re-evaluated within the following 24–48 h.

Differences between quantitative variables were tested by the Mann–Whitney U-test or by repeated measures analysis of variance (ANOVA), as appropriate. The relationship between non-parametric variables was tested by Spearman rank correlation. Stepwise regression analysis was used to evaluate the predictive ability of multiple independent continuous variables. All tests were two-tailed. A P-value of < 0.05 was considered statistically significant. The logistic regression model was used to determine the factors significantly related to the deterioration of graft function over time. The significant predictors were next fitted in a multivariate model. The P-value of < 0.05 was used to evaluate the predictive ability of multiple independent continuous variables. All tests were two-tailed. A P-value of < 0.05 was considered statistically significant. The logistic regression model was used to determine the factors significantly related to the deterioration of graft function over time. The significant predictors were next fitted in a multivariate model. The risk is expressed as the odds ratio (OR) and 95% confidence interval (CI). The Statview software package (version 5.0; SAS Inc.) was used for all analyses.

Results

Patients showed 100% graft and patient survival at 12 months and an extremely low rate of AR (2.5%). DGF occurred in three patients (7.7%). Serum creatinine levels (mg/dl) during 1 year follow-up were 1.49 ± 0.35 at 1 month, 1.47 ± 0.34 at 3 months, 1.42 ± 0.28 at 6 months, 1.37 ± 0.32 at 9 months and 1.32 ± 0.26 at 12 months.

Blood samples for C₂ monitoring were obtained within the requisite time window (120 ± 15 min) in 86–93% of patients during the 12 months of follow-up, which apparently demonstrates the feasibility of this monitoring approach, given an adequate organization of nursing staff and information of patients.

None of the patients experienced CsA-related nephrotoxicity with C₂ levels within the target range. Two patients had 20% increase of sCr with C₂ definitely above 2000 ng/ml.

During the first days after grafting and specifically at days 3–7 after the start of CsA therapy, a sizable percentage of patients failed to reach C₂ levels of 1600 ng/ml, in spite of daily Neoral doses of 12–14 mg/kg (Table 2). The percentage of patients below the target range values of C₂ remained elevated during the first 3 months, despite frequent dosage adjustments (Table 2).

An observational study on a very large population of adult renal transplants has demonstrated that the change in sCr over the first year strongly predicts long-term renal allograft survival [11]. Thus, we wondered whether CsA exposure, measured as peak blood levels (C₂) throughout 12 month follow-up, would affect post-transplant renal graft function, defined as ΔsCr (difference between sCr at 12 months and sCr measured at 6 months). Patients with negative ΔsCr (i.e. an amelioration of sCr levels at 12 months) showed significantly higher levels of C₂ over time (Figure 1). Of note, baseline sCr levels were not different between the two groups (at hospital discharge: 1.51 ± 0.32 vs 1.46 ± 0.39 mg/dl in negative ΔsCr vs positive ΔsCr patients; P = 0.66). Particularly, during the first 6 months, mean C₂ levels above the mean target level (1400 ng/ml) turned out to be associated with the highest decrease of sCr (i.e. the highest negative ΔsCr) at 12 months (Mann–Whitney U-test: Z = 3.36; P = 0.0008).

Patient variability has been advocated to favour both acute and chronic kidney allograft rejection. Therefore, we measured the %CV in each patient and sought for a relationship with the change of sCr over time. This study showed an inverse association between ΔsCr (12–6 months) and the %CV (Spearman rank

Table 2. C₀ and C₂ levels and CsA relative absorption [measured as dose- and weight (mg/kg)-corrected C₂] during 1 year follow-up. At each time-point, the percentage of patients with out-of-range C₂ levels is reported.

<table>
<thead>
<tr>
<th>Day</th>
<th>C₀ (ng/ml)</th>
<th>C₂ (ng/ml)</th>
<th>C₂/dose/kg (ng/ml⁻¹ kg⁻¹)</th>
<th>Pts above C₂ range (%)</th>
<th>Pts below C₂ range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 3</td>
<td>276 ± 69</td>
<td>1597.5 ± 356.6</td>
<td>117.9 ± 46.8</td>
<td>7.7</td>
<td>51.3</td>
</tr>
<tr>
<td>Day 7</td>
<td>349 ± 129</td>
<td>1722.9 ± 355.8</td>
<td>172.7 ± 64.9</td>
<td>17.9</td>
<td>41</td>
</tr>
<tr>
<td>Day 14</td>
<td>324 ± 107</td>
<td>1716 ± 474</td>
<td>201.9 ± 63.5</td>
<td>28.2</td>
<td>35.9</td>
</tr>
<tr>
<td>Day 21</td>
<td>354 ± 90</td>
<td>1783 ± 521</td>
<td>210.3 ± 90.6</td>
<td>28.2</td>
<td>38.4</td>
</tr>
<tr>
<td>Day 28</td>
<td>338 ± 105</td>
<td>1670 ± 418</td>
<td>264.1 ± 77.9</td>
<td>25.6</td>
<td>33.3</td>
</tr>
<tr>
<td>Month 3</td>
<td>283 ± 96</td>
<td>1363 ± 390</td>
<td>294.7 ± 122.4</td>
<td>17.9</td>
<td>30.7</td>
</tr>
<tr>
<td>Month 6</td>
<td>235 ± 68</td>
<td>1071 ± 262</td>
<td>299.4 ± 105.6</td>
<td>12.8</td>
<td>15.4</td>
</tr>
<tr>
<td>Month 9</td>
<td>196 ± 53</td>
<td>952 ± 267</td>
<td>291.8 ± 96.1</td>
<td>15.4</td>
<td>5.1</td>
</tr>
<tr>
<td>Month 12</td>
<td>145 ± 60</td>
<td>860 ± 176</td>
<td>279.1 ± 103.6</td>
<td>12.8</td>
<td>2.5</td>
</tr>
</tbody>
</table>
correlation: $\rho = 0.585$; $P = 0.0003$). ROC analysis revealed an inflection point of $\%CV$ vs a positive $\Delta sCr$ (i.e. a deterioration of kidney graft function over time) at 19.5% [sensitivity (95% CI): 0.76 (0.50–0.93); specificity (95% CI): 0.77 (0.54–0.92); AUC under ROC curve: 0.815 (95% CI: 0.671–0.959)]. For descriptive purposes, patients were then separated into two groups (CV $\leq 9.5\%$ or $> 19.5\%$): at 12 months, patients with CV $\leq 19.5\%$ (43.6%) showed a significantly better graft function, as measured by both sCr and $\Delta sCr$ (Figure 2).

The logistic regression model was used to determine the factors significantly related to a positive $\Delta sCr$ (i.e. worsening of graft function). A range of clinical, laboratory and demographic variables, such as arterial hypertension, $C_0$ and $C_2$ levels, $\%CV$, HLA matching between the donor, cold ischaemia time, DGF, age and gender were selected as factors. None of these factors, with the exception of $C_2$ and $\%CV$, showed any significant correlation with the dependent nominal variable. As a matter of fact, the population studied exhibited rather homogeneous values for many of the variables tested (i.e. incidence of hypertension, HLA MM, cold ischaemia time), while some variables (AR, DGF) were negligible and this might help explain the lack of correlation. The significant predictors were next fitted in a multivariate model. Both mean $C_2$ and $\%CV$ maintained their relationship with the dependent variable ($C_2$: OR = 0.994, CI = 0.988–1.00; $\%CV$: OR = 1.359, CI = 1.080–1.710; likelihood ratio: 18.107; $P < 0.0001$).

Thirty-three patients (84.6%) accepted to undergo protocol kidney graft biopsy, which was performed 11–13 months (median: 12.2 months) after transplantation. Serum creatinine in biopsied patients was $1.35 \pm 0.39$ mg/dl (non-biopsied patients: $1.28 \pm 0.18$ mg/dl; $P$ not significant). No evidence of borderline changes, suspicious for acute rejection, could be observed. Chronic tubulointerstitial lesions compatible with the diagnosis of chronic allograft nephropathy (CAN) were present in 54.5% of patients. CAN lesions were scored as mild in 36.3% and moderate in 18.2% of patients, with none showing severe lesions. Chronic vascular lesions were present in 45.4% of biopsied patients and were mild in nine patients, moderate in five and severe in one out of 33 patients. The severity of histological lesions was correlated positively with the $\%CV$ and negatively with $C_2$ mean levels (Table 3): the higher the $\%CV$, and the lower the mean $C_2$, the higher the histological score. Stepwise regression analysis confirmed that both variables independently predicted the severity of chronic histological lesions ($R = 0.69$; $R^2 = 0.475$; $P < 0.0001$). Of note, there was no difference in graft score at implantation between groups (i.e. patients with negative vs those with positive $\Delta sCr$). Finally, donor age failed to correlate with the degree of kidney graft damage ($P = 0.8$), showing a trend, although not significant, towards a correlation with vascular damage ($P = 0.08$).

In contradistinction, CsA trough levels failed to correlate with either 1 year graft function or the severity of histological lesions and definitely underestimated intrapatient variability in CsA exposure (data not shown).

Discussion

Emerging evidence over the past few years has shown that there is a robust relationship between Neoral...
absorption profile, measured by either AUC0–4 or C2, and the probability of AR, whereas the relationship between C0 and subsequent AR is weak and fails to reach statistical significance [2,3,6–10]. Adequate exposure to CsA within the first 3–7 days post-transplantation has been advocated to be critically important in preventing subsequent rejection, with C2 threshold ranging from 1500 to 1700 ng/ml [8–10], even in patients with basiliximab immunoprophylaxis [8]. Indeed, Morris et al. [16] reported a 0% rejection rate when C2 exceeded 1200 ng/ml. In sharp contrast, Perico et al. [17] have reported very recently that CsA trough levels measured at day 2 post-transplant would be the strongest predictor of acute graft rejection over a 6 month follow-up period, while C2 levels considered alone would have no predictive values at all; C0 levels of 300–440 ng/ml being associated with the lowest risk of rejection. In the cohort studied here, a sizable percentage of patients were below the target C2 range during the first week after transplantation, 18% being <1200 ng/ml (and 60% patients had C0 levels of <300 ng/ml at day 3 after the start of CsA therapy): none of them had AR throughout the follow-up. We suspect that basiliximab immunoprophylaxis might minimize the risk of relative underexposure to CsA in the early post-transplant period, although contrasting evidence exists in the literature [8,9].

Actually, studies designed to explore the impact of C2 monitoring immediately after transplantation on long-term kidney graft function are virtually absent. Mahalati et al. [18] reported that higher early AUC0–4 was associated with lower sCr at 3 months, while Clase et al. [10] found that adequate exposure within the first 3 days post-transplantation is critically important in preventing subsequent rejection, but seemingly does not influence sCr at 6 months. Finally, Pescevitz et al. [9] observed that higher mean C2 levels were not related to higher CsA nephrotoxicity, as expressed by sCr levels at either weeks 4 or 24 post-transplantation [9].

Our results demonstrate that negative ΔsCr (i.e. an amelioration of kidney graft function between 6 and 12 months) is associated with higher C2 levels, within the target range values chosen. Interestingly, C2 levels achieved during the first 6 months after transplant appear to strongly influence graft function at the end of follow-up, which seemingly confirms the critical role of adequate immunosuppression in the early months after engraftment.

Studies by Kahan et al. [19] revealed that both exposure and the degree of day-to-day variability in exposure were predictive of the incidence of acute and chronic rejection in renal transplant recipients. Moreover, data from a prior international multicentre study in de novo renal transplantation, during which kinetics were evaluated from 2 weeks to 3 months post-transplant, have suggested that absorption might stabilize between 1 and 2 months after commencing therapy [20]. We, therefore, measured the %CV of CsA relative absorption between 2 and 12 months in each patient and found that renal transplant recipients with CV >19.5% displayed higher sCr and lower ΔsCr at the end of follow-up.

One year histological alterations have been shown to predict graft survival, even when the graft function is still normal, with the progression of the lesion rather than the intensity of alterations at a single given time-point being the most meaningful predictor [12]. Then, it is critical to identify the clinical risk factors that lead to a high CAN score at 1 year, in order to devise possible intervention trials. In the cohort studied here, the progression of renal lesions during the first year after engraftment was significantly correlated with both mean C2 levels and the %CV of CsA relative absorption. The results point to inadequate CsA exposure as one of the clinical risk factors for CAN and indirectly support the role of immune-mediated mechanisms in the pathogenesis of chronic allograft damage.

In conclusion, higher C2 levels, within the recently proposed target range values [13], seem to be associated with better renal function and structure. Lower C2 threshold values in the early post-transplant period seemingly do not increase the incidence of AR, at least in Caucasian patients receiving basiliximab immunoprophylaxis. Finally, serial measurements of C2 are a simple and practical strategy to reveal the variability of oral absorption of CsA and allow the identification of subjects potentially at higher risk for chronic graft dysfunction. We are aware, however, that the findings presented here were attained retrospectively and in a limited sample of patients and that the above conclusions should be interpreted with caution until results from larger prospective studies are made available.

Conflict of interest statement. None declared.

References


16. Morris RG, Russ GR, Cervelli MJ, Juneja R, McDonald SP, Mathew TH. Comparison of trough, 2-hour, and limited AUC blood sampling for monitoring cyclosporin (Neoral) at day 7 post-renal transplantation and incidence of rejection in the first month. *Ther Drug Monit* 2002; 24: 479–486


Received for publication: 19.2.04
Accepted in revised form: 6.5.04