Abstract. Accurate monitoring of cyclosporine (CsA) dosage is still a problem, because measurement of the area under the curve (AUC)—the most appropriate indicator of exposure to CsA—requires a number of blood samples to be taken over 12 h, which makes monitoring difficult in day-to-day clinical practice. This study investigated whether a limited sampling strategy in human kidney transplantation reflected the actual AUC better in patients given Neoral® than in those being treated with Sandimmune®. Stepwise multiple regression analysis of CsA blood levels recorded after Neoral administration to 20 renal transplant patients showed the best results in AUC prediction with three sampling points (1.5, 8, 11 h after Neoral dosing; r = 0.992 with an associated error in AUC prediction ranging from −8.0 to 8.8%). Because blood sampling at 8 and 11 h is not feasible in routine clinical practice, sample points from 0 to 3 h after Neoral dosing, i.e., up to the time of maximum mean blood CsA concentration plus 2 SD, were considered. The best results were obtained with a three-point strategy (0, 1, and 3 h after Neoral dosing; error in prediction: −9.0 to 7.2%), which gave an excellent correlation between measured and predicted AUC (r = 0.989). A similar analysis after Sandimmune given to the same patients always resulted in poor AUC prediction with a wide associated error. These findings indicate that with Neoral, but not Sandimmune, a limited strategy of three-point sampling taken early after dosing allows an excellent and perfectly reliable prediction of the actual AUC. (J Am Soc Nephrol 8: 647–652, 1997)
nephic profile that correlate best with AUC in renal transplant patients given Neoral as a part of their immunosuppressive therapy; (2) to define the best limited sampling strategy to predict AUC after Neoral administration, to facilitate routine monitoring of CsA in routine outpatient transplant clinic visits; (3) to compare data with Neoral and with Sandimmune given to the same patients in the same limited sampling strategy.

Materials and Methods

Patients
Twenty patients (six female, 14 male) who were regularly followed-up at the Division of Nephrology, Ospedali Riuniti, Bergamo, Italy, were studied. They had received kidney transplants at least 6 months before starting the study and had stable CsA dosages for more than 1 month. All patients had stable renal function as documented by changes in creatinine clearance rates of less than 30% over the last 3 months and no clinical proteinuria (<0.5 g/24 h). The study protocol was described in detail to all patients before admission, and informed consent to perform the study was obtained in each instance.

Study Protocol
The study design was an open, sequential, within-patients evaluation. Patients entered a 2-wk stabilization period, during which they received Sandimmune as the trial drug at the same previous maintenance daily dose, and blood CsA trough levels were repeatedly measured for determining individual therapeutic windows. At the end of this period, each patient’s 12-h CsA pharmacokinetic profile was measured after the morning dose of Sandimmune. The next day, the patients were shifted to Neoral at a 1:1 dose ratio. The Neoral daily dose was adjusted to maintain the CsA trough level in the established individual therapeutic window during the next 2 wk, and the CsA pharmacokinetics were again evaluated after 2 more wk at the fixed Neoral dose.

The pharmacokinetic profiles were based on analysis of blood samples collected from the antecubital vein 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h after the morning Neoral or Sandimmune administration. The CsA blood concentration-time profile was recorded for all patients, together with trough level (C_{trough}) and time (T_{max}) to reach the maximum concentration, C_{max}. The area under the blood concentration curve from time equals zero to the last sampling point (12 h) (AUC_{0→12}) was calculated by trapezoidal rule.

Analytical Procedures
Blood CsA analysis was performed with HPLC (17). Isocratic liquid chromatographic separation was carried out on a Model 342 high-pressure liquid chromatograph equipped with a Model 163 UV detector (Beckman, Fullerton, CA) operating at 214 nm. The column was a 150 mm × 4.6 mm i.d., 5 μm Ultrasphere C-8 (Beckman) and was heated to 72°C in a Model 101 LV Oven (Perkin-Elmer, Norwalk, CT). The mobile phase consisted of acetonitrile:methanol:water:ammonium sulfate (0.757 M) in proportions of 380:340:280:1. The eluent was pumped throughout the column at a flow rate of 1.0 ml/min. CsA and the internal standard, cyclosporin D (CsD), eluted at 7.8 and 9.4 min, respectively. A Chromatopac C-R3A integrator (Shimadzu, Kyoto, Japan) was used for the detector signal output. The samples were vortexed for 10 s to lyse the blood cells. One milliliter of heptane was then added, and the samples were vortexed for 10 s. Then 8 ml of diethyl ether was added, and the tubes were then vortexed for 30 s. The organic phase was clarified by centrifugation for 10 min. The ether layer was decanted into a clean test tube and washed with 1 ml sodium hydroxide (0.1 M) by vortexing for 10 s. After centrifugation for 5 min, the ether layer was transferred into a clean tube and evaporated to dryness at 40°C in a water bath under a gentle nitrogen stream. The residue was redissolved in 200 μl of eluent by vortexing for a further 30 s with heptane (1 ml). The sample was finally centrifuged for 5 min, and a 25-μl aliquot of the lower aqueous layer was injected onto the HPLC column for analysis.

Statistical Analyses
Multiple and stepwise regression analyses were carried out using AUC as the dependent variable, and the blood concentrations were grouped by time as the independent variables to find the minimum number of time points that could be used to provide a good estimate of AUC.

Agreement between the predicted and measured AUC was estimated using regression, and the prediction error was calculated as:

\[
\text{% prediction error} = \frac{(\text{predicted AUC} - \text{measured AUC})}{\text{measured AUC}} \times 100.
\]

Results
Pharmacokinetic profiles after Neoral administration were more consistent and reproducible than those after Sandimmune. Specifically, after conversion from Sandimmune to Neoral while the C_{trough} was unchanged (Sandimmune: 97 ± 40 ng/ml; Neoral: 99 ± 40 ng/ml), the mean AUC increased from 2794 ± 828 to 3376 ± 1011 ng·h/ml (average increase, 25%; P < 0.01, paired t test). The rate of CsA absorption differed between the two formulations: T_{max} was earlier (1.7 ± 0.8 versus 2.7 ± 1.6 h, P < 0.01) and C_{max} was higher (878 ± 309 versus 646 ± 193 ng/ml, average increase 51%, P < 0.01) for Neoral formulation as compared with Sandimmune.

Stepwise regression analysis indicated that, for Neoral, the best results in AUC prediction were obtained by using a three-point strategy. Particularly, the highest correlation between measured and predicted AUC (r = 0.992) was found with sampling points at 1.5, 8, 11 h after Neoral dosing. In this setting, the associated error in AUC prediction ranged from −8.0% to 8.8%. By contrast, with Sandimmune, comparable results (r ≥ 0.990) were obtained only using a five-point strategy (1, 2, 3, 6, and 7 h after Sandimmune dosing), with an associated error in AUC prediction ranging from −5.6% to 5.9%. Applying a three-point strategy to the Sandimmune pharmacokinetic profile, the regression analysis still showed a high correlation between measured and predicted AUC (r = 0.987), but the error in AUC prediction yielded values ranging from −13.6% to 7.6%. As shown in Table 1, with the same number of blood sampling points in any instances, the model predicted the measured AUC better with Neoral than with Sandimmune administration.

Collection of three blood samples, the last of which was followed by hydrochloric acid (1 ml of 0.2 M solution). The samples were vortexed for 10 s to lyse the blood cells. One milliliter of heptane was then added, and the samples were vortexed for 10 s. Then 8 ml of diethyl ether was added, and the tubes were then vortexed for 30 s. The organic phase was clarified by centrifugation for 10 min. The ether layer was decanted into a clean test tube and washed with 1 ml sodium hydroxide (0.1 M) by vortexing for 10 s. After centrifugation for 5 min, the ether layer was transferred into a clean tube and evaporated to dryness at 40°C in a water bath under a gentle nitrogen stream. The residue was redissolved in 200 μl of eluent by vortexing for a further 30 s with heptane (1 ml). The sample was finally centrifuged for 5 min, and a 25-μl aliquot of the lower aqueous layer was injected onto the HPLC column for analysis.

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obtained at 11 h after Neoral administration, is, however, difficult in the routine patient’s follow-up. Considering the peculiar CsA pharmacokinetic profile in patients receiving Neoral, we proceeded to stepwise regression analysis using only sampling points up to 5 h after drug dosing, which would include the absorption and the distribution phase of the drug. With sampling points from 0 to 5 h, the best AUC prediction by stepwise regression analysis was obtained at 1, 3, and 5 h, with a correlation coefficient of 0.994 and an associated error in AUC prediction ranging from -5.1% to 9.2%. To further shorten the time of the last blood sampling, we analyzed the pharmacokinetic profile from 0 to 3 h after Neoral administration. This interval corresponds to the time required to reach the mean maximum CsA blood concentration plus 2 SD [1.7 + (2 × 0.8 h)]. Again, the best results were obtained using a three-point strategy, as demonstrated by the following equation:

\[ \text{AUC} = 5.189 \times [0 \text{ h}] + 1.267 \times [1 \text{ h}] + 4.150 \times [3 \text{ h}] + 135.079 \]

As shown in Figure 1, the stepwise regression analysis gave a correlation coefficient between measured and predicted AUC of 0.989, whereas the associated error in AUC prediction ranged from -9.0% to 7.2%. Calculating the 95% confidence interval for these data, a further index of the validity of the above equation, the analysis gave a zero error value inside such interval (CI, -1.91% to 2.55%; average error 0.32%).

We then applied the same equation to predict CsA AUC after Sandimmune administration. As reported in Figure 2, the values were more dispersed around the line of identity. This resulted in a lower correlation between measured and predicted AUC \( r = 0.872 \), with a very large error in AUC prediction (range, -31.2 to 66.4%). The errors in AUC prediction with different models that analyzed the data obtained from pharmacokinetic profiles after Neoral or Sandimmune administration are summarized in Figure 3.

Given the fact that time 0 blood collection (the trough level) should be performed exactly 12 h after the evening dose of CsA, but that only a few patients met this requirement, we have reanalyzed our data up to 3 h without considering time 0 sampling. By stepwise regression analysis, the best AUC prediction was obtained with three blood samplings according to the following equation:

\[ \text{AUC} = 1.542 \times [1 \text{ h}] - 0.686 \times [2 \text{ h}] + 5.834 \times [3 \text{ h}] + 176.923 \]

This 1-, 2-, and 3-h point strategy gave a correlation coefficient between measured and predicted AUC of 0.984, with an
associated error in AUC prediction ranging from 14.13 to
-12.70%.

We have also considered a two-point sampling approach, such as 0 and 2h, 0 and 4h, or 2 and 6h after dosing, as suggested by some investigators (16,18,19). With this two-point blood sampling strategy, the correlation coefficient between measured and predicted AUC was 0.931, 0.839, and 0.949, respectively.

**Discussion**

The data presented here show that a simplified sampling strategy with three time points of blood collection at 0, 1, and 3 h after Neoral administration allows adequate and accurate prediction of the daily exposure to CsA of patients given Neoral as a part of their immunosuppressive therapy. Until now, routine monitoring of CsA drug therapy is performed by measuring blood/plasma CsA trough concentration, but with the latter method, the percentage of failure in predicting CsA exposure is elevated (12,13). The measurement of the AUC was introduced as an alternative to trough-level monitoring of CsA therapy. The AUC, calculated from the individual pharmacokinetic profile, is obviously more informative and is a better indicator of drug exposure, and, ultimately, is a better predictor of the clinical outcome. Indeed, AUC monitoring provides a better prediction of the patient risk in rejection of transplanted kidney or in CsA-induced episodes of toxicity. On the other hand, AUC estimation from the complete 12-h pharmacokinetic profile after CsA dosing to guide dosage decision is difficult to apply in the day-to-day outpatient clinic follow-up, particularly because of the discomfort involved for the patient. The possibility of measuring the CsA AUC within a reasonable percentage of error (less than 9%) by using only three very early blood samples after Neoral dosing indicates that, with this novel CsA formulation, drug therapy and patient exposure to the drug could be monitored accurately with minimum effort. Indeed, three blood samples collected within 3 h after Neoral administration are definitely less uncomfortable for the patient because he can save time and, more importantly, the physician can reduce the total amount of blood collected, compared with the complete pharmacokinetic profile. This strategy is also less taxing for the transplant center in terms of the effort required of the staff for blood sampling and sample analysis and overall results in reduction of costs. The reliability of the proposed approach is further supported by the fact that the error in AUC prediction using three very early sampling points after CsA dosing is identical to that obtained with the equation derived, considering three points on the overall pharmacokinetic profile, namely those at 1.5, 8, and 11 h. These results are at variance with those we have previously shown in patients receiving the conventional Sandimmune formulation, in which a limited sampling strategy resulted in a very high error in AUC prediction as compared with measured AUC (13). This has been also confirmed in the study presented here, in which a lower correlation coefficient between measured and predicted AUC was found in patients given Sandimmune than in the same patients receiving Neoral. This was also associated with a larger error in AUC prediction. One possible explanation is that Neoral gives a more consistent and reproducible CsA pharmacokinetic profile in the same patients in different occasions (15) because it is more rapidly, completely, and
uniformly absorbed because of its microemulsion formulation (14–16).

A complete pharmacokinetic profile includes blood sampling from time 0 to 12 h after CsA dosing (12). This point strategy assumes that time 0 is the trough level, the minimum CsA concentration 12 h after the evening dose of the drug. Usually, however, this is difficult to achieve, and only a few patients usually meet this requirement. To avoid the bias of the trough point blood sampling, we also analyzed our data according to a limited sampling strategy up to 3 h after dosing, excluding the time 0 point. Although in this case (1, 2, and 3 h points by stepwise regression analysis) the correlation coefficient between measured and predicted AUC was very similar to that obtained with 0, 1, 3 h points after Neoral dosing, the associated error in AUC prediction was wider.

Prediction of CsA AUC using a limited sampling strategy in patients given Neoral has been recently proposed by Foradori and coworkers (20), who found a high correlation ($r = 0.910$) between measured and predicted AUC with three sampling points at 1, 2.5, and 5 h after dosing. Similarly, other investigators (21) have documented the best AUC prediction with blood sampling at 0, 1, and 2 h in patients receiving the new CsA formulation. However, when we applied the equation derived from the above-mentioned studies to our patients, we found a very large associated error (ranging from -25.2 to 17.6% [Figure 3]), despite the fact that there was a good correlation between measured and predicted AUC ($r = 0.938$). On the other hand, other investigators have found the best AUC prediction by regression analysis with a two-point blood sampling strategy, namely 0 and 2 h, 0 and 4 h, or 2 and 6 h after Neoral dosing (16,18,19), but this was not confirmed by our results. These discrepancies may be the consequence of the analysis performed on different sets of data and/or related to differences in the analytical method employed for CsA blood determination (HPLC versus fluorescence polarization immunoassay).

In summary, the findings presented here indicate that in kidney transplant recipients, (1) a limited blood sampling strategy early after CsA dosing is feasible in patients given Neoral but not Sandimmune; (2) three sampling points at 0, 1, and 3 h after Neoral administration accurately predict the actual daily exposure of patient to CsA, as measured by the AUC$_{0\rightarrow12}$. Thus the peculiar pharmacokinetic profile of Neoral, related to its microemulsion formulation, offers for the first time the opportunity of adequate day-to-day monitoring of CsA exposure, with minimum effort for clinicians and staff nurses and no discomfort for the patient.

Better monitoring of transplanted patients by using this approach might ultimately potentiate the immunosuppressive effect of CsA and minimize the risk of side effects, possibly contributing to prolongation of the long-term survival of the graft.

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References


