Efficacy of Area Under the Curve Cyclosporine Monitoring in Renal Transplantation

Mary M. Meyer, Myrna Munar, Joseph Udeaja, and William Bennett

ABSTRACT

Previous studies suggest that area-under-the-curve (AUC) pharmacokinetic monitoring is superior to trough level monitoring for proper cyclosporin A (CSA) dosing, but AUC monitoring is expensive and unwieldy. The utility of a simplified AUC monitoring method was evaluated for predicting AUC on the basis of three timed levels. CSA pharmacokinetic profiles were studied in 27 renal transplant patients at steady state early (days), late (months), and in some patients, serially posttransplantation. Whole-blood RIA levels were obtained at 2, 4, 6, 10, 12, 14, and 24 h after a once-daily CSA dose. The 6- and 24-h levels were the best single-level predictors of AUC ($r = 0.77$ and $0.76$, respectively). The best model predictive of AUC curves used three time points at 2, 6, and 24 h postdose: AUC predicted = $8.6 \times (24 \text{ h}) + 1.4 \times (2 \text{ h}) + 6.2 \times (6 \text{ h}) + 1.57 \text{ mg} \times \text{h/L}; r^2 = 0.986, P = 0.00001$. The greatest pharmacokinetic variability occurred between 0 and 10 h postdose (absorption and distribution) between patients and even within individual patients monitored serially over time. The 12- to 24-h postdose portion (elimination) of the curve was consistently flat and uniform among patients. AUC were not consistent in individual patients over time. An AUC of more than 13 mg x h/L correlated with nephrotoxicity, whereas a value of 8 mg x h/L correlated with protection from rejection in first-transplant recipients. This AUC, however, was not able to prevent rejection in reengrafted or highly sensitized patients.

Key Words: Area under the curve, cyclosporine pharmacokinetics, renal transplant, trough level monitoring

Since the introduction of cyclosporin A (CSA) into clinical practice for transplant and nontransplant indications, physicians and pharmacologists have been frustrated in their efforts to target a therapeutically effective but nontoxic regimen for oral CSA administration. In most centers, trough level monitoring is used; however, the optimization of CSA dosing by this method is difficult because of the narrow therapeutic window that balances the adequacy of dosage to prevent rejection with avoidance of nephrotoxicity.

Trough level monitoring and maintenance of this single CSA level within a narrow "therapeutic range" correlates poorly with clinical events such as rejection or nephrotoxicity in clinical renal transplantation. Trough level monitoring does not take into account the extreme patient variability observed in CSA absorption, metabolism by the intestinal and liver P450IIIa microsomal oxidase system, volume of distribution, enterohepatic circulation, hepatic clearance, and tissue drug accumulation.

Previous studies have shown that formal CSA area-under-the-curve (AUC) determination, but not trough level monitoring, correlated significantly with the oral dose of CSA (total milligrams or milligrams per kilogram). Grevel et al. (4) reported that periodic formal AUC monitoring can reduce the number of dosage adjustments required in individual patients by a factor of 3. However, such AUC monitoring requires the collection of multiple postdose blood samples, which is expensive, labor intensive, and too inconvenient for routine clinical practice. Therefore, we evaluated the efficacy and reproducibility of a simplified practical method of AUC determination with only three blood samples.

METHODS

Patient Population

CSA pharmacokinetic profiles were studied at steady state in 28 adult (19 men, 9 women; range, 20 to 63 yr) renal transplant recipients at three different times after transplantation. Ten patients were studied once, 16 patients were studied twice, 1 patient was studied three times, and 1 patient was studied...
The patients were clinically stable, and all had normal cholesterol and no abnormalities in hepatic enzymes. Hematocrit rose from (initial) post-transplant study (Group 1) to the final study time but was stable in Groups 2 and 3. No medication changes were made during the course of the study. Except for one patient treated with diltiazem, none of the patients were being treated with medication known to interact with CSA. A three-sample AUC was used to predict AUC calculated from seven sampling points. To develop the three-point model, initial CSA AUC studies were performed at steady state in 10 renal transplant recipients (Group 1, perioperative period). The model was then tested in another group of 17 renal transplant recipients who were between 2 and 31 mo posttransplantation (Group 2, early transplant period; Group 3, late transplant period) (Table 1).

**Group 1 (Perioperative Period)**. Fourteen patients were studied perioperatively. All patients received OKT3 prophylaxis through postoperative day (POD) 11. Solumedrol was administered at a dose of 250 mg on POD 0, and 125 mg was given twice daily on POD 1, tapering rapidly to 0.5 mg of prednisone per day. Azathioprine was administered at 5 mg/kg on POD 0 and at 2 mg/kg per day thereafter. CSA (7 mg/kg) was begun on POD 5, and the dosage was adjusted to maintain a whole-blood CSA concentration of 250 to 350 ng/mL by whole-blood monoclonal RIA. Pharmacokinetic monitoring was performed at steady state within 8 to 24 days postoperatively (11 patients were studied on POD 8).

**Group 2 (Early Transplant Period)**. Serial CSA pharmacokinetic monitoring was performed in 17 (2 from Group 1) renal transplant patients who had serum creatinine levels of less than 2.0 mg/dL and who were within 6.5 mo of transplantation. CSA was administered once daily between 7:00 and 8:00 a.m. and was followed by a meal.

**Group 3 (Late Transplant Period)**. Serial CSA pharmacokinetic monitoring was performed in 14 (2 from Groups 1 and 2) stable renal patients who had serum creatinine levels of less than 3.1 and who were within 7.5 to 31 mo of transplantation. CSA was administered once daily between 6:00 and 7:00 p.m. and was followed by a meal. The groups did not differ demographically.

**Pharmacokinetic Monitoring**

Timed blood samples were collected at steady state before the dose and at 2, 4, 6, 10 to 12, 14, and 24 h after a once-daily dose of CSA. Steady state was assumed if the patient received the same CSA dose for at least 5 days. For serial studies, the CSA was taken at the same time for all studies and was always accompanied by a meal. CSA levels were determined by whole-blood monoclonal RIA (Incstar Corp., Stillwater, MN). The within- and between-assay coefficients of variation were both less than 10% (10% and 6.4 to 9%, respectively). The lower limit of detection was 25 ng/mL.

**Statistical Analysis**

The peak concentration ($C_{\text{max}}$) and the time to peak ($T_{\text{max}}$) were the observed values. Area under the serum concentration versus time curve (AUC) was calculated by the linear trapezoidal rule and was extrapolated to infinity ($AUC_{\infty}$). The AUC was divided by 24 h to obtain the average steady-state CSA concentration during the dosing interval ($C_{\text{ss}}$). Noncompartmental analysis was used to calculate total body clearance ($CL/F$, where $CL$ is clearance and $F$ is an oral bioavailability factor), apparent volume of distribution ($V_{\text{area}}/F$), and mean residence time (MRT) according to the following equations: $CL/F = dose/AUC_{\infty}$, $V_{\text{area}}/F = CL/\beta$, and MRT = $AUC_{\infty}/\beta$, where $\beta$ is the terminal slope of the linear least-squares regression line of a semilogarithmic plot of serum concentration versus time data and $AUMC$ is the area under the moment curve. Half-life ($t_{1/2}$) was calculated by the equation: $t_{1/2} = 0.693/\beta$. Pearson product-moment correlations were calculated to evaluate the linear relationship between CSA levels and dose and CSA levels and AUC. Multiple regression analysis was then used to identify the minimum number of CSA levels required to predict CSA AUC.

Predicted CSA AUC were compared with the corresponding measured CSA AUC with prediction error, where:

\[
\text{prediction error} = \frac{\text{predicted AUC} - \text{measured AUC}}{\text{measured cyclosporine AUC}}
\]

Patient demographic data, episodes of rejection, nephrotoxicity (by histologic criteria or by rises in serum creatinine, which declined with CSA dose reduction), liver function abnormalities, lipid profiles, and interactive drug therapy were noted in all patients. Analysis of variance (ANOVA) was performed to assess differences in demographics, laboratory data, and AUC among the three patient groups. Differences in AUC among patients with or without rejection (by rise in serum creatinine, which responded to heightened immunosuppression, or by histologic criteria) were evaluated by the use of ANOVA. Significance was defined as $P \leq 0.05$. SPSS/PC (Chicago, IL) was used for statistical analysis.

**RESULTS**

**Correlation of Level With Dose and AUC**

There were poor correlations between the area under the CSA blood concentration over time curve (AUC/24 h) and single whole-blood CSA levels at any
### TABLE 1. CsA pharmacokinetics for the early and late transplant periods

<table>
<thead>
<tr>
<th>Patient</th>
<th>No. of Days Post-transplantation</th>
<th>f&lt;sub&gt;u&lt;/sub&gt; (h)</th>
<th>MRT (h)</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; (L)</th>
<th>CL (mL/min)</th>
<th>Predicted AUC 0–24 (mg·h·L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>AUC 0–24 (mg·h·L&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
</table>

#### Early Transplant Period

**Group 1 (N = 14)**

- AND 19 16.9 18.8 1,223.54 1,188 9.5 7.1
- CAR<sup>b</sup> 8 16.9 14.2 1,127.51 1,539.17 3.1
- CLO<sup>b</sup> 8 22.4 19.6 1,874.69 1,740.17 3.2
- COL<sup>b</sup> 9 24.7 23.4 650.75 496.67 10.5
- EGG<sup>b</sup> 8 22.4 29.3 2,199.22 1,522.83 2.5
- EST<sup>b</sup> 9 19.8 19.2 513.21 485.33 13.9
- GAR<sup>b</sup> 13 16.9 20.3 537.88 479.33 14.7
- HAI 8 15.1 12.7 592.74 993 8.1 8.9
- MUS<sup>b</sup> 8 16.1 26.9 393.36 259 17.8
- NEE 9 18.7 19.7 1,556.42 1,435 5.7 5.9
- RAM<sup>b</sup> 8 16.9 17.5 1,099.12 1,154.67 6.5
- RMZ 24 21.0 28.9 747.02 455.83 12.3 12.3
- TIL<sup>b</sup> 7 16.1 11.9 622.52 1,007.67 7.1
- WAL<sup>b</sup> 8 19.8 13.4 863.04 1,223.33 4.8
- Mean 10 18.8 19.7 1,000.07 984.29 8.4
- SD 5 2.8 5.5 529.18 452.95 4.6

**Group 2 (N = 18)**

- BLA 184 8.7 12.1 873.5 1,382.33 6.2 7.1
- COL 53 7.5 9.7 349.17 717.67 9.4 9.6
- CRU 118 7.7 11.7 502.16 825.82 12.7 9.2
- EGG 130 7.2 7.7 317.14 870.83 8.0 9.1
- DON 63 8.4 11.7 529.29 874.75 8.0 8.9
- DON 96 19.3 22.5 771.49 613.5 8.9 9.5
- FON 183 15.07 18.8 471.22 457.17 10.3 13.4
- FON 197 14.4 18.2 676.81 680.33 7.6 9.0
- KRU 194 26.7 28.9 1,299.14 792.34 6.1 6.3
- NEE 52 13.9 18.6 819.46 803.83 7.6 8.8
- OST 169 9.1 13.6 572.64 792.63 7.9 7.9
- OST 193 8.5 11.8 366.93 600.5 8.9 10.9
- RAM 158 8.9 11.8 787.97 1,292 6.1 6.7
- RAM 165 6.1 7.9 490.69 1,297.69 7.7 6.3
- TUH 111 12.6 16.8 456.48 499.96 9.3 8.8
- TUH 144 6.2 11.0 374.32 660.5 6.6 7.8
- WIN 120 7.8 10.3 433.18 832.55 5.4 8.5
- WIN 153 10.3 13.6 765.52 1,066.5 4.1 4.5
- Mean 138 11 14.3 603.17 836.73 7.8 8.3
- SD 47 5.1 5.3 241.53 259.35 1.9 2.0

#### Late Transplant Period

**Group 3 (N = 15)**

- ABA 319 11.7 19.9 409.93 372 5.0 6.0
- BLA 212 13.6 15.9 844.16 984.5 7.6 9.3
- CLR 370 14.4 17.0 807.4 872.8 4.4 4.3
- CLR 377 8.1 11.9 507.34 817 5.3 5.4
- HAI 218 2.1 3.2 2,441.99 1,465.41 5.9 5.1
- HAII 225 9.9 14.3 747.82 977.83 8.1 6.4
- HAI 291 5.2 12.2 721.44 940.5 6.4 8.0
- HAL 298 5.7 11.9 547.6 888.04 9.4 8.7
- OLS 347 9.4 17.1 697.05 746.83 4.5 6.1
- PLA 942 10.2 17.4 836.88 884.5 5.5 6.5
- PLA 956 12.2 17 603.9 651.82 6.9 8.7
- RAM 245 6.7 12.5 560.45 854.67 8.5 10.3
- ROB 662 5.7 12.3 466.28 723.22 7.9 9.9
- ROB 669 13.9 16.5 530.74 591.67 8.7 10.3
TABLE 1.—Continued

<table>
<thead>
<tr>
<th>Patient</th>
<th>No. of Days Post-transplantation</th>
<th>( t_n ) (h)</th>
<th>MRT (h)</th>
<th>( V_{ss} ) (L)</th>
<th>CL (mL/min)</th>
<th>Predicted AUC (mg h/L)</th>
<th>AUC 0-24 (mg h/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>438</td>
<td>9.2</td>
<td>14.2</td>
<td>765.93</td>
<td>840.77</td>
<td>6.7</td>
<td>7.5</td>
</tr>
<tr>
<td>SD</td>
<td>251</td>
<td>3.6</td>
<td>3.9</td>
<td>236.89</td>
<td>236.89</td>
<td>1.6</td>
<td>2.0</td>
</tr>
</tbody>
</table>

\( \text{MRT, mean residence time; CL, clearance; } V_{ss}, \text{ volume of distribution at steady state as calculated by}\ (\text{CL/KEL, where KEL is the elimination rate constant; predicted AUC, AUC predicted by the three-point model; AUC 0-24, actual AUC calculated from seven points.} \)

postdosage time (2, 4, 6, 10, 12, 14, 24 h, peak), whether the CSA dose was examined with total milligrams or normalized for body weight (milligrams per kilogram) (Table 2). The linear relationship between dose and CSA level was worse when the CSA dose was normalized for body weight (which is the standard dosing method for CSA administration) than when the total dose in milligrams was used. A number of levels were significantly correlated with AUC. The 6- and 24-h postdose levels showed the best correlation with AUC \((r = 0.77, P < 0.01; r = 0.76; P < 0.01, \text{respectively})\).

Figure 1 shows the linear relationship between CSA AUC and the 6- and 24-h postdose levels. A majority of the points lie in close proximity to the regression line with \( r \) values of 0.77 and 0.76, respectively, and a highly significant \( P \) value of 0.0001. The best model with which to predict CSA AUC on a single daily dosage regimen incorporated three levels (2, 6, and 24 h) according to the following equation \((r^2 = 0.986; P < 0.00001):\)

\[
\text{predicted AUC} = 8.6 \times [24 \text{ h}] + 1.4 \times [2 \text{ h}]
+ 6.2 \times [6 \text{ h}] + 1.57 \text{ mg} \times \text{h/L}
\]

TABLE 2. Correlation coefficients between CsA levels, dose, and AUC

<table>
<thead>
<tr>
<th>CSA Dose (mg)</th>
<th>CSA Dose (mg/kg)</th>
<th>AUC 0-24 mg x h/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 h</td>
<td>0.20 (0.18)</td>
<td>0.16 (0.29)</td>
</tr>
<tr>
<td>4 h</td>
<td>0.29 (0.05)</td>
<td>0.05 (0.75)</td>
</tr>
<tr>
<td>6 h</td>
<td>0.28 (0.06)</td>
<td>-0.04 (0.80)</td>
</tr>
<tr>
<td>10 h</td>
<td>0.28 (0.06)</td>
<td>0.36 (0.02)</td>
</tr>
<tr>
<td>12 h</td>
<td>0.43 (0.37)</td>
<td>-0.18 (0.24)</td>
</tr>
<tr>
<td>14 h</td>
<td>0.10 (0.52)</td>
<td>-0.04 (0.77)</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (Peak)</td>
<td>0.38 (&lt;0.01)</td>
<td>0.05 (0.72)</td>
</tr>
<tr>
<td>24 h (Trough-2)</td>
<td>0.14 (0.36)</td>
<td>0.08 (0.59)</td>
</tr>
<tr>
<td>( C_{\text{avg}} )</td>
<td>0.36 (0.01)</td>
<td>1.00 (&lt;0.01)</td>
</tr>
<tr>
<td>AUC 0-24</td>
<td>0.36 (0.05)</td>
<td>0.07 (0.65)</td>
</tr>
</tbody>
</table>

\( \text{* Pearson product-moment correlation } r \text{ (P value). } N = 46. \)
Predicted CSA AUC (mgxhr/L) vs Actual CSA AUC (mgxhr/L)

- Regression Line
- Line of Identity

Figure 2. Prediction of actual AUC with three CSA levels (three-point prediction) at 2, 6, and 24 h post-CSA dose: predicted AUC = 8.6 x (24 h) + 1.4 x (2 h) + 6.2 x (6 h) + 1.57 mg x h/L.

levels but rapid clearance. One case was underpredicted by more than 25% and tended to have an earlier peak level (2 h). In the majority of cases (32 of 35; 91%), the prediction error was less than 25%, and in 27 (77%) of 35 patients, the model underpredicted the AUC.

AUC Profiles

Individual AUC profiles were examined in detail, and Figure 3 shows selected patient profiles comparing individual differences and serial differences over time. A majority of patients reached peak CSA value at or before 6 h after the administration of CSA: 2 h, 29 patients; 4 h, 6 patients; 6 h, 10 patients; 8 h, 1 patient.

The greatest pharmacokinetic variability between patients and within a single patient over time occurred within the first 10 h of receiving a single daily dose of CSA. This was depicted in Figure 4, which illustrates a greater degree of variability in peak levels when compared with either the 12- or 24-h levels. In all patients, the 12- to 24-h portion of the pharmacokinetic curve was uniform and flat. There was a very poor correlation between initial AUC studies (AUC 1) and AUC studies repeated in the same patient 1 wk to 8 mo later (AUC 2) (Figure 5, CSA AUC-1 versus CSA AUC-2). There were seven episodes in which the CSA level peaked twice in 24 h, implying enterohepatic recirculation. In all cases, prediction errors were less than 25% (Table 3).

Clinical Correlation

Four of 27 grafts failed, with one patient death due to overwhelming infection. Two of the graft losses were related to CSA nephrotoxicity. The fourth patient was a retransplant patient with 100% panel reactivity and did not reach peak AUC levels, but rapid clearance. One case was underpredicted by more than 25% and tended to have an earlier peak level (2 h). In the majority of cases (32 of 35; 91%), the prediction error was less than 25%, and in 27 (77%) of 35 patients, the model underpredicted the AUC.

Figure 3. Serial AUC performed at various times between 10 days and 8 mo posttransplantation. Two selected patients.

Figure 4. Interpatient variability of CSA levels at different times after dosage administration.

Figure 5. Comparison of intrapatient variability of AUC at different times after transplantation (AUC-1 versus AUC-2) (N = 18).
reactive antibody (PRA) who developed rejection refractory to a steroid pulse followed by antilymphocyte therapy.

Two patients developed chronic rejection within a year of transplantation. Both patients were observed to have low AUC (<6 mg x h/L). One of these patients was a regrafted patient with a PRA of 100%, documented noncompliance, and the lowest recorded AUC was a regrafted patient with a PRA of 100%, documented noncompliance, and the lowest recorded AUC was

The biopsy revealed focal cortical necrosis, fibrin thrombi in the glomerular capillaries, and tubular calcification consistent with, but not diagnostic of, CSA toxicity. CSA was discontinued, but allograft nephrectomy was required 16 days later and was found to have widespread thomboses.

Patient C (EST) (AUC = 13.9 mg x h/L). The patient received a second allograft and developed high CSA levels while on diltiazem. The patient developed a mild steroid responsive rejection on POD 21 but did not develop nephrotoxicity and remained rejection free thereafter.

Patient D (FON) (AUC = 13.4 mg x h/L). The patient developed elevated bilirubin and serum glutamic-oxaloacetic transaminase, myalgias, arthralgias, and no evidence of nephrotoxicity. There were no rejection episodes.

No significant differences in AUC were found among patients with or without rejection (P < 0.05) (Figure 6). However, when patients were grouped according to the number of transplants (first cadaveric or living related versus reengraftment), there was a significant difference in AUC between those who rejected and those who did not (Figure 7). Predicted AUC by use of the three-point model showed similar results (Table 4).

Corresponding differences were observed with Cmax levels. However, no significant differences in the 12-h, 24-h, or Cmax levels were found between patients who rejected compared with those who did not (Table 4).

All first-graft recipients who experienced an episode of rejection had an AUC value of less than 6 mg x h/L before the rejection episode, whereas all but one recipient of first grafts, who experienced no rejection, had AUC values of more than 6 mg x h/L, with a mean value of 8.7. Only one recipient of a second graft experienced no episode of rejection. AUC was significantly higher in first-transplant patients without rejection compared with those with rejection (P < 0.05).

Thus, an AUC of 8 in a first-transplant recipient appeared to correlate with protection from rejection without nephrotoxicity. Our data suggest that a higher AUC must be targeted for regrafted patients, because rejection occurred despite higher mean AUC

<table>
<thead>
<tr>
<th>Patient</th>
<th>Actual AUC</th>
<th>Predicted AUC</th>
<th>Prediction Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAM</td>
<td>6.65</td>
<td>6.10</td>
<td>-0.08</td>
</tr>
<tr>
<td>HAI</td>
<td>5.12</td>
<td>5.90</td>
<td>0.15</td>
</tr>
<tr>
<td>NEE</td>
<td>8.76</td>
<td>7.60</td>
<td>-0.13</td>
</tr>
<tr>
<td>DGN</td>
<td>8.98</td>
<td>8.00</td>
<td>-0.11</td>
</tr>
<tr>
<td>HAL</td>
<td>8.01</td>
<td>6.40</td>
<td>-0.20</td>
</tr>
<tr>
<td>HAL</td>
<td>8.70</td>
<td>9.40</td>
<td>0.08</td>
</tr>
<tr>
<td>ABA</td>
<td>5.97</td>
<td>5.00</td>
<td>-0.19</td>
</tr>
</tbody>
</table>

Figure 6. Comparison of AUC of patients who never rejected (N = 10) with those who rejected within 3 mo of transplantation near the time of measurement of AUC (N = 23) and with those whose AUC was measured at a time remote from rejection (N = 7).
Efficacy of AUC CsA Monitoring

Figure 7. Comparison of AUC in patients grouped according to number of transplants (Tx). First transplant with no rejection (11 patients, 17 AUC measurements), mean ± SE = 8.72 ± 0.83; first transplant with rejection (4 patients, 6 AUC measurements), mean ± SE = 4.92 ± 0.45; reengraftment with rejection (5 patients, 6 AUC measurements), mean ± SE = 8.23 ± 2.32.

values. Three of the five regrafted patients were highly sensitized and had PRA levels of 100%.

An AUC of more than 13 mg x h/L correlated with CSA nephrotoxicity (microangiopathic hemolytic anemia, fibrin thrombi in glomeruli, and/or primary nonfunction), although there were only four cases. Calcium channel blockers may be protective of CSA nephrotoxicity despite a high AUC, as suggested by Patient C above. Lower initial CSA doses (7 mg/kg) may be responsible for the low incidence of CSA toxicity (Table 4).

DISCUSSION

Stiller and Keown (5) showed that, in renal transplant recipients, there was no significant difference in CSA levels between those with and without rejection or nephrotoxicity. For their patients, CSA levels appeared to have no predictive value. They went so far as to suggest that CSA levels should only be obtained to confirm the absorption of the drug and to evaluate patient compliance. Others have found a fair correlation of nonselective polyclonal RIA methods of determining trough levels with adverse clinical events (6–8).

However, most clinicians are not comfortable with ignoring the blood level of a potentially toxic drug. They are equally unwilling to expend the considerable time and money necessary to obtain full pharmacokinetic monitoring of CSA, despite the demonstrated superiority of AUC monitoring over trough level monitoring (4).

Grevel et al. (9) have reported a model-derived AUC, using three times (2, 6, and 14 h), that correlated with a formal seven-point AUC:

\[
AUC = 2.91 \times (2 \text{ h}) + 5.95 \times (6 \text{ h}) + 11.68 \times (14 \text{ h}) + 153; \ \ r^2 = 0.963
\]

Those authors also showed, as we have (10), only fair correlations between a 24-h postdose trough sample and the AUC in 77 stable renal transplant patients receiving a single daily dose of CSA. However, in their data and ours, no single concentration within the seven-point profile could accurately predict AUC.

Johnston et al. (11) also developed an algorithm to predict AUC that incorporated three blood levels:

\[
\text{predicted AUC} = 4.3 \times [3.5 \text{ h}] + 5.5 \times [8 \text{ h}] + 3.1 \times [10 \text{ h}] - 333; \ \ r^2 = 0.99
\]

Correlation was less with a single 5-h level. Our findings confirmed that a three-level algorithm was highly predictive of the AUC derived from seven levels, but it tended to underpredict the actual AUC. A closer approximation of predicted AUC will be obtained with further study of a larger patient population.

Other investigators have noted that a 5- or 6-h postdose level is the best single, although imperfect, predictor of AUC (12). This time is more likely to reflect the interindividual and intraindividual varia-

<table>
<thead>
<tr>
<th>Event</th>
<th>Actual AUC</th>
<th>Predicted AUC</th>
<th>Cmax</th>
<th>Cmax</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Transplant, Rejection (N = 6)</td>
<td>4.92 ± 0.5a</td>
<td>4.20 ± 0.4a</td>
<td>205a</td>
<td>381</td>
<td>142</td>
<td>81</td>
</tr>
<tr>
<td>Retransplant, Rejection (N = 6)</td>
<td>8.23 ± 2.3</td>
<td>7.67 ± 2.5</td>
<td>343</td>
<td>107</td>
<td>246</td>
<td>138</td>
</tr>
<tr>
<td>1st Transplant, No Rejection (N = 17)</td>
<td>8.72 ± 0.8a</td>
<td>8.48 ± 0.9a</td>
<td>363a</td>
<td>268</td>
<td>287</td>
<td>160</td>
</tr>
<tr>
<td>Retransplant, No Rejection (N = 1)</td>
<td>7.11</td>
<td>5.78</td>
<td>296</td>
<td>1053</td>
<td>202</td>
<td>107</td>
</tr>
</tbody>
</table>

\[ a \] Difference between rejection versus no rejection in first-transplant patients; \( P < 0.05 \), ANOVA.
bility in CSA absorption, as demonstrated by the erratic behavior of the initial portion of the pharmacokinetic curve over time.

In the patients studied here, we were able to target a level that predicted nephrotoxicity and a level that was protective against rejection in first-transplant recipients. The proper AUC target in regrafted patients will require further study but is likely to be higher than in first-graft recipients.

The incidence of CSA nephrotoxicity was low and may reflect the efficacy and low toxicity of our immunosuppression protocol. CSA is not initiated until POD 5 at a low initial dose of 7 mg/kg. Experimental data suggest that once-daily dosing may be less nephrotoxic than twice-daily dosing. In addition, calcium channel blockers are used preferentially in our patients for the treatment of hypertension in allograft recipients (16 of 27 on nifedipine; 1 of 27 on diltiazem); this may ameliorate or reduce the incidence of CSA nephrotoxicity (13,14).

Regrafted patients tended to be more highly sensitized and may require higher targeted AUC in addition to antilymphocyte prophylaxis at the time of transplantation. Low AUC may be associated with chronic "vascular" rejection with extensive interstitial scarring. The two patients with the lowest AUC developed chronic rejection within 1 yr of transplantation. One patient had a low AUC because of documented noncompliance. The second patient had no episodes of acute rejection and had a serum creatinine level of less than 1.5 until a biopsy for a sudden inexorable rise in creatinine revealed extensive scarring and no acute cellular component.

Despite controlling for dosing time of day and association with meals, we found that pharmacokinetic profiles varied markedly over time (short or long interval) in individual patients.

Many investigators have studied the effect of food and meal composition on CSA absorption with conflicting results (15,16).

Awni et al. (17–19) showed that the metabolism of CSA varied insignificantly over time but that factors that altered the binding and distribution of CSA, such as rising hematocrit, plasma proteins, and especially lipoproteins, correlated with rising AUC and reduced CSA clearance because of the increased retention of the bloodbound portion.

Various factors have been identified (15,20–26) that have an effect on CSA pharmacokinetic profiles (Table 5).

In this study, stable patients several months post-transplantation had variability in AUC over time, despite relatively stable hematocrit, lipoprotein level, cholesterol level, and association with meals (but not meal composition). The variability in serial pharmacokinetic profiles has been observed by others (17–19,21,22) and is not fully understood. The multiplicity of factors that affect CSA absorption and distribution is as complex and difficult to control as patient compliance. Nevertheless, the observation that CSA pharmacokinetics can vary markedly over time, even in a compliant patient, has been noted repeatedly and must be taken into consideration when determining the frequency of obtaining levels and the necessity for making dosage adjustments in a clinically stable patient. At our institution, routine CSA trough level monitoring costs the hospital more than one million dollars per year and is the single most costly laboratory test in the institution. Serial AUC monitoring based on three levels obtained in a controlled fashion in a clinic setting may reduce the variability of some of the "patient factors" that contribute to variability. The patient’s clinic visit can be timed so that a trough sample is obtained at the time of arrival, followed by the administration of CSA and the 2- and 6-h levels. Blood samples are batched and charged to the patient as an adjusted single fee, comparable to the fee for a sample level. Once the target AUC is achieved, only occasional three-point AUC monitoring is necessary, unless a clinical event or dosage adjustment dictates a repeat full AUC study. Our goals are to reduce the number of levels obtained (and therefore the cost of CSA monitoring), as well as the misleading information provided by single trough levels (Table 6).

Once-daily CSA dosing is used routinely at our institution, so results observed in this study may not apply to twice-daily CSA dosing. However, our data showed that the 12-h level, which is used for twice-daily dosing, correlated poorly with AUC.

The variability of both trough level and AUC monitoring has prompted investigators to look for biologic assays of in vitro CSA immunosuppressive efficacy. Such experimental assays include binding to immunophilin (27), intranuclear parent CSA concentrations correlating with the degree of inhibition of [3H]thymidine incorporation, and the development of a rapid functional assay of the state of immune reac-

<table>
<thead>
<tr>
<th>Table 5. Variables in CSA pharmacokinetic profiles</th>
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<tbody>
<tr>
<td><strong>Timing of Meals and Meal Composition (25)</strong></td>
</tr>
<tr>
<td><strong>Bile Acid Administration, Bile Flow (23,25)</strong></td>
</tr>
<tr>
<td><strong>Gastric Emptying (21)</strong></td>
</tr>
<tr>
<td><strong>Body Weight (20)</strong></td>
</tr>
<tr>
<td><strong>Time After Transplant (24,25)</strong></td>
</tr>
<tr>
<td><strong>Lipoprotein Levels (21,25)</strong></td>
</tr>
<tr>
<td><strong>High-Fat Meals (15)</strong></td>
</tr>
<tr>
<td><strong>Impairment of Liver Function (25)</strong></td>
</tr>
<tr>
<td><strong>Age (24,25,28)</strong></td>
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<tr>
<td><strong>Circadian Variation (22,26)</strong></td>
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<tr>
<td><strong>Drug Interactions (24,25)</strong></td>
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<tr>
<td><strong>Drug Accumulation at the Tissue Level (24)</strong></td>
</tr>
<tr>
<td><strong>Diarrhea, Enteritis (25)</strong></td>
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</tbody>
</table>
Efficacy of AUC CsA Monitoring

TABLE 6. Cost comparison of trough level and AUC monitoring of CSA levels

<table>
<thead>
<tr>
<th>Current cost of CSA trough level monitoring per patient for first 6 posttransplant months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single CSA level: $54.50</td>
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<tr>
<td>Titr weekly, Months 1 to 2</td>
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<tr>
<td>Biweekly, Months 3 to 6</td>
</tr>
<tr>
<td>Other clinical events (rejection, infection, dosage adjustment)</td>
</tr>
<tr>
<td>Three-point AUC (2)</td>
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<tr>
<td>Clinical Event</td>
</tr>
<tr>
<td>Baseline steady state repeat</td>
</tr>
<tr>
<td>AUC (Establish and confirm validity of three-point algorithm)</td>
</tr>
<tr>
<td>Cost of batch CSA levels</td>
</tr>
<tr>
<td>(7) $84.00 126.50</td>
</tr>
<tr>
<td>(3) $36.00 78.50</td>
</tr>
<tr>
<td>Dosage adjustment</td>
</tr>
<tr>
<td>Three-point AUC (2)</td>
</tr>
<tr>
<td>Cost: $1,744.00</td>
</tr>
<tr>
<td>Estimated additional cost: $545.00</td>
</tr>
<tr>
<td>Total: $2,289.00</td>
</tr>
<tr>
<td>Cost: $763.00</td>
</tr>
<tr>
<td>Actual cost at our institution: $253.00</td>
</tr>
<tr>
<td>Cost: $327.00</td>
</tr>
<tr>
<td>Actual cost at our institution: $157.00</td>
</tr>
<tr>
<td>Total Cost: $1,417.00</td>
</tr>
<tr>
<td>Total cost of batch CSA levels: $667.00</td>
</tr>
</tbody>
</table>

4. **Grevel J, Welsh MS, Kahan BD:** Cyclosporine monitoring renal transplantation: Area under the curve monitoring is superior to trough-level monitoring. Ther Drug Monitoring 1989;11:246–248.
5. **Stiller C, Keown P:** Failure of 125K-tracer selective monoclonal antibody levels on a whole blood matrix to predict rejection or nephrotoxic episodes in renal transplant patients under antilymphocyte globulin and prednisone therapy. Transplantation 1990;22:1253–1254.

It is in the extreme case of the largest of living forms, the whale, that the mutational alteration has operated most spectacularly in the production of multiple renuli. And here is the most curious paradox: The resolution of this greatest of the adjustments of evolutionary structural-functional renal equilibrium is attained not by complexity of configuration but by simplification. As an earlier analyst has shown, each renulus of the whale is in its general structural configuration a complete, perfect, and most simple unipapillary kidney; the nephrons, which compose the aggregate are also of the simplest type. The result of the evolutionary processes seems therefore to have come full circle, and if the term kidney is to be used, then the literal statement must be made that, in any exact sense of the word, the problem has been solved in the whale not by 2 but by 14,000 kidneys.