

# Cyclosporin $C_2$ and $C_0$ Concentration Monitoring in Stable, Long-Term Heart Transplant Recipients Receiving Metabolic Inhibitors

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**Background:** Cyclosporin (CsA) dose selection is complicated by significant pharmacokinetic variability between patients. Although therapeutic drug monitoring (TDM) has proven to be a useful tool for dose individualization, the search for an effective and practical measure of clinical effect has uncovered a number of options. Monitoring the CsA concentration in a blood sample taken 2 hours after the dose ( $C_2$ ) has been utilized but has not been rigorously evaluated in all clinical situations. The aim of this study was to evaluate  $C_2$  and trough ( $C_0$ ) CsA concentrations as surrogate markers of area under the concentration–time curve (AUC) in stable, long-term heart transplant recipients receiving CsA alone or with diltiazem and/or ketoconazole.

**Methods:** CsA blood concentration–time data were collected at steady state for 47 stable heart transplant recipients after the morning dose of Neoral. CsA concentration in whole blood was quantitated using the EMIT immunoassay. Patients were stratified into 4 groups, depending on the long-term concomitant administration of drugs known to inhibit CsA metabolism, as part of their routine therapy: Group A ( $n = 11$ ), CsA alone; Group B ( $n = 10$ ), CsA with slow-release diltiazem; Group C ( $n = 13$ ), CsA with ketoconazole; and Group D ( $n = 12$ ), CsA with a combination of diltiazem and ketoconazole.

**Results:** In Group A,  $C_2$  correlated poorly with  $AUC_{0-5}$  ( $r^2 = 0.197$ ;  $p = 0.17$ ), whereas  $C_0$  (trough blood sample) showed a stronger correlation ( $r^2 = 0.710$ ;  $p = 0.001$ ). Correlations of  $C_0$  and  $C_2$  with  $AUC_{0-5}$  were the same, but weaker in patients receiving CsA and diltiazem ( $r^2 = 0.650$ ;  $p = 0.005$ ); however,  $C_2$  correlated strongly with  $AUC_{0-5}$  in patients receiving ketoconazole ( $r^2 = 0.870$ ;  $p < 0.0001$ ) or ketoconazole with diltiazem ( $r^2 = 0.898$ ;  $p < 0.0001$ ).  $C_0$  was a poor predictor of  $AUC_{0-5}$  in the latter 2 groups.

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**Conclusions:**  $C_2$  showed a strong correlation with  $AUC_{0-5}$  in cardiothoracic transplant recipients receiving CsA with ketoconazole, but not with CsA alone or diltiazem. TDM using  $C_2$  as an estimate of AUC requires further evaluation before being applied in long-term, stable cardiac transplant patients, as it may lead to inappropriate dose adjustment of CsA in patients receiving concomitant metabolic inhibitors. *J Heart Lung Transplant* 2003;22:715–722.

Optimizing cyclosporin (CsA) therapy remains challenging because of the narrow therapeutic window and variable pharmacokinetics of the drug. There are large inter- and intraindividual variabilities in CsA pharmacokinetics with a 12-fold interindividual variability in absorption and a 7-fold variability in clearance.<sup>1</sup> This situation is complicated by the concomitant administration of drugs that alter CsA exposure, disease states (e.g., cystic fibrosis), the time after transplantation, the organ transplanted, diet, race and age.<sup>2</sup> Reducing the intraindividual variability of CsA exposure has been shown to reduce episodes of chronic rejection and healthcare costs in renal transplant recipients.<sup>3</sup> Furthermore, CsA pharmacokinetics have been reported to have a marked impact on renal transplant graft outcome, and acute rejection episodes.<sup>1</sup> Therefore, therapeutic drug monitoring of CsA has become an accepted strategy to optimize dose; however, conventional methods using trough whole blood concentrations ( $C_0$ ) as the surrogate marker of effect and toxicity have been challenged.<sup>4</sup>

Recent drug monitoring strategies have focused on management of the variable absorption of CsA.<sup>5</sup> Area under the concentration–time curve (AUC) is an accepted measure of drug exposure and the concept of “absorption profiling,” with associated clinical benefits, has been proposed.<sup>5</sup> Various techniques have been used to predict AUC in patients receiving CsA. The limited-sampling strategy, using  $\geq 2$  CsA concentrations taken after the dose to estimate AUC from a derived formula has been suggested but has not been accepted universally because it is impractical in routine care and costly.<sup>4</sup> As limited-sampling protocols were developed the focus of sample collection was in the first 4 hours after the dose, during which most of the variability in AUC occurs.<sup>5</sup>

An alternative approach using an abbreviated AUC profile for the first 4 hours post-dose is similarly impractical; however, the strategy of estimating an abbreviated  $AUC_{0-4}$  using a single CsA whole blood concentration collected 2 hours post-dose ( $C_2$ ) is attracting much interest.  $C_2$  monitoring

is a new concept with only a few clinical trials showing clinical benefit.<sup>6</sup> In addition, one study in lung transplant recipients found little evidence to support the concept that measuring  $C_2$  provides a better indication of the response to CsA than traditional  $C_0$  monitoring.<sup>7</sup> The available evidence that  $C_2$  is a better predictor than the conventional  $C_0$  monitoring is complicated by the use of non-specific assays to quantitate cyclosporine.<sup>8,9</sup> The cross-reactivity of metabolites is different for the various immunoassay techniques used to quantitate CsA in blood. There is substantial and variable overestimation of CsA concentrations in some patients, depending on the method used,<sup>8</sup> and the metabolite concentration in blood can vary over the dosing interval, a problem exacerbated by other drugs such as diltiazem.<sup>10</sup> Furthermore, the utility of  $C_2$  monitoring in transplant recipients receiving metabolic inhibitors that alter the pharmacokinetics and metabolism of CsA has not been evaluated.

Diltiazem, in particular, is commonly co-administered with CsA because of the beneficial effects of lowering blood pressure and reduction in coronary artery disease.<sup>11</sup>

The arrival of  $C_2$  monitoring offers new hope that the optimization of CsA therapy could be improved; however, further studies are required before a shift from trough monitoring occurs. The aim of this study was to evaluate the utility of  $C_2$  monitoring and its ability to predict  $AUC_{0-5}$  in stable heart transplant recipients receiving metabolic inhibitors of CsA.

## MATERIALS AND METHODS

CsA blood concentration–time data used in this analysis were derived from a study by Akhlaghi et al.<sup>12</sup> In the study, samples were collected at steady state from 47 stable heart transplant recipients at 0, 0.5, 1, 2, 3, 5, 7 and 12 hours after the morning dose of CsA (Neoral, Novartis Pharmaceuticals). CsA concentrations in whole blood were quantitated using an enzyme multiplied immunoassay technique (EMIT 2000 assay, Syva/Behring, Inc, San Jose, CA). Heart transplant recipients were stratified into

**TABLE I** Pharmacokinetic parameters by group for stable heart transplant recipients

	Group			
	A (CsA)	B (CsA + Diltiazem)	C (CsA + ketoconazole)	D (CsA + diltiazem + ketoconazole)
C <sub>0</sub> (µg/liter)	179 ± 64	171 ± 61	201 ± 48	179 ± 68
C <sub>2</sub> (µg/liter)	1,088 ± 165	1,022 ± 237	503 ± 110	627 ± 209
C <sub>max</sub> (µg/liter)	1,147 ± 307	1,222 ± 501	503 ± 110	659 ± 224
C <sub>max</sub> (%CV)	27	41	22	34
t <sub>max</sub> (hours)	1.5 ± 0.5	1.4 ± 0.7	2.2 ± 0.5	2.4 ± 0.5
t <sub>max</sub> (%CV)	33	50	23	21
Dose (mg/day)	302 ± 68	243 ± 55	66 ± 35	87 ± 60
AUC <sub>0-5</sub> (µg/h · liter)	3,356 ± 641	3,304 ± 655	1,980 ± 414*	2,406 ± 675*
AUC <sub>0-12</sub> (µg/h · liter)	4,912 ± 935	4,748 ± 924	3,703 ± 824*	4,386 ± 1,082

Data expressed as mean ± SD.

\*Area under the concentration–time curve (AUC) data significantly different when compared with Group A (Student's *t*-test).

4 treatment groups, depending on the long-term concomitant administration of drugs known to inhibit CsA metabolism, as part of their routine therapy.<sup>9</sup> Group A (*n* = 11 patients) received CsA alone (no long-term metabolic inhibitors), whereas the other patients received CsA administered with a slow-release formulation of diltiazem (Group B, *n* = 11; 120 mg/day), with ketoconazole (Group C, *n* = 14; 200 mg/day), or with a combination of diltiazem and ketoconazole (Group D, *n* = 12; diltiazem 120 mg/day and ketoconazole 200 mg/day) for at least 24 months.

#### Data Analysis

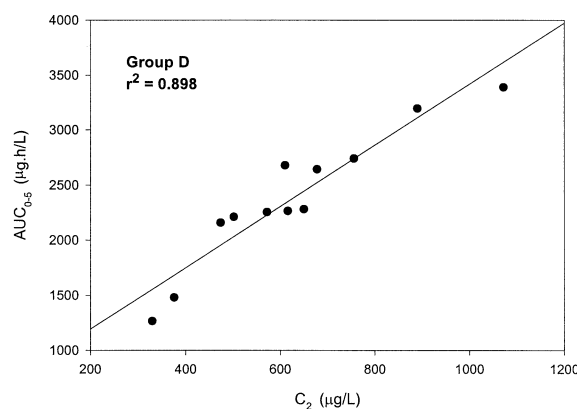
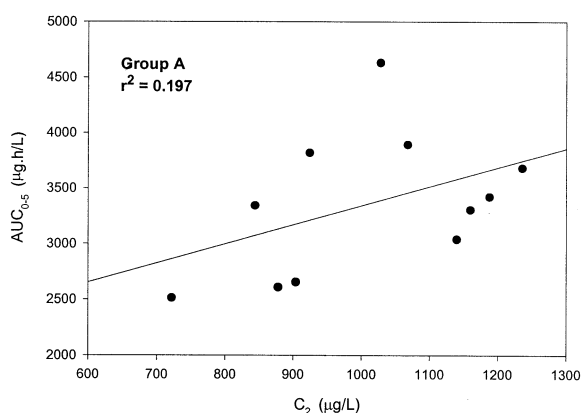
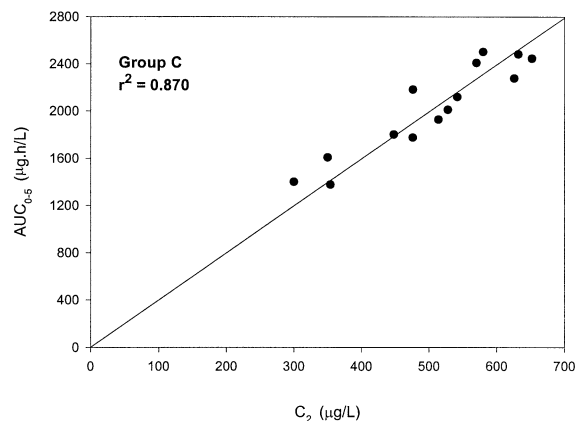
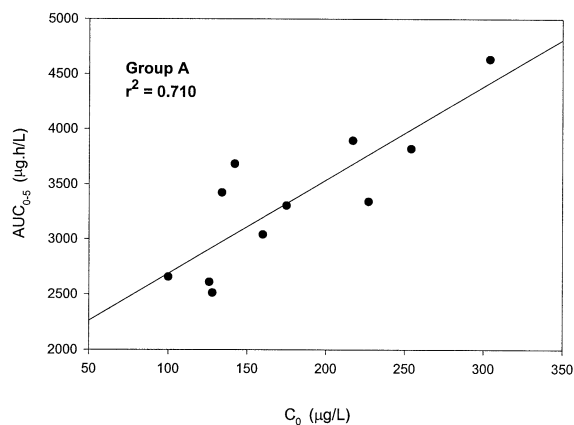
The pharmacokinetic parameters for this study have been reported previously.<sup>12</sup> The AUC was calculated for the first 5 hours after the dose and for the 12-hour dosage interval, at steady state, using the trapezoidal rule (TOPFIT, Version 2.0, Department of Pharmacokinetics and Drug Metabolism, Birkenfelder, Germany). AUC was calculated for the first 5 hours instead of the first 4 hours, as other investigators have reported, due to the sample collection protocol in the original pharmacokinetic study.<sup>12</sup> CsA C<sub>2</sub> and C<sub>0</sub> were obtained from the observed data.

#### Statistics

Descriptive statistics (including mean and standard deviation), comparative statistics (Student's *t*-test) and linear regression analyses were performed using SIGMASTAT for Windows (Version 2.03, SPSS, Inc, Chicago, IL). All *p*-values were based on 2-tailed tests and *p* < 0.05 was considered statistically significant.

#### RESULTS

Demographic characteristics of patients and pharmacokinetic parameters for transplant recipients in the various treatment groups have been published previously.<sup>12</sup> Table I provides a summary of the pharmacokinetic parameters evaluated in this study. There was no statistical difference in trough CsA concentration between the 4 study groups as the CsA dose was adjusted clinically using the CsA trough concentration. The addition of ketoconazole to the CsA regimen appeared to reduce the variability of the rate of CsA absorption. The time to maximum concentration (*t*<sub>max</sub>) was delayed in the patients receiving CsA and ketoconazole (Groups C and D) and the variability decreased: the coefficient of variation for *t*<sub>max</sub> was 33% for Group A; 50% for Group B; 23% for Group C; and 21% for Group D (Table I). In addition, although there was no statistical difference between mean C<sub>2</sub> CsA concentration and C<sub>max</sub>, regression analysis comparing individual C<sub>2</sub> with C<sub>max</sub> showed variability between the groups. C<sub>2</sub> correlated poorly with C<sub>max</sub> in Groups A and B (CsA only, *r*<sup>2</sup> = 0.211; CsA plus diltiazem, *r*<sup>2</sup> = 0.442); however, there was a strong correlation between C<sub>2</sub> and C<sub>max</sub> in the groups that received ketoconazole (Group C: CsA plus ketoconazole, *r*<sup>2</sup> = 0.955; Group D: CsA plus ketoconazole and diltiazem, *r*<sup>2</sup> = 0.952). C<sub>max</sub> variability was greatest in patients receiving CsA and diltiazem (Group B CV was 41%, Group D CV was 34%; Table I). The lower dose of CsA in Groups C and D reflects the inhibitory effect of ketoconazole on CsA metabolism.<sup>13</sup> The AUC<sub>0-5</sub> of Groups C and D was significantly lower than that of Group A (CsA alone).



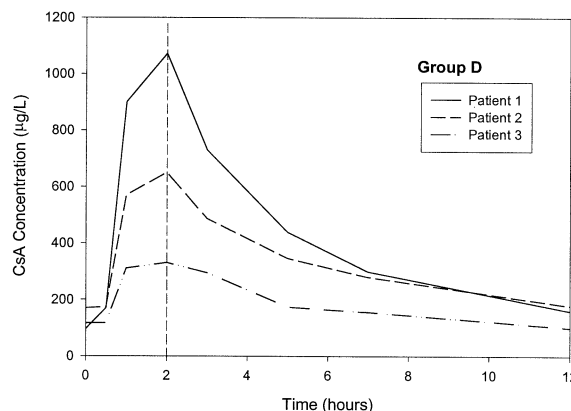
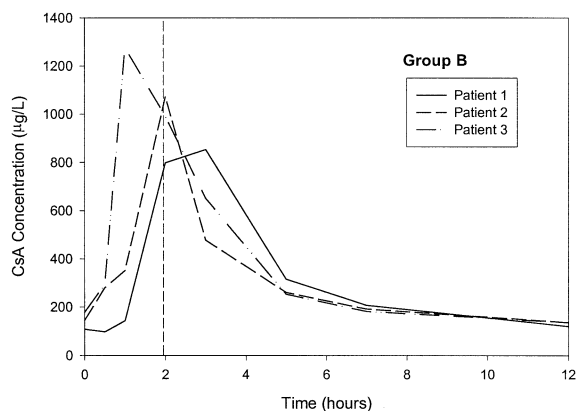
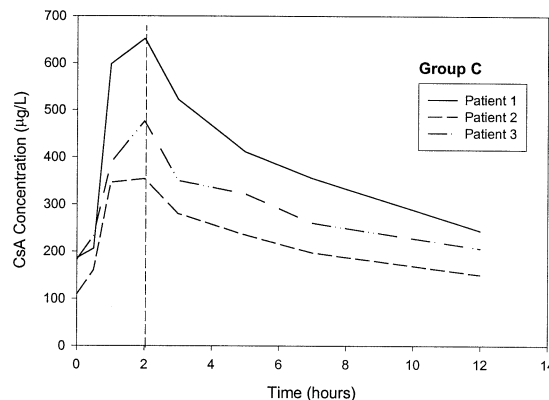
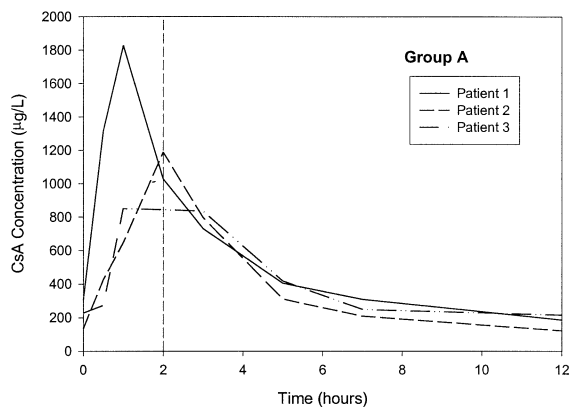
**FIGURE 1** Correlations between CsA whole blood concentrations and AUC<sub>0-5</sub> for 11 stable heart transplant recipients receiving CsA alone (Group A).

Surprisingly, total exposure to CsA during the 12-hour dosing interval (AUC<sub>0-12</sub>; Table I) was significantly lower for Group C (CsA plus ketoconazole) when compared with Group A (CsA alone). The C<sub>0</sub> values for Groups C and D were not significantly lower compared with Groups A and B. This indicates that the observed decrease in CsA exposure in Groups C and D was not reflected by the C<sub>0</sub> CsA concentrations, the commonly used index of CsA dosing (Table I). However, the C<sub>2</sub> data point did appear to be a more predictive surrogate marker of CsA exposure seen in the statistically lower C<sub>2</sub> CsA concentrations (Table I) in Groups C and D, which reflected correspondingly lower values for AUC<sub>0-5</sub>.

The relationship between C<sub>0</sub>, C<sub>2</sub> and AUC<sub>0-5</sub> is presented in Figures 1 and 2, whereas CsA concentration-time profiles are presented in Figures 3 and 4. In patients receiving CsA alone (Group A), C<sub>2</sub> showed a poor correlation with AUC<sub>0-5</sub> ( $r^2 = 0.197$ ;

**FIGURE 2** Correlations between CsA whole blood concentrations and AUC<sub>0-5</sub> in stable heart transplant recipients receiving CsA plus ketoconazole (Group C) and CsA plus both diltiazem and ketoconazole (Group D).

$p = 0.17$ ), whereas C<sub>0</sub> showed a stronger correlation with AUC<sub>0-5</sub> ( $r^2 = 0.710$ ;  $p = 0.001$ ). In patients receiving CsA and diltiazem (Group B), both C<sub>0</sub> and C<sub>2</sub> had the same but weaker correlation with AUC<sub>0-5</sub> ( $r^2 = 0.650$ ;  $p = 0.005$ ). The C<sub>2</sub> reflected AUC<sub>0-5</sub> most accurately in Groups C ( $r^2 = 0.870$ ;  $p < 0.0001$ ) and D ( $r^2 = 0.898$ ;  $p < 0.0001$ ), for which ketoconazole was co-administered with CsA (Figure 2). C<sub>0</sub> was a poor marker of AUC<sub>0-5</sub> in Groups C ( $r^2 = 0.176$ ) and D ( $r^2 = 0.022$ ). These data indicate that, when variability in the rate of CsA absorption decreases, the C<sub>2</sub> timepoint becomes the more effective marker of AUC<sub>0-5</sub> (Figures 3 and 4). When comparing 3 representative patients from Group A (Figure 3), the C<sub>2</sub> time points for Patients 1 and 2 were similar (1,028 and 1,188 µg/liter),



**FIGURE 3** Typical CsA concentration–time profiles for stable heart transplant recipients receiving CsA (Group A) and CsA plus diltiazem (Group B).

**FIGURE 4** Typical CsA concentration–time profiles for 3 stable heart transplant recipients receiving CsA plus ketoconazole (Group C) and CsA plus both diltiazem and ketoconazole (Group D).

whereas  $AUC_{0-5}$  varied by approximately 30% (4,632 and 3,422  $\mu\text{g}/\text{h}\cdot\text{liter}$ ). Similarly, when Patients 2 and 3 were compared, the  $C_2$  marker differed by approximately 30% (1,188 and 844  $\text{ng}/\text{ml}$ ), but the  $AUC_{0-5}$  values were the same (3,422 and 3,343  $\mu\text{g}/\text{h}\cdot\text{liter}$ ). The same pattern was seen in Group B (CsA plus diltiazem), and is shown in Figure 3. Patients 1 and 2 had the same  $AUC_{0-5}$  but different  $C_2$  concentrations (1,072 and 798  $\text{ng}/\text{ml}$ ). Patients 2 and 3 had the same  $C_2$  CsA concentration, but  $AUC_{0-5}$  varied by 30% (3,365 and 2,485  $\mu\text{g}/\text{h}\cdot\text{liter}$ ). This unpredictable pattern was not observed when ketoconazole was added to the CsA dosing regimen, where  $C_2$  became an efficient marker of  $AUC_{0-5}$  (Figure 4). These data suggest that the  $C_0$  timepoint is an ineffective marker of CsA exposure and that the  $C_2$  timepoint is affected by the variable absorption of CsA and becomes a better predictor of

$AUC_{0-5}$  when a metabolic inhibitor of CsA reduces this variability.

Other surrogate markers ( $C_1$  and  $C_3$ ) were assessed; however,  $C_1$  correlated poorly with  $AUC_{0-5}$  in all groups ( $r^2 = 0.716$ ), whereas  $C_3$  followed a pattern similar to that of  $C_2$ , with poor correlation in Groups A and B ( $r^2 = 0.317$  and  $0.015$ ) and stronger correlation in Groups C and D ( $r^2 = 0.922$  and  $0.855$ ).

## DISCUSSION

Adjusting CsA dose to maintain efficacy and minimize toxicity is difficult. Early clinical experience has demonstrated that sub-therapeutic blood concentrations of CsA were associated with an increased incidence of acute rejection.<sup>14</sup> Further studies in kidney and liver transplant recipients reported a strong association between CsA exposure and graft survival.<sup>1,15,16</sup> In addition, Kahan<sup>17</sup> reported that

high variability of drug exposure was a risk factor for chronic rejection. The pharmacokinetic measure in these studies most closely associated with post-transplant clinical events was AUC.

Conventional TDM strategies (i.e., trough concentration monitoring) have proven inadequate as a measure of CsA exposure, and a number of alternate strategies have been suggested (limited sampling strategies, bayesian), but have not been widely accepted.

Conventional TDM strategies (trough monitoring) have failed to realize clinical benefits because CsA exhibits substantial variability in the rate and extent of absorption as well as clearance.<sup>1</sup> The search for an ideal pharmacokinetic measure for AUC, a measure of CsA exposure, has persisted because TDM of CsA is essential to optimize CsA dose to maintain efficacy and reduce drug-related toxicity.<sup>4</sup> TDM using the CsA concentration in a whole blood sample collected 2 hours after the dose ( $C_2$ ) is currently being promoted as a practical and effective marker to adjust CsA dose.<sup>5</sup>

The pharmacokinetic parameters of CsA depend on diet,<sup>18,19</sup> disease state,<sup>20</sup> concomitant medication,<sup>21</sup> race,<sup>22</sup> time post-transplant<sup>2</sup> and CsA dose.<sup>23</sup> Factors that alter the rate of absorption, such as fat intake,<sup>18</sup> disease state (cystic fibrosis) and other drugs (particularly CYP3A4 inhibitors, the dominant metabolic enzyme system for CsA), could affect the ability of  $C_2$  to accurately predict CsA exposure, leading to inappropriate dose adjustments.

The data from this study confirm that  $C_0$  is a poor marker of CsA exposure. However, we were unable to reproduce the strong correlation between  $C_2$  and  $AUC_{0-5}$  previously reported in stable heart transplant recipients receiving CsA, even though the same immunoassay technique was used to quantitate CsA whole blood concentrations in both studies.<sup>24</sup> Although the sample size in this study was small, the different outcomes between each group are distinct. It is difficult, therefore, to explain the differences between our results and the strong correlation found by Cantarovich et al, because important information about diet, race, etc, that affect CsA absorption were not reported. Composition of diet and timing of dose in relation to food has an unpredictable effect on the absorption of CsA and may contribute to variability, especially after discharge from hospital.<sup>25</sup> In addition, the study by Cantarovich et al,<sup>24</sup> in stable heart transplant recipients, examined 60 data points from 30 patients evaluated on 2 separate occasions and the dose was

adjusted on the second admission using  $C_2$  as the TDM marker. It has been suggested that this practice introduces an inherent bias<sup>7</sup> and may produce a falsely high correlation between  $C_2$  and  $AUC_{0-4}$ .

Furthermore, these data suggest that the failure of  $C_2$  as a predictor of CsA AUC is dependent on the variability of the rate of absorption of CsA.  $C_2$  appears to be an effective predictor of AUC when the metabolic inhibitor, ketoconazole, is added to the therapeutic regimen. Ketoconazole appears to decrease the variability of the rate of absorption without having a significant effect on the variability of the extent of absorption. The data show reduced variability in  $t_{max}$  with ketoconazole and stronger correlation of  $C_{max}$  with  $C_2$  when ketoconazole is co-administered. This latter point is important because  $C_{max}$  has been shown to be associated with the period of maximum calcineurin inhibition.<sup>25</sup> Although the Neoral formulation of CsA showed a significantly better pharmacokinetic profile compared with the earlier formulation, Sandimmune, the microemulsion formulation still demonstrated substantial pharmacokinetic variability.<sup>1</sup> The absorption "rate" constant ( $k_a$ ) for Neoral reported in previous studies showed significant variability, ranging from 0.3 to 5.5 per hour.<sup>25,27</sup> Furthermore, in another study, 15% of patients receiving Neoral exhibited a significant delay in absorption of CsA.<sup>28</sup> This inherent pharmacokinetic variability is exacerbated by fat content in the diet and other drugs such as diltiazem.<sup>18,21</sup> Klausner et al examined individual concentration-time profiles in renal transplant recipients and demonstrated marked shifts in  $t_{max}$  in response to the fat content in the diet of some patients.<sup>18</sup> Surprisingly, these investigators reported low variability within the low-fat group (16% CV) and the high-fat group (8.4% CV), suggesting that a controlled diet might reduce the variability in absorption of CsA.

The interaction of CsA with diltiazem is complex and may involve cytochrome P450 3A4 in the gut and liver as well as *p*-glycoprotein in the gut wall.<sup>21,29</sup> There may also be a gender difference in CsA metabolism when diltiazem is co-administered with CsA.<sup>21</sup> The failure of  $C_2$  to correlate with AUC in the patients receiving diltiazem may be explained by the variable rate of absorption seen in this study and studies performed by others.<sup>21</sup> However, another confounding factor in this group of patients may relate to the lack of specificity of the assay used to quantitate CsA in whole blood. The variability and extent of interference from CsA metabolites in

the available immunoassays have been reported recently.<sup>8,9</sup> In addition, metabolite concentrations have been shown to increase, over the dosing interval, in patients receiving diltiazem.<sup>10</sup> It is of interest to note, in the same study, that adding ketoconazole to the regimen suppressed CsA metabolite formation across the dosing interval. The lack of specificity in the present assays, a problem exacerbated by diltiazem, also raises questions about the applicability of target ranges developed using a particular assay technique. Target ranges developed using an immunoassay with significant cross-reactivity to CsA metabolites would not be applicable in a center using a more specific technique and could lead to inappropriate dose adjustment in some patients at that center. Although the lack of immunoassay specificity may explain the results seen in patients receiving diltiazem and/or ketoconazole it does not explain the results seen in patients who received CsA alone. Reports of concentration–time data of CsA and metabolites indicate that the proportion of metabolites to CsA is greater at  $C_0$  compared with  $C_2$ .<sup>10,30</sup> However, the poorer correlation of  $C_2$  with  $AUC_{0-5}$  in the patients who received CsA alone suggests that other factors are involved.

TDM of CsA to allow optimal dosing of the drug is difficult but vital. The  $C_2$  predictor of CsA exposure offers new hope, but its utility appears vulnerable in some clinical settings and requires further testing before becoming the gold standard of CsA TDM in all situations. There is an urgent need to examine the effect of metabolite interference on the performance of  $C_2$  using more specific chromatographic assays, particularly in patients receiving CsA and diltiazem. Furthermore, although  $C_2$  correlated well with AUC when ketoconazole was co-administered with CsA, we advise caution with dose adjustment using  $C_2$  in patients receiving CsA/diltiazem combination therapy or when other interacting drugs are utilized (e.g., short-term fluconazole or itraconazole) and where  $C_2$  has not been evaluated. Finally, we suggest that  $C_2$  performance in controlled trials to this point may not reflect  $C_2$  performance in uncontrolled, outpatient situations. Our data suggest that stricter control of diet in transplant outpatients, although difficult, may be a necessary requirement to allow critical dose adjustment using  $C_2$ . We suggest that future studies evaluating  $C_2$  report details of diet, race, gender and other factors affecting the absorption of CsA to allow proper assessment of the performance of  $C_2$ .

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