

## Association Between Cyclosporine Concentrations at 2 Hours Post-dose and Clinical Outcomes in De Novo Lung Transplant Recipients

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**Background:** The objective of this study was to investigate the relationship between cyclosporine (CsA) pharmacokinetic parameters and clinical outcomes after lung transplantation.

**Methods:** Data from 48 lung or heart/lung transplant recipients originally recruited to a randomized, prospective clinical trial of Sandimmune vs Neoral and followed for 12 months were included in this study. CsA dosing was based on the trough concentration. CsA concentrations at 0 (C0), 2 (C2), and 6 (C6) hours post-dosing were obtained at 1, 2, 3, 4, 13, 26, 39, and 52 post-operative weeks. Based on their average C2 levels in the first post-transplant month, patients were stratified retrospectively into Low C2 (<1,000 µg/liter, *n* = 18), Intermediate C2 (1,000–1,500 µg/liter, *n* = 16) and High C2 (>1,500 µg/liter, *n* = 14) Groups.

**Results:** Cyclosporine C2 was the best single-point determinant ( $r^2 = 0.934$ ) for area-under-the-concentration-time curve (AUC<sub>0-6 hours</sub>) compared with C0 ( $r^2 = 0.267$ ) or C6 ( $r^2 = 0.304$ ). The mean ± SD values of CsA C2 and AUC<sub>0 to 6 hours</sub> in the first year post-transplant were significantly lower in patients with >2 rejection episodes compared with those with ≤2 rejection episodes (C2: 875 ± 546 µg/liter vs 1,114 ± 633 µg/liter, *p* = 0.01; AUC<sub>0-6 hours</sub>: 4,036 ± 1,904 µg × hour/liter vs 4,870 ± 2,182 µg × hour/liter; *p* = 0.01) whereas C0 and C6 did not differ. Patients in the Intermediate C2 Group were free from rejection episodes for a significantly longer duration (*p* < 0.001) and had significantly higher predicted forced expiratory volume in 1 second (%) values (*p* < 0.001) compared with the Low and High C2 Groups. The percentage of increase in serum creatinine concentration by the end of first month post-transplant was significantly higher in the Intermediate C2 Group (*p* < 0.003).

**Conclusions:** CsA C2 concentrations correlated better with the incidence of multiple rejections after lung transplantation than did C0 or C6. C2 concentrations between 1,000 and 1,500 µg/liter within the first post-operative month may be associated with better graft outcomes and improved pulmonary function and worsened renal function. *J Heart Lung Transplant* 2005;24:2120–8. Copyright © 2005 by the International Society for Heart and Lung Transplantation.

Lung transplantation is a common life-saving intervention for the treatment of end-stage lung disease. The success of lung transplantation, however, is still lower than that for other solid organ transplants, and the survival half-life for single- or double-lung transplant recipients is only 4.4 years.<sup>1</sup>

Bronchiolitis obliterans syndrome (BOS), an end-stage and potentially fatal lung disease, which by the fifth year after transplantation develops in approxi-

mately 50% of all adult lung transplant recipients,<sup>1</sup> is one of the major factors contributing to poor long-term survival after lung transplantation.<sup>2</sup> Acute rejection episodes in the early post-operative period are the most significant risk factor for the development of BOS after lung or heart-lung transplantation.<sup>3</sup> Optimizing immunotherapy is therefore imperative for preventing acute rejection and improving long-term results in lung transplantation.

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Approximately 40% of all lung transplant recipients receive cyclosporine (CsA) as part of the maintenance immunosuppressive regimen at 1 and 5 years after transplantation.<sup>1</sup> Therapeutic monitoring is a necessity with CsA therapy because of its variable pharmacokinetics, narrow therapeutic index, and significant side effects, including nephrotoxicity.

Although CsA dosing is generally based on trough concentrations (C<sub>0</sub>), the correlation between C<sub>0</sub> and post-transplant outcomes is poor.<sup>4</sup> The area-under-the-concentration-time curve (AUC) is a better measure of total exposure to CsA and its immunosuppressive activity.<sup>5</sup> Routine monitoring of CsA AUC is impractical, but pharmacokinetic studies performed with the microemulsified formulation of CsA (Novartis Pharmaceuticals, Frimely, UK) showed that the AUC<sub>0 to 4 hours</sub> was a more sensitive marker of CsA absorption than was C<sub>0</sub> and furthermore demonstrated that the 2-hour post-dose sampling point (C<sub>2</sub>) was the most accurate single-point measure of CsA AUC<sub>0 to 4 hours</sub>.<sup>6</sup>

Importantly, CsA C<sub>2</sub> concentrations are predictive of clinical outcomes in kidney,<sup>7</sup> liver,<sup>8</sup> and heart<sup>9,10</sup> transplant recipients. A recent randomized, multicenter, open-label clinical trial in de novo liver transplant recipients also showed that the efficacy of immunosuppression was similar in patients treated with Neoral (Novartis Pharmaceuticals) using C<sub>2</sub> monitoring or with tacrolimus, with the exception of a significantly higher incidence of diabetes mellitus in the tacrolimus group.<sup>11</sup>

Although the reliability of CsA C<sub>2</sub> monitoring has been established for kidney, liver, and heart transplant recipients, its use in lung transplantation remains to be tested and validated. In a previous randomized, prospective clinical trial that compared outcomes after use of Neoral or Sandimmune (Novartis Pharmaceuticals) in de novo lung transplant recipients, we detected no association between any single-point CsA measurement and clinical outcomes.<sup>12</sup> However, the analysis in that study was focused on comparing the pharmacodynamics of the 2 CsA preparations and was not optimized to examine the relationship of pharmacokinetic parameters with clinical outcomes.<sup>12</sup> In this study, we have reanalyzed the pharmacokinetics data from the clinical trial to investigate the relationship between CsA C<sub>0</sub>, C<sub>2</sub>, C<sub>6</sub>, and AUC<sub>0 to 6 hours</sub> in the first post-operative month and clinical outcomes in the first year after transplantation.

## MATERIALS AND METHODS

### Patients and Study Design

The original clinical study was a 1-year randomized, prospective, open-label trial that compared the efficacy and safety of the microemulsified formulation of CsA (Neoral) ( $n = 27$ ) vs the conventional formulation

(Sandimmune) ( $n = 21$ ) in 48 consecutive lung transplant recipients.<sup>12</sup> Dosing for CsA was based on C<sub>trough</sub> (C<sub>0</sub>) monitoring. The original study was approved by local ethics committees and was conducted in accordance with guidelines established by the Declaration of Helsinki. All patients gave written informed consent. The Institutional Review Board at the University of Rhode Island approved the data analysis.

All patients received intravenous (IV) methylprednisolone (500 mg) and rabbit anti-thymocytes globulin induction immunosuppressive therapy followed by maintenance triple therapy with oral CsA, azathioprine, and prednisolone. An initial 50-mg oral dose of Sandimmune or Neoral was administered on Day 1 post-transplantation, with a 50-mg increase at each dosing period until target trough concentrations of CsA were reached (300 to 400  $\mu\text{g/liter}$  at Months 1 and 2; 200 to 300  $\mu\text{g/liter}$  between Months 3 and 12). Cyclosporine dose adjustment throughout the entire follow-up period was based on trough concentrations, hence physicians and histopathologists were blinded to randomization of the patients to the Sandimmune or Neoral arms of the study and were not aware of the cyclosporine C<sub>2</sub> or C<sub>6</sub> levels. Patients with cystic fibrosis received their daily dose of CsA at 8-hour intervals concomitantly with pancreatic enzyme supplements. Laboratory and clinical evaluations were performed at baseline (pre-transplant) and at the end of weeks 1, 2, 3, 4, 13, 26, 39, and 52.

To investigate the effect of CsA C<sub>2</sub> in the first month post-transplantation on clinical outcomes, the patients were stratified into 3 groups by their average CsA C<sub>2</sub> concentration during Month 1 as follows: Low (<1,000  $\mu\text{g/liter}$ ;  $n = 18$ ), Intermediate (1,000 to 1,500  $\mu\text{g/liter}$ ;  $n = 16$ ), and High (>1,500  $\mu\text{g/liter}$ ;  $n = 14$ ).

### Pharmacokinetic Analysis

Abbreviated blood CsA concentration-time profiles were obtained by measuring CsA concentrations predose and at 2 and 6 hours post-dose at weeks 1, 2, 3, 4, 13, 26, 39, and 52 after transplantation. The CsA AUC<sub>0 to 6 hours</sub> was calculated with the linear trapezoidal rule.<sup>13</sup> CsA concentrations in whole blood were measured by an accredited clinical laboratory with a homogeneous Enzyme Multiplied Immunoassay Technique (EMIT 2000, Dade-Behring Diagnostic UK, Ltd, Milton Keynes, UK).

### Clinical Outcomes

**Acute rejection.** Episodes of clinically significant acute rejection were diagnosed from the presence of characteristic histopathologic changes in transbronchial biopsies, as defined by International Society of Heart and Lung Transplantation criteria,<sup>14</sup> combined with the requirement for augmented immunosuppression. According to the transplant protocol, a transbronchial biopsy was carried out at the end of first post-operative

month and at other times when indicated clinically and read by a histopathologist who was blinded to trial randomization or CsA concentration levels. In the absence of histopathologic confirmation, acute rejection was diagnosed from characteristic clinical, radiologic, and functional criteria as well as an appropriate response to augmented immunosuppression. Rejection episodes were treated with intravenous doses of methylprednisolone (500 to 1,000 mg), usually administered over 3 consecutive days.

**Lung function.** The forced expiratory volume in 1 second (FEV<sub>1</sub>) was measured at each post-transplantation visit in the lung function laboratory. In addition, the predicted FEV<sub>1</sub> was calculated to normalize FEV<sub>1</sub> values for height and gender according to the Quanjer equation.<sup>15</sup> The results of lung function tests performed by patients at home using a portable spirometer were also recorded.

**Infection.** Episodes of infection were diagnosed from culture, serology, or diagnostic histopathology combined with appropriate antibiotic therapy. Alternatively, in the absence of microbiologic confirmation, infection was diagnosed from the presence of fever (maximum temperature  $\geq 38^\circ\text{C}$ ) combined with characteristic clinical manifestations such as changes in radiologic results and appropriate antibiotic treatment.

**Blood pressure.** Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded, and mean arterial pressure (MAP) was calculated according to the following equation:  $\text{MAP} = [(2 \times \text{DBP}) + \text{SBP}]/3$ .

**Renal function.** In addition to the routine measurement of serum creatinine, creatinine clearance was measured by 24-hour urine collection at 1, 2, 3, 4, 13, 26, 39, and 52 weeks post-transplant and was also calculated using routinely measured serum creatinine with the Cockcroft-Gault formula. Plasma cystatin C concentrations were measured as an indicator of glomerular filtration rate.

### Statistical Analysis

The results from the Sandimmune and Neoral arms of the original study were pooled. Data were analyzed with SPSS (version 10.1.0) statistical software (SPSS Inc, Chicago, IL) and the results were double checked with SAS (version 9.0) statistical software (SAS Inc, Cary, NC) for Windows (Microsoft, Redmond, WA). A *p* value of  $<0.05$  was considered statistically significant. The results of all normally distributed data are reported as mean  $\pm$  SD.

To test for normal distribution, all continuous variables were subjected to Shapiro-Wilk's test and also evaluated for the degree of skewness and kurtosis. If all 3 criteria showed the variable to be non-normally

**Table 1.** Baseline Demographic and Clinical Characteristics

Total (n)	48
Gender (n [%])	
Male	26 (54)
Female	22 (46)
Age (yrs) (mean $\pm$ SD [range])	42 $\pm$ 13 (19–66)
Weight (kg) (mean $\pm$ SD)	61 $\pm$ 13
CsA formulation (%)	
Sandimmune	21 (44)
Neoral	27 (56)
Type of transplant (%)	
Heart-lung transplant	21 (44)
Single lung Transplant	18 (38)
Double lung transplant	9 (19)
Primary diagnosis (%)	
Emphysema	13 (27)
Cystic fibrosis	8 (17)
Eisenmenger's syndrome	6 (13)
Bronchiectasis	6 (13)
Other	15 (31)
Baseline serum creatinine ( $\mu\text{mol/liter}$ ) (mean $\pm$ SD)	89.9 $\pm$ 19.9

distributed, data were transformed to natural logarithm values, subjected to appropriate statistical tests, and reported as the back-transformed values of the mean (geometric mean) with 95% confidence intervals (CI). If transformation did not result in a normal distribution of the data, appropriate nonparametric tests were used.

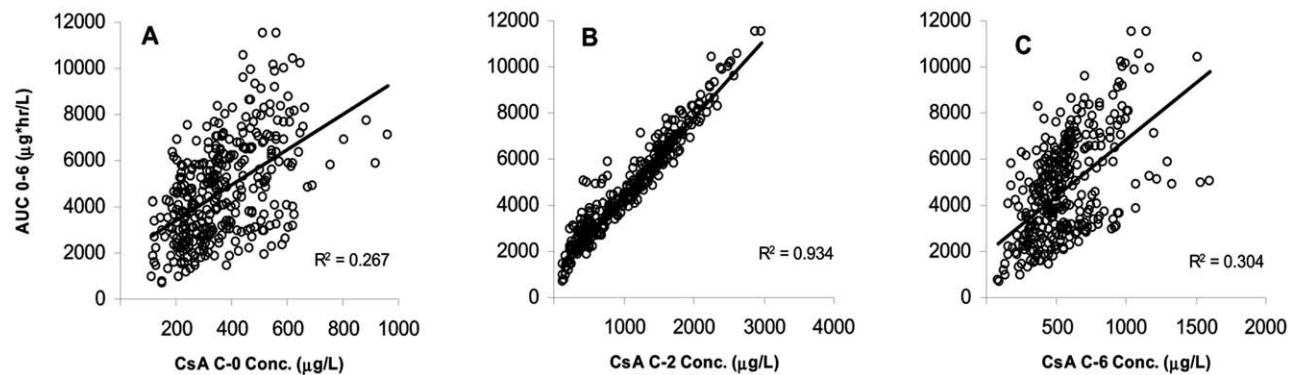
Pearson's correlation coefficient was calculated to determine the association between 2 continuous variables, and the chi-square test was used to assess differences in proportions. The independent samples *t*-test was used to compare the means of 2 groups, and the Mann-Whitney *U*-test was used if the variable was not normally distributed.

The 1-way analysis of variance (ANOVA) procedure was used to perform ANOVA for parametric data, and the Kruskal-Wallis *H*-test was used for nonparametric data. Scheffé's post-hoc multiple comparison test was performed after parametric 1-way ANOVA to identify pair-wise differences between groups. The Kaplan-Meier procedure followed by the log-rank (Mantel-Cox) test was performed to characterize freedom from rejection or infection episodes between different groups of patients.

## RESULTS

### Baseline Demographic and Clinical Characteristics

The demographic characteristics of the patient population are summarized in Table 1. Data were available for 48 patients. The median duration of follow-up was 380 days, with the exception of 2 patients who died from infection at Days 23 and 51 post-transplant. Emphysema and cystic fibrosis were the most common diagnosis requiring lung transplantation.



**Figure 1.** Association of cyclosporine (CsA) concentrations predose (A) and at 2 (B) and 6 (C) hours post-dose with area-under-the-time curve of 0 to 6 hours ( $AUC_{0\text{ to }6\text{ hours}}$ .)

The mean oral doses of prednisolone and azathioprine were  $24.8 \pm 19.1$  mg/day and  $93 \pm 38$  mg/day, respectively, at the first post-transplant month and were reduced to  $8.8 \pm 4.1$  mg/day and  $61 \pm 29$  mg/day, respectively, by 12 months post-transplant. The Low, Intermediate and High CsA C2 Groups included 3, 4, and 1 patient with cystic fibrosis, respectively.

#### Correlation of Single-Point CsA Concentrations with $AUC_{0\text{ to }6\text{ hours}}$

A total of 343 abbreviated CsA profiles were available. Of these, 36 were obtained from 11 patients in whom the CsA dose was reduced because of the concomitant administration of itraconazole (400–600 mg/day) at some time during the 12-month period. These profiles were included in the overall analysis, because after dose adjustment, CsA C0 and C2 were similar with or without itraconazole administration. The association between CsA C0, C2, or C6 with CsA  $AUC_{0\text{ to }6\text{ hours}}$  is shown in Figure 1. CsA C2 provided the best single-point determinant for  $AUC_{0\text{ to }6\text{ hours}}$  ( $r^2 = 0.934$ ). This relationship between C2 and  $AUC_{0\text{ to }6\text{ hours}}$  was also observed when the data for Sandimmune and Neoral were examined separately ( $r^2 = 0.88$  for Sandimmune and  $r^2 = 0.95$  for Neoral).

#### Association between CsA Concentration and Allograft Rejection

Over the first 12 months, 83 episodes of treated acute rejections were recorded, with a median of 2 episodes (range, 0–7) per patient. Multiple rejection episodes, defined as  $>2$  episodes of treated acute rejection, occurred in 7 patients, with a similar frequency in both arms (Neoral, 3; Sandimmune, 4;  $p = 0.68$ ). The mean first-year CsA C2 and  $AUC_{0\text{ to }6\text{ hours}}$  were significantly lower in patients with  $>2$  rejection episodes compared with those with  $\leq 2$  rejection episodes (C2:  $875 \pm 546$  µg/liter vs  $1,114 \pm 633$  µg/liter,  $p = 0.01$ ;  $AUC_{0-6\text{ hours}}$ :  $4,036 \pm 1904$  µg × hour/liter vs  $4,870 \pm 2,182$  µg ×

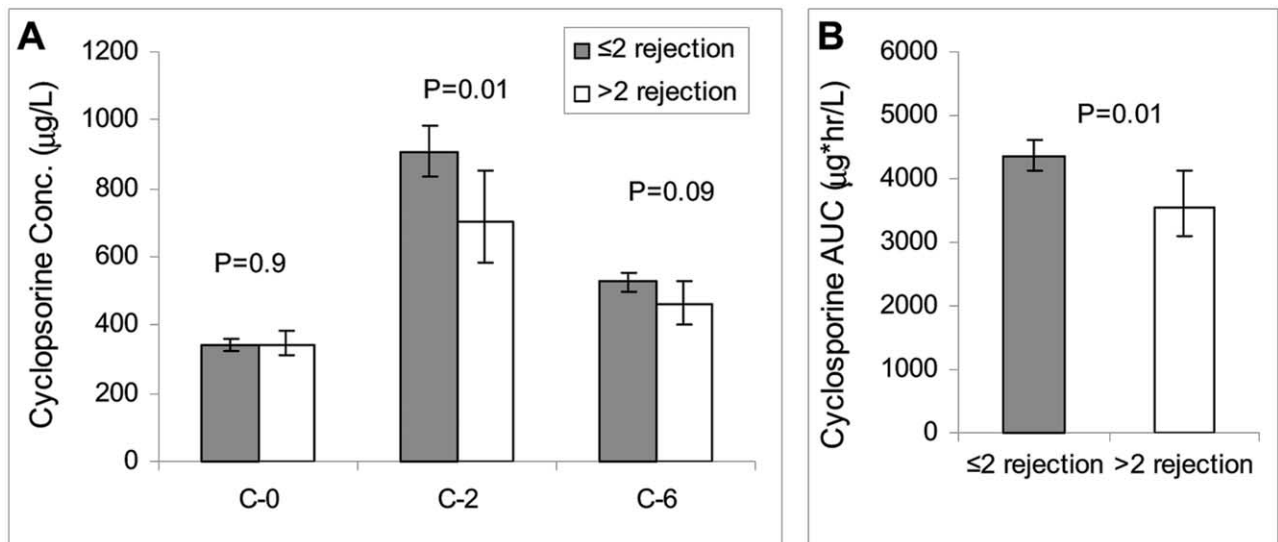
hour/liter;  $p = 0.01$ ) whereas mean first-year C0 and C6 did not differ between the 2 groups (Figure 2).

We next compared rejection-free periods in patients stratified according to mean C2 in the first post-operative month (Figure 3). The mean rejection-free duration was 197 days (95% CI, 194–200 days) for the Low C2 Group, 226 days (95% CI, 223–229 days) for the Intermediate C2 Group, and 191 days (95% CI, 188–194 days) for the High C2 Group, indicating that patients in the Intermediate C2 Group remained free of rejection for a significantly longer period compared with the Low or the High C2 Groups ( $p < 0.0001$ ). No differences in rejection-free times were noted between the Low and High C2 Groups. The 3 groups did not differ significantly ( $p = 0.4$ ) in the average number of rejections per patient (Table 2).

#### Association between CsA Concentration and Other Clinical Outcomes

**Infections.** A total of 71 episodes of infections were observed, with a range of 0 to 5 episodes per patient. These included 53 episodes of bacterial infection, 10 of fungal infection, and 8 of viral infection. There was no significant difference in the number of infections between the Sandimmune and Neoral arms of the study ( $p = 0.96$ ). No single-point concentration of CsA (C0, C2, or C6) or the  $AUC_{0\text{ to }6\text{ hours}}$  differed significantly between patients who experienced  $>2$  episodes of infection and those who experienced  $\leq 2$  episodes of infections. In addition, there was no significant difference in the number of infection episodes among patients stratified according to first-month C2 (Table 2).

**Pulmonary function.** In total, 645  $FEV_1$  observations were available with a mean value of  $2.20 \pm 0.91$  liter, which corresponded to a predicted  $FEV_1$  value of  $67\% \pm 25\%$ . Home spirometry yielded an additional 5,775 measurements, with a mean  $FEV_1$  value of  $2.20 \pm 0.85$  liter. The predicted  $FEV_1$  was  $70.1\% \pm 25.5\%$  for



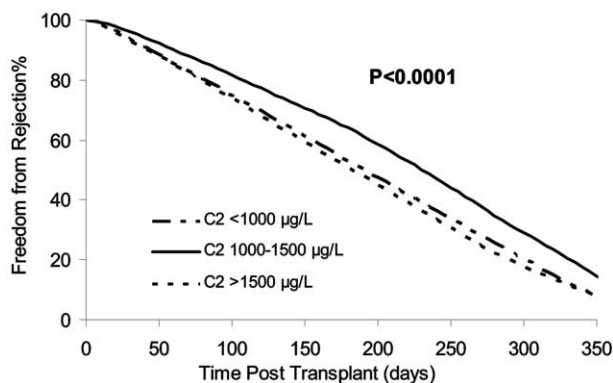
**Figure 2.** (A) Mean and 95% confidence intervals of 12-month cyclosporine concentrations at 0, 2, and 6 hours post-dose in patients with or without multiple rejection episodes. (B) Mean and 95% confidence intervals of cyclosporine area-under-the-time curve of 0 to 6 hours ( $AUC_{0 \text{ to } 6 \text{ hours}}$ ) in patients with or without multiple rejection episodes.

patients with  $\leq 2$  rejections compared with  $49.2\% \pm 15.2\%$  for patients with  $>2$  rejections ( $p < 0.0001$ ). All lung function parameters, including  $FEV_1$ ,  $FEV_1\%$  predicted and home spirometry results, differed significantly among the 3 C2 groups (Table 2) (Figure 4). Lung function in the Intermediate C2 Group was superior to that in the other 2 groups, as evidenced by significantly higher values for all 3 lung function parameters in this group. The results of lung function tests were similar in patients with mean C2 concentrations  $<1,000 \mu\text{g/liter}$  or  $>1,500 \mu\text{g/liter}$ . There was no significant difference in the proportion of patients who received heart-lung, single-lung, or double-lung transplants ( $p = 0.23$ ) (Table 2).

**Renal function.** The mean 12-month serum creatinine concentration, based on 1,693 observations (17–77 per

patient), was  $125 \pm 54.8 \mu\text{mol/liter}$ . The median creatinine clearance was  $58.0 \pm 19.8 \text{ ml/min}$  (range, 10–136 ml/min). No significant correlation was observed between any of the kidney function indices (routinely measured serum creatinine, measured or calculated creatinine clearance, and serum cystatin C concentration) with CsA C0, C2, or C6 level or  $AUC_{0 \text{ to } 6 \text{ hours}}$ . The mean serum creatinine concentration during the first year and the mean percent increase in serum creatinine concentration from baseline during the first month were significantly higher in patients with intermediate C2, whereas the measured or the calculated creatinine clearance value did not differ among the 3 groups ( $p = 0.09$ ) (Table 2). Serum creatinine concentration at 1 year post-transplant was  $147 \pm 64 \mu\text{mol/liter}$  for the Low,  $160 \pm 52 \mu\text{mol/liter}$  for the Intermediate and  $145 \pm 37 \mu\text{mol/liter}$  for the High Groups ( $p = 0.42$ ). The mean percentage increase in serum creatinine from baseline remained significantly higher in the Intermediate Group at 3, 6, and 12 months post-transplant; however, the concentrations of cystatin C were not different among the 3 groups.

**Blood pressure.** In total, 1,891 measurements of systolic (SBP) and diastolic blood pressure (DBP) were available, with 11 to 127 measurements per patient. The mean calculated MAP was 98.1 mm Hg. A linear trend was noted among all 3 measures of blood pressure and the CsA C2 strata ( $p < 0.001$ ); with increasing CsA C2, a corresponding increase was observed in SBP, DBP, and MAP. However, none of the measured CsA pharmacokinetic parameters, including C2 and  $AUC_{0 \text{ to } 6 \text{ hours}}$ , correlated significantly with any of the blood pressure indices.



**Figure 3.** Kaplan-Meier plot for freedom from allograft rejection in groups stratified by first-month C2. The  $p$  value reflects the level of significance between the intermediate group (C2, 1,000–1,500  $\mu\text{g/liter}$ ) and the other 2 groups.

**Table 2.** Post-transplant Clinical Outcomes According to Mean First-Month Cyclosporine C2

	Patient Groups			<i>p</i>
	Low C2 <1000 μg/L ( <i>n</i> = 18)	Intermediate C2 1000–1500 μg/L ( <i>n</i> = 16)	High C2 >1500 μg/L ( <i>n</i> = 14)	
Sandimmune/Neoral (S/N)	16 S/2 N	4 S/12 N	1 S/13 N	<0.001
Type of transplant (HL/SL/DL) <sup>#</sup>	7 HL/6 SL/5 DL	10 HL/4 SL/2 DL	4 HL/8 SL/2 DL	0.23
CsA dose (mg/kg/day)	7.38 ± 4.08	7.42 ± 4.59	6.26 ± 4.13*	<0.001
Clinically measured C0 (μg/liter)	355 ± 154	365 ± 162	361 ± 147	0.60
CsA C2 concentration (μg/liter) (CV%)	631 ± 210 (33.0%)	1204 ± 108 (8.9%)	1799 ± 213 (11.8%)	<0.00001
AUC <sub>0-6 hr</sub> (μg · hr/liter)	3444 ± 1599	4901 ± 1759	6109 ± 2264	<0.00001
Rejections/patient ( <i>n</i> )	1.67 ± 1.19	1.56 ± 1.63	2.00 ± 1.51	0.40
Infections/patient ( <i>n</i> )	1.67 ± 1.49	1.31 ± 1.25	1.57 ± 1.45	0.76
FEV <sub>1</sub> (Liter)	2.01 ± 0.75	2.53 ± 1.03 <sup>†</sup>	1.86 ± 0.76	<0.001
Predicted FEV1 (%)	64.0 ± 24.8	74.4 ± 26.9 <sup>†</sup>	58.7 ± 20.7	<0.001
FEV <sub>1</sub> (portable spirometer) (liter)	1.95 ± 0.83	2.61 ± 0.81 <sup>†</sup>	1.93 ± 0.42	<0.001
Scr (μmol/L)	120 ± 63	135 ± 55 <sup>†</sup>	119 ± 42	<0.001
% increase in Scr <sup>§</sup>	18.6 ± 46.6	35.8 ± 61.7 <sup>†</sup>	16.8 ± 34.6	<0.003
Creatinine Clearance (mL/min)	58.1 ± 20.5	59.4 ± 19.4	56.5 ± 19.3	0.09
SBP (mm/Hg)	127 ± 15	131 ± 17	142 ± 20	<0.001
DBP (mm/Hg)	78 ± 12	80 ± 11	85 ± 13	<0.001
MAP (mm/Hg)	94 ± 12	97 ± 12	104 ± 14	<0.001

AUC, area under the curve; CsA, cyclosporine; CV: coefficient of variation; DBP, diastolic blood pressure; FEV<sub>1</sub>, Forced expiratory volume in 1 second; MAP, mean arterial pressure; SBP, systolic blood pressure; Scr, serum creatinine.

\*Significantly lower than the other two groups.

<sup>#</sup>HL: Heart-Lung Transplant; SL: Single-Lung Transplant; DL: Double-Lung Transplant.

<sup>†</sup>Significantly different from the other two groups.

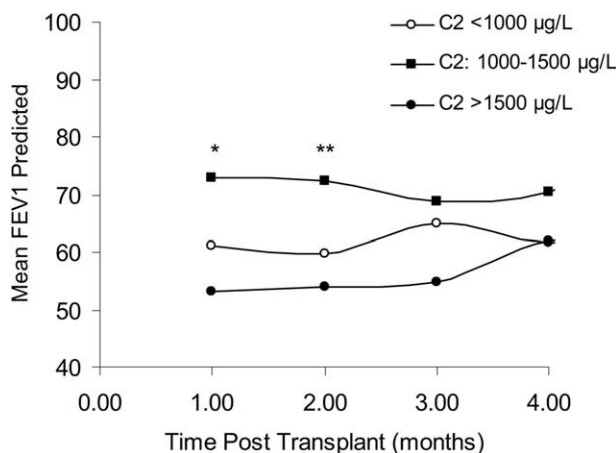
<sup>§</sup>Percent increase in serum creatinine in the first post transplant month from baseline.

## DISCUSSION

The need to optimize immunosuppression in lung transplantation is highlighted by the increased incidence of acute rejection and poorer outcomes after lung transplantation compared with other solid organ transplants.<sup>2</sup> The immunosuppressive activity of CsA is highly dependent on systemic exposure, which most commonly is measured with the C0. Variability in CsA

C0 is a significant risk factor for acute lung rejection in the early post-transplant period.<sup>16</sup> Recently, C2 has emerged as a more accurate and consistent single-point predictor of exposure to CsA than C0,<sup>17,18</sup> and a number of clinical studies have documented the close association between CsA C2 and post-transplant outcomes in kidney,<sup>7,19–21</sup> liver,<sup>22–24</sup> and heart<sup>10</sup> transplant recipients. Little data are available on CsA monitoring after lung transplantation, however, and the optimal concentration range for CsA C2 has not been established for lung transplant recipients.

To our knowledge, this report is one of the first studies to characterize the relationship between CsA concentrations and clinical outcomes after lung transplantation. We show that in de novo lung transplant recipients, CsA C2 is superior to C0 and C6 as a single-point estimate of CsA exposure, as measured by AUC<sub>0 to 6</sub> hours. Importantly, both C2 and AUC<sub>0 to 6</sub> hours, but not C0 or C6, demonstrated a significant association with clinical outcomes, including acute rejection episodes and lung function. A higher frequency of rejection was associated with a significantly lower mean 12-month C2 and AUC<sub>0 to 6</sub> hours, whereas there was no association of rejection frequency with the mean C0 or C6. Clinical outcomes were related to the mean C2 in the first post-operative month. Patients in the intermediate C2 group (1,000 to 1,500 μg/liter) had a significantly longer rejection-free



**Figure 4.** Pulmonary function over the first 6 months post-transplantation in patients stratified by first-month C2 (\**p* < 0.05 and \*\**p* < 0.01; level of difference between C2: 1,000–1,500 μg/liter). FEV<sub>1</sub>, forced expiratory volume in 1 second.

period and better lung function compared with patients whose mean C2 was  $<1,000 \mu\text{g/liter}$  or  $>1,500 \mu\text{g/liter}$ .

The pharmacokinetic and clinical data for this study were obtained from a prospective trial in 48 de novo lung transplant recipients who received either Neoral or Sandimmune and who were maintained on similar trough concentrations of CsA. The analysis from that study showed that both C2 and  $\text{AUC}_{0 \text{ to } 6 \text{ hours}}$ , but not C0 or C6, were significantly higher with the use of Neoral compared with Sandimmune and also that Neoral significantly reduced intra- and inter-patient variability in these parameters. Despite these differences, C2 was the parameter that associated best with  $\text{AUC}_{0 \text{ to } 6 \text{ hours}}$  for both formulations. Furthermore, the 2 groups were similar with respect to clinical outcomes such as acute rejection episodes, infections, and laboratory variables. Therefore, in the current analysis, we combined data from both arms of the original study, which provided us with a total of 341 abbreviated CsA profiles obtained at various intervals in the first post-operative year. The availability of a wide range of C2 concentrations also allowed us to analyze clinical outcomes in 3 groups of patients stratified by the mean C2 in the first post-operative month, as adequate immunosuppression during the first post-operative month is critical to subsequent graft outcomes.

Our finding that C2, but not C0 or C6, was a strong predictor of exposure to CsA ( $\text{AUC}_{0-6 \text{ hours}}$ ) ( $r^2 = 0.934$ ) in lung transplant recipients confirms a similar relationship reported in renal and liver transplantation.<sup>18</sup> An examination of CsA concentrations and clinical outcomes revealed that only the C2 and  $\text{AUC}_{0 \text{ to } 6 \text{ hours}}$  showed an association with multiple rejection episodes. Thus, mean 12-month C2 and  $\text{AUC}_{0 \text{ to } 6 \text{ hours}}$  were significantly higher in patients who experienced  $\leq 2$  rejection episodes compared with those who experienced multiple rejection episodes; by contrast, the mean C0 and C6 did not differ in the 2 patient groups. As a result of multiple allograft rejections, the lung function, as characterized by  $\text{FEV}_{1\text{s}}$ , in these patients were compromised. A previous analysis of the data from this trial had suggested that C0 but not C2 or C6 was associated with the risk of acute rejection; however, those data were based on a limited analysis of only 17% of the pharmacokinetics profiles,<sup>12</sup> whereas clinically measured C0 concentrations were measured at 1,496 occasions. The current findings support the results of another recent study that demonstrated a trend toward fewer and less severe rejection episodes after C2 monitoring in 2 sequential groups of 18 de novo double-lung transplant recipients monitored with C2 vs C0.<sup>25</sup>

Our results also suggest that a CsA C2 between 1,000 and 1,500  $\mu\text{g/liter}$  in the first post-transplant month is associated with significantly better graft outcomes and pulmonary function than higher or lower C2. Thus,

compared with the Low and High C2 Groups, the Intermediate C2 Group remained rejection free for a significantly longer period and also had significantly higher values for all 3 indices of lung function, although the frequency of first-year rejection was similar in the 3 groups. It is important to note that in this study, patients with an average C2  $>1,500 \mu\text{g/liter}$  had a proportionately higher number of rejections and poorer lung function compared with patients in the Intermediate Group. Patients within the High C2 Group may have been over-immunosuppressed because most received Neoral, which increases CsA exposure, and because monitoring was based on C0. Over-immunosuppression, theoretically, may trigger episodes of rejection by lowering innate lung immunity, which increases susceptibility to infection and hence the risk of rejection.<sup>2</sup>

Thirty-two of 83 rejections occurred in this study during or within 4 days of an infection episode; however, the patients with high cyclosporine C2 levels did not have higher number of infection episodes. This observation may also explain the unusual finding of an absence of any difference in post-transplant clinical outcomes between Sandimmune and Neoral in the original study.<sup>12</sup>

There was no significant relationship between C2 and episodes of infection, a finding similar to that of Morton et al<sup>25</sup> in their trial of C0 vs C2 monitoring. However, in another study in 15 lung transplant recipients, patients diagnosed with cytomegalovirus infection had significantly higher mean CsA C2, compared with those without cytomegalovirus infection.<sup>26</sup> We did not examine this relationship because only 8 patients in our study had a viral infection.

Renal function, as judged by serum creatinine concentration, was poorer in patients with intermediate C2 than in the Low and High C2 Groups. Both the serum creatinine level and the percent increase in serum creatinine concentration from baseline were significantly higher in patients with intermediate CsA C2, although neither creatinine clearance nor cystatin C concentrations differed among the 3 groups. In a study of 15 stable lung transplant recipients, Glanville et al<sup>27</sup> found that switching patients with abnormal renal function from C0 monitoring to C2 monitoring with a target of 300 to 600  $\mu\text{g/liter}$  improved renal function significantly and also reduced the dose of CsA by approximately 50%.

The data from this study have been used to develop a population pharmacokinetics model to predict the apparent clearance (CL/F) and concentrations of CsA in lung transplant recipients.<sup>28</sup> We have found that the apparent clearance of CsA is influenced by the CsA formulation, concomitant administration of itraconazole, presence of cystic fibrosis, and patient

weight. In a clinical setting, this model would be useful for predicting the apparent clearance of CsA and, therefore, for calculating initial or maintenance doses of CsA.

## CONCLUSION

This study shows that CsA C2 was the best single-point predictor of  $AUC_{0 \text{ to } 6 \text{ hours}}$  in de novo lung transplant recipients. Both CsA C2 and  $AUC_{0 \text{ to } 6 \text{ hours}}$  showed an association with multiple rejection episodes. Furthermore, an average CsA C2 of between 1,000 and 1,500  $\mu\text{g/liter}$  in the first post-transplant month was associated with a significantly longer rejection-free period and better lung function; however, this concentration range may also associate with poorer renal function. The results of this study suggest that measuring C2 may provide a more accurate method for monitoring CsA exposure after lung transplantation. The usefulness of this method must be further evaluated in a randomized, prospective clinical trial.

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