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IMPROVED CLINICAL OUTCOMES FOR LIVER TRANSPLANT RECIPIENTS USING CYCLOSPORINE MONITORING BASED ON 2-HR POST-DOSE LEVELS (C_2)[1]

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Background. **A prospective, open-label, study was conducted at 29 centers in 9 countries, involving 307 de novo liver transplant patients to compare the clinical usefulness of monitoring 2-hr post-dose cyclosporine (CsA) levels (C_2) with conventional trough cyclosporine blood levels (pre-dose) (C_0).**

Methods. **Neoral oral therapy was initiated at 15 mg/kg/day and dose adjusted according to predetermined C_2 or C_0 target level ranges. The primary efficacy variable was treatment failure at 3 months, where evaluation was based on a composite endpoint of biopsy-proven rejection, treatment for rejection, graft loss, death, or premature withdrawal/discontinuation from the study.**

Results. **Baseline characteristics were similar between groups. Graft loss at 12 weeks (retransplantation or death) occurred in 6.8% C_2 and in 7.0% C_0 patients. Overall incidence of treated acute rejection was lower for C_2 (23.6%) than C_0 patients (31.6%) (*P* 0.144, Cochran-Mantel-Haenszel [CMH] test). In hepatitis C virus (HCV)-negative patients, the incidence of rejection in the C_2 group was significantly less than in the C_0 group (21.2% vs. 33.0%; *P*<0.05), whereas in HCV-positive patients, the rejection rate was similar in both groups (26.7% for C_2 group vs. 27.3% for C_0 group: *P* 0.81). C_2 patients (n 16) who reached minimum target CsA levels by day 3 had a notably low incidence of rejection (12.5%), whereas there was no difference in the incidence of rejection in C_0 patients, irrespective of time to reach target level. For biopsy-proven acute rejections (21.6% for C_2 vs. 30.4% for C_0), the incidence of moderate and severe histological diagnosis was significantly lower in the C_2 group than in the C_0 group (47% vs. 73%; *P* 0.01). Safety profiles were similar between the two groups, with few patient withdrawals due to adverse events (9.5% for C_2; 7.0% for C_0).**

Conclusions. **Using C_2 monitoring, the overall incidence of acute cellular rejection was lower compared with the C_0 group, and the histological severity of acute rejections was shown to be significantly milder for the C_2 group, indicative of good long-term prognosis. These data demonstrate that the use of C_2 monitoring is superior to C_0 and results in a reduction in the incidence and severity of acute cellular rejection without detrimental effect on the drug safety profile.**

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Renewed interest has been shown in methods of therapeutic drug monitoring to manage CsA dosing for organ transplant recipients. Cyclosporine monitoring has been performed traditionally by measurement of predose blood concentrations (C_0), which does not correlate well with drug exposure as measured by AUC, based on 12-hr pharmacokinetic analysis (AUC_{0-12}) (*8*), and does not predict freedom from rejection (*9*, *10*).

AUC_{0-12}, a far more accurate measurement of drug exposure, is too inconvenient, costly, and difficult to perform on a routine basis. The absorption phase for Neoral occurs usually within 2 hr after administration of the drug (*10*), characterized by a rapid rise in blood CsA concentrations and high degree of inter-patient variability. The latter is dependent upon the individual patient's ability to absorb CsA from the gastrointestinal tract (*11*, *12*). Recent studies in renal, liver, and heart transplantation have demonstrated that monitoring strategies using abbreviated sampling techniques to estimate AUC (*13*) or CsA levels 2 hr after dosing (C_2) may be more effective methods of optimizing Neoral immunosuppression (*10*, *14*). More specifically, these studies showed that there is a strong association between freedom from graft rejection and AUC or peak Neoral levels (C_{max}) up to 15 days after liver transplantation (*10*). Full pharmacokinetic profiles (AUC_{0-12}) have shown that C_2 values were very close to calculated C_{max} and thus supports the use of C_2 as a surrogate marker for C_{max}. Not only does C_{max} correlate quite well with AUC but some of the toxicity may be directly attributable to the peak level, e.g., some patients report the development of tingling or headache 2 hr after taking their dose of Neoral. Monitoring C_2 levels, therefore, may offer a better way to individualize therapy and a more precise method for optimizing Neoral dosing, in both the early and later stages posttransplantation.

The present study was undertaken to compare C_2 (used as a surrogate marker of C_{max}) versus conventional predose cyclosporine sampling to determine whether C_2 monitoring can improve clinical outcomes in terms of minimizing graft rejection after liver transplantation.

MATERIALS AND METHODS

A randomized, open-label, parallel group, comparative study involving 307 primary liver transplant patients was undertaken at 29 centers in 9 countries (Argentina, Brazil, Canada, France, Hungary, Italy, Spain, U.K. and U.S.A.). Patients were followed for 3 months to record the monitoring of CsA levels according to randomization and to assess the clinical and laboratory parameters indicative of efficacy, graft function, and safety. The primary efficacy variable was treatment failure at 3 months, where evaluation was based on a composite endpoint of biopsy-proven rejection, treatment for rejection, graft loss, death, or premature withdrawal/discontinuation from the study by a univariate analysis. Secondary efficacy variables were time to treatment failure, time to first biopsy-proven or treated rejection, severity of rejection over the 3-month posttransplant period, effect of hepatitis C virus (HCV) infection on the incidence of rejection, and effect of time to reach C_2 and C_0 target levels on the incidence of rejection.

The treatment protocol was approved by local ethics committees, and written, informed consent was obtained from all patients. Patient care complied with the Declaration of Helsinki guidelines.

First-time, single-organ transplant recipients aged 18–70 years were eligible for enrollment. Patients were excluded if they had a second or subsequent organ transplants; had received a liver from an ABO-incompatible cadaveric donor; were in receipt of split livers; had known malignancy; were scheduled to receive induction therapy with any antibody preparation or the immunosuppressive com- pound, mycophenolate mofetil, because at the time of initiation of the study, MMF was an investigational drug for liver patients; were in acute liver failure; had a severe coexisting disease such as significant cardiac disease, chronic renal failure, active systemic infection, or other unstable medical condition; had received any investigational drugs within the preceding month; were pregnant or lactating; or were tested positive for human immunodeficiency virus.

Patients were randomized to be monitored according to C_2 (sample taken 2 hr after Neoral administration) or according to conventional "trough" monitoring (preceding the morning dose) for 3 months. Dose adjustments were made based on local laboratory values for C_0 and C_2. Duplicate samples collected on days 7, 14, and 84 posttransplantation were frozen and sent to a central laboratory and analyzed using a monoclonal specific radioimmunoassay (mRIA, INCSTAR). Half of the study centers (n 15) accounting for 173 patients used the monoclonal-specific fluorescence polarization immunoassay (TDx, ABBOTT); the monoclonal specific radioimmunoassay (mRIA, Diasorin) was used in 4 study sites that recruited 70 patients, and EMIT (homogeneous enzyme immunoassay, SYVA) was used in 7 centers that recruited 62 patients. Both C_2 and C_0 levels were measured in all patients, but the investigator was blinded to the C_0 value in group 1 (C_2) patients and to the C_2 value in group 2 (C_0) patients. Training of laboratory personnel consisted of site initiation and information sessions on study conduct. The use of colored labels clearly identified the post-dose blood samples for potential dilutions. Phlebotomists were instructed to take blood samples within 15 min of the 2 hr post-dose time, because this range was verified to keep minimal variability. After randomization into one of the treatment groups, Neoral therapy was initiated as soon as possible, and not more than 12 hr after liver transplantation, as a single dose of 7.5 mg/kg of Neoral solution given orally (soft gelatin capsules) or via a nasogastric tube. Administration by nasogastric tube continued until the patient was able to swallow capsules or drink the oral solution. Patients were maintained at a dose of 15 mg/kg/day, given as equally divided doses at 12-hr intervals, for the first 48 hr. After 48 hr, dose adjustments were made according to the predefined C_2 or C_0 ranges, weeks 0–12: group 1—C_2 levels: 850–1400 ng/ml; Group 2—C_0 levels: 250–400 ng/ml. Patients were monitored daily for the first 14

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days to make dose adjustments to ensure that they attained and remained within the predetermined C_2 or C_0 range, provided that renal function permitted. Thereafter, monitoring was at weeks 3, 4, 8, and 12. Continuous i.v. infusion of Sandimmune was instituted in patients who failed to show acceptable absorption of Neoral (i.e., not reaching the target levels) within 5 days. Additional immunosuppressive medication (steroids azathioprine) was used if deemed necessary by the investigator. The C_0 target was as per local practice determined by an advisory committee, whereas the selected target range for C_2 was based on data derived from a previous study (*10*).

The diagnosis of graft rejection was based on biochemical evidence of liver dysfunction and a positive biopsy result. If feasible, a core biopsy was examined locally by a blinded pathologist in all suspected cases of rejection before the commencement of antirejection therapy. All patients with an increase in their levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, or bilirubin levels underwent Doppler studies and had an ultrasound examination. If these tests were normal, a biopsy was performed. The diagnosis of rejection was based on the following histological features: (a) a mixed, but predominantly mononuclear portal inflammation; (b) bile duct inflammation/damage; and (c) subendothelial inflammation of portal veins or terminal hepatic venules (*15*). Graft rejection was treated according to local center policy.

Safety and tolerability assessments were recorded as per previous protocols (*11*) and included physical examination at baseline and week 12, as well as chemistry and hematology laboratory evaluations at day 2 and at weeks 1, 2, 3, 4, 8, and 12.

The intent-to-treat (ITT) population comprised all randomized patients who had their scheduled transplant, received at least one dose of the study drug regimen, had their CsA level monitored at least once, and who had at least one post-baseline efficacy assessment. The safety population comprised all subjects randomized to treatment, who received at least one dose of study medication, and had at least one post-baseline safety measurement. Clinical efficacy was measured by biopsy-proven rejection with severity grading, graft and patient survival, and any treatment for rejection.

Based on results from previous randomized trials in liver transplantation with Neoral dosing based on C_0 monitoring (*16*), this study was designed with a 80% power to detect, at a 5% significance level, and a 16% difference in rejection from 45% (C_0 group) to 29% (C_2 group). The chi-square and the CMH tests were used to compare incidences between the two arms. Time to treatment failure, time to graft loss or subject death, and time to the first biopsy-proven or treated rejection was summarized using the Kaplan-Meier estimator over the 3-month posttransplant period, and the log-rank and Wilcoxon tests were applied for the Kaplan-Meier analyses. For each group comparison, the Breslow-Day test was used to analyze the monitoring group by center interaction.

RESULTS

The safety population comprised 307 patients, with 306 patients included in the ITT population. Patient demographic profiles were comparable between the two cohorts (Table 1). Evaluation of laboratory data at baseline relating to hepatic, renal, and other organ system functions showed no significant differences between groups (Table 2). One patient was not assessed for efficacy before withdrawal and thus did not meet one of the criteria for inclusion in the ITT population. A total of 213 patients (C_2 104, C_0 109) completed the 12-week protocol (Fig. 1) with discontinuation rates of 30.2% (45/149) in the C_2 group and 31.0% (49/158) in the C_0 group. Primary reasons for patient withdrawals are shown in Table 3.

A total of 16/148 (10.8%) patients in the C_2 group and 89/158 (56.3%) patients in the C_0 population reached the required CsA range by day 3; the average time to reach

TABLE 1. Pretransplant diagnosis and patient demographic profiles

	C_2 (n 148)	C_0 (n 158)
Mean age: years SD	49.7 11.42	48.9 11.03
Gender		
Male	99 (66.9%)	108 (68.4%)
Race		
Caucasian	137 (92.6%)	152 (96.2%)
Black	4 (2.7%)	1 (0.60%)
Other	7 (4.7%)	5 (3.20%)
Primary disease requiring liver transplantation		
Primary biliary cirrhosis	12 (8.1%)	14 (8.9%)
Primary sclerosing cholangitis	10 (6.8%)	10 (6.3%)
Chronic active hepatitis	55 (37.2%)	60 (38.0%)
Alcoholic cirrhosis	38 (25.7%)	33 (20.9%)
Other	33 (22.3%)	41 (25.9%)
Viral serology (organ recipient)		
Hepatitis B		
Negative	133 (89.9%)	135 (85.4%)
Positive	12 (8.1%)	14 (8.9%)
Not tested	3 (2.0%)	9 (5.7%)
Hepatitis C		
Negative	99 (66.9%)	100 (63.3%)
Positive	46 (30.4%)	56 (34.8%)
Not tested	3 (2.0%)	2 (1.3%)

minimum target CsA levels was significantly longer for the C_2 group (mean 6.5 days) than for the C_0 group (mean 4.0 days, P 0.0001) (Fig. 2). Specific analysis was performed on the mean daily dose of Neoral at different time intervals within the first month after transplantation, when the majority of acute rejection episodes occurred. During this period, patients in the C_2 group received, on average, a higher dose of Neoral than the patients in the C_0 group: 12.9 mg/kg/day in C_2 group vs. 10.3 mg/kg/day in C_0 group (P 0.001). However, there was no difference in dose between those patients without rejection and those who had a rejection within the interval or any time thereafter (P 0.5).

The incidence of acute rejection and the histological diagnosis of biopsy-proven episodes at the end of 12 weeks are shown in Table 4. Kaplan-Meier analysis of time to acute rejection events shows that, compared with patients in the C_0 group, fewer patients in the C_2 group had early rejections (Wilcoxon P 0.054; log-rank P 0.085); only 3 patients had rejection events in the first week and 12 in the second week posttransplant, compared with 19 patients in the first week and 12 in the second week for the C_0 group (Fig. 3). In the C_2 group, the time to reach the target range minimum (850 ng/ml) had a marked effect on the incidence of acute rejection, with a rejection rate of 12.5% for the minority of patients (n 16) who achieved C_2 target by day 3, whereas patients reaching target by day 7 (n 95) had a rejection rate of 26.3% (P 0.03). For patients reaching C-2 target by day 10, the rejection rate was 33.5% (n 37). In contrast, the time to reach the target range minimum had no effect on the incidence of cellular rejection in the patients monitored by C_0 levels (Fig. 4).

Furthermore, for all biopsy-proven rejections, moderate and severe histological diagnosis was significantly lower (P 0.01) in the C_2 group compared with the C_0 group (Table

days to make dose adjustments to ensure that they attained and remained within the predetermined C_2 or C_0 range, provided that renal function permitted. Thereafter, monitoring was at weeks 3, 4, 8, and 12. Continuous i.v. infusion of Sandimmune was instituted in patients who failed to show acceptable absorption of Neoral (i.e., not reaching the target levels) within 5 days. Additional immunosuppressive medication (steroids±azathioprine) was used if deemed necessary by the investigator. The C_0 target was as per local practice determined by an advisory committee, whereas the selected target range for C_2 was based on data derived from a previous study (*10*).

The diagnosis of graft rejection was based on biochemical evidence of liver dysfunction and a positive biopsy result. If feasible, a core biopsy was examined locally by a blinded pathologist in all suspected cases of rejection before the commencement of antirejection therapy. All patients with an increase in their levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, or bilirubin levels underwent Doppler studies and had an ultrasound examination. If these tests were normal, a biopsy was performed. The diagnosis of rejection was based on the following histological features: (a) a mixed, but predominantly mononuclear portal inflammation; (b) bile duct inflammation/damage; and (c) subendothelial inflammation of portal veins or terminal hepatic venules (*15*). Graft rejection was treated according to local center policy.

Safety and tolerability assessments were recorded as per previous protocols (*11*) and included physical examination at baseline and week 12, as well as chemistry and hematology laboratory evaluations at day 2 and at weeks 1, 2, 3, 4, 8, and 12.

The intent-to-treat (ITT) population comprised all randomized patients who had their scheduled transplant, received at least one dose of the study drug regimen, had their CsA level monitored at least once, and who had at least one post-baseline efficacy assessment. The safety population comprised all subjects randomized to treatment, who received at least one dose of study medication, and had at least one post-baseline safety measurement. Clinical efficacy was measured by biopsy-proven rejection with severity grading, graft and patient survival, and any treatment for rejection.

Based on results from previous randomized trials in liver transplantation with Neoral dosing based on C_0 monitoring (*16*), this study was designed with a 80% power to detect, at a 5% significance level, and a 16% difference in rejection from 45% (C_0 group) to 29% (C_2 group). The chi-square and the CMH tests were used to compare incidences between the two arms. Time to treatment failure, time to graft loss or subject death, and time to the first biopsy-proven or treated rejection was summarized using the Kaplan-Meier estimator over the 3-month posttransplant period, and the log-rank and Wilcoxon tests were applied for the Kaplan-Meier analyses. For each group comparison, the Breslow-Day test was used to analyze the monitoring group by center interaction.

RESULTS

The safety population comprised 307 patients, with 306 patients included in the ITT population. Patient demographic profiles were comparable between the two cohorts (Table 1). Evaluation of laboratory data at baseline relating to hepatic, renal, and other organ system functions showed no significant differences between groups (Table 2). One patient was not assessed for efficacy before withdrawal and thus did not meet one of the criteria for inclusion in the ITT population. A total of 213 patients (C_2=104, C_0=109) completed the 12-week protocol (Fig. 1) with discontinuation rates of 30.2% (45/149) in the C_2 group and 31.0% (49/158) in the C_0 group. Primary reasons for patient withdrawals are shown in Table 3.

A total of 16/148 (10.8%) patients in the C_2 group and 89/158 (56.3%) patients in the C_0 population reached the required CsA range by day 3; the average time to reach minimum target CsA levels was significantly longer for the C_2 group (mean 6.5 days) than for the C_0 group (mean 4.0 days, $P<0.0001$) (Fig. 2). Specific analysis was performed on the mean daily dose of Neoral at different time intervals within the first month after transplantation, when the majority of acute rejection episodes occurred. During this period, patients in the C_2 group received, on average, a higher dose of Neoral than the patients in the C_0 group: 12.9 mg/kg/day in C_2 group vs. 10.3 mg/kg/day in C_0 group ($P=0.001$). However, there was no difference in dose between those patients without rejection and those who had a rejection within the interval or any time thereafter ($P>0.5$).

TABLE 1. Pretransplant diagnosis and patient demographic profiles

	C_2 (n=148)	C_0 (n=158)
Mean age: years±SD	49.7±11.42	48.9±11.03
Gender		
Male	99 (66.9%)	108 (68.4%)
Race		
Caucasian	137 (92.6%)	152 (96.2%)
Black	4 (2.7%)	1 (0.60%)
Other	7 (4.7%)	5 (3.20%)
Primary disease requiring liver transplantation		
Primary biliary cirrhosis	12 (8.1%)	14 (8.9%)
Primary sclerosing cholangitis	10 (6.8%)	10 (6.3%)
Chronic active hepatitis	55 (37.2%)	60 (38.0%)
Alcoholic cirrhosis	38 (25.7%)	33 (20.9%)
Other	33 (22.3%)	41 (25.9%)
Viral serology (organ recipient)		
Hepatitis B		
Negative	133 (89.9%)	135 (85.4%)
Positive	12 (8.1%)	14 (8.9%)
Not tested	3 (2.0%)	9 (5.7%)
Hepatitis C		
Negative	99 (66.9%)	100 (63.3%)
Positive	46 (30.4%)	56 (34.8%)
Not tested	3 (2.0%)	2 (1.3%)

The incidence of acute rejection and the histological diagnosis of biopsy-proven episodes at the end of 12 weeks are shown in Table 4. Kaplan-Meier analysis of time to acute rejection events shows that, compared with patients in the C_0 group, fewer patients in the C_2 group had early rejections (Wilcoxon $P=0.054$; log-rank $P=0.085$); only 3 patients had rejection events in the first week and 12 in the second week posttransplant, compared with 19 patients in the first week and 12 in the second week for the C_0 group (Fig. 3). In the C_2 group, the time to reach the target range minimum (850 ng/ml) had a marked effect on the incidence of acute rejection, with a rejection rate of 12.5% for the minority of patients (n=16) who achieved C_2 target by day 3, whereas patients reaching target by day 7 (n=95) had a rejection rate of 26.3% ($P<0.03$). For patients reaching C-2 target by day 10, the rejection rate was 33.5% (n=37). In contrast, the time to reach the target range minimum had no effect on the incidence of cellular rejection in the patients monitored by C_0 levels (Fig. 4).

Furthermore, for all biopsy-proven rejections, moderate and severe histological diagnosis was significantly lower ($P=0.01$) in the C_2 group compared with the C_0 group (Table

TABLE 2. Main laboratory values for C_2 and C_0 groups at baseline, week 4, and week 12

Parameter (mean values)	Baseline		Week 4		Week 12	
	C_2	C_0	C_2	C_0	C_2	C_0
Serum creatinine (mol/L)	90.0	91.8	123.4	115.7	120.7	116.7
Calculated creatinine clearance (ml/min)	98.3	103.1	71.1	74.4	73.0	71.0
Serum alkaline phosphatase (U/L)	305.9	303.2	216.8	241.8	183.0	181.4
Serum total bilirubin (mol/L)	84.0	78.0	65.9	53.5	34.0	27.8

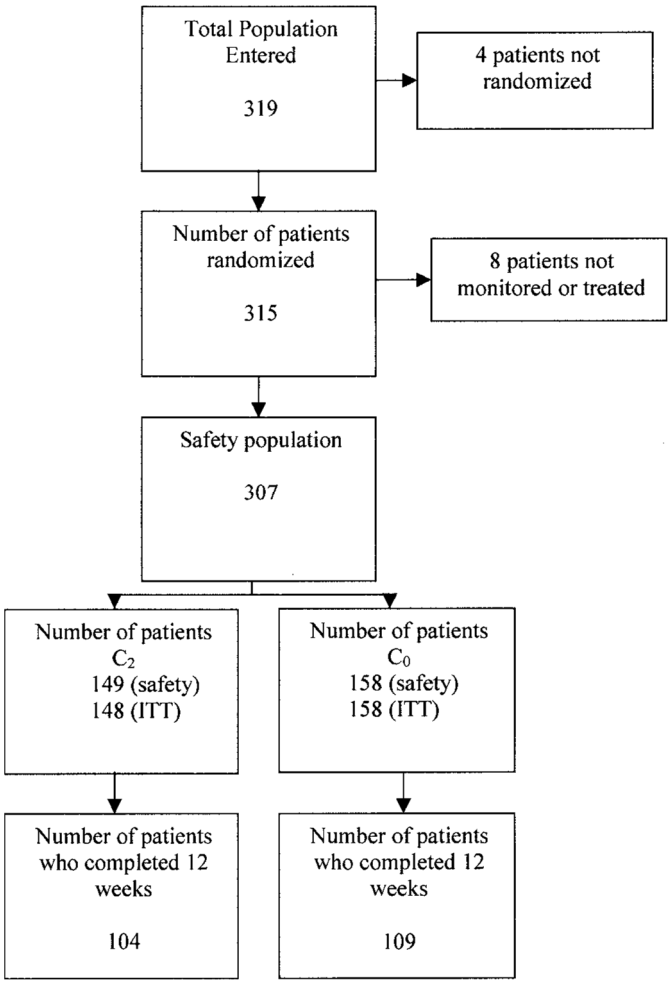

FIGURE 1. Trial profile.

TABLE 3. Primary reasons for patient withdrawal

	C_2 (n 148)	C_0 (n 158)	Total (n 306)
Adverse event	14 (9.5%)	11 (7.0%)	25 (8.2%)
Graft loss	1 (0.7%)	0 (0.0%)	1 (0.3%)
Death	6 (4.1%)	7 (4.4%)	13 (4.2%)
Withdrawal of consent	2 (1.4%)	0 (0.0%)	2 (0.7%)
Protocol violation	2 (1.4%)	7 (4.4%)	9 (2.9%)
CRF-defined treatment failure	7 (4.7%)	7 (4.4%)	14 (4.6%)
Discontinuation of study medication for more than 14 days	6 (4.1%)	9 (5.7%)	15 (4.9%)
Inadmissible immunosuppressants	3 (2.0%)	2 (1.3%)	5 (1.6%)
Switch of monitoring methods	3 (2.0%)	0 (0.0%)	3 (1.0%)
Other	0 (0.0%)	6 (3.8%)	5 (2.0%)

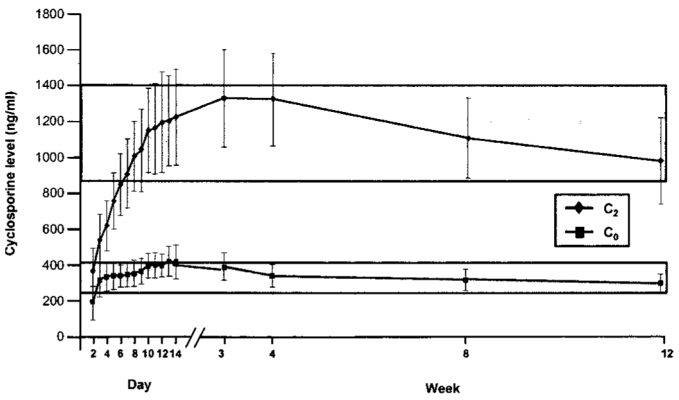

FIGURE 2. Ability to achieve and maintain CsA target levels. Data is the mean SD for each treatment group. On day 3, only 18% of patients monitored by C_2 achieved target, whereas 57% of patients monitored by C_0 achieved target by day 3. The upper shaded area represents the target range for the C_2 group, and the lower shaded area represents the target range for the C_0 group.

4). The incidence of acute rejection for patients who were hepatitis C-positive at entry was similar for both groups (C_2 12/46 [26.1%], C_0 16/56 [28.6%], P 0.81). However, for hepatitis C-negative patients, who accounted for approximately two-thirds of each study group, acute rejection was significantly lower (P 0.05) in the C_2 group than in the C_0 group (21/99 [21.2%] vs. 33/100 [33.0%]), respectively.

Death occurred in 12/148 (8.1%) patients in the C_2 group and in 9/158 (5.7%) patients in the C_0 group; graft loss (retransplantation) was recorded in 3/148 (2.0%) and 4/158 (2.5%) of patients in the C_2 and C_0 groups, respectively.

Steroid dosage was tapered to 20 mg/day within the first 2 weeks and maintained at a level not less than 10 mg/day during the first 3 months, then according to local practice.

When azathioprine was used, the dosage was maintained at 1–2 mg/kg/day for the duration of the study (3 months). The majority of patients in both C_2 (87/148 [58.8%]) and C_0 (98/158 [62.0%]) groups received triple therapy with Neoral plus steroids and azathioprine. For these patients, the rate of rejection was similar for both groups: 19/87 (21.8%) in the C_2 group vs. 26/98 (26.5%) in the C_0 group, P 0.496 (Fisher's exact test). For patients on dual therapy with Neoral and steroids, (C_2 group 61/148 [41.2%] and C_0 group 60/158 (38.0%]), the rate of rejection was lower for the C_2 group with 16/61 (26.2%) vs. 24/60 (40.0%) for the C_0 group, P 0.125 (Fisher's exact test). At any given time in the first 2 weeks after transplantation, the maximum number of patients re-

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Parameter (mean values)	Baseline		Week 4		Week 12	
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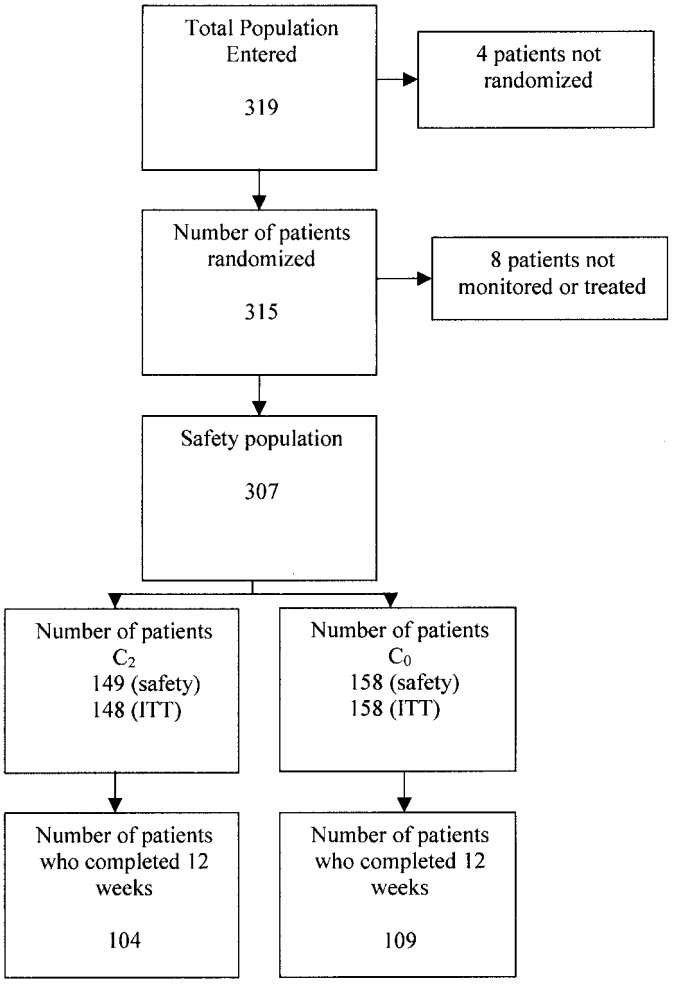

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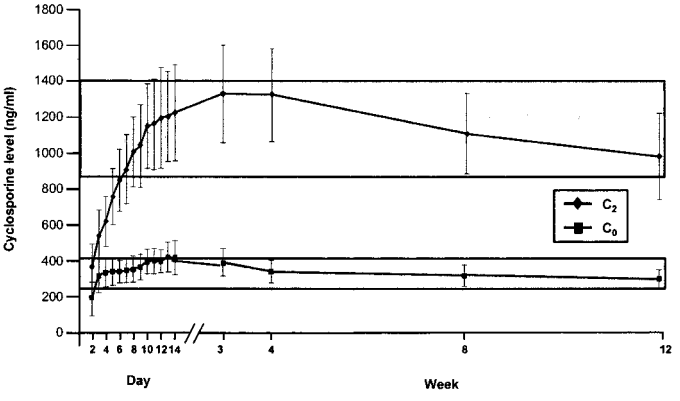
Death	6 (4.1%)	7 (4.4%)	13 (4.2%)
Withdrawal of consent	2 (1.4%)	0 (0.0%)	2 (0.7%)
Protocol violation	2 (1.4%)	7 (4.4%)	9 (2.9%)
CRF-defined treatment failure	7 (4.7%)	7 (4.4%)	14 (4.6%)
Discontinuation of study medication for more than 14 days	6 (4.1%)	9 (5.7%)	15 (4.9%)
Inadmissible immunosuppressants	3 (2.0%)	2 (1.3%)	5 (1.6%)
Switch of monitoring methods	3 (2.0%)	0 (0.0%)	3 (1.0%)
Other	0 (0.0%)	6 (3.8%)	5 (2.0%)



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Death occurred in 12/148 (8.1%) patients in the C_2 group and in 9/158 (5.7%) patients in the C_0 group; graft loss (retransplantation) was recorded in 3/148 (2.0%) and 4/158 (2.5%) of patients in the C_2 and C_0 groups, respectively.

Steroid dosage was tapered to 20 mg/day within the first 2 weeks and maintained at a level not less than 10 mg/day during the first 3 months, then according to local practice. When azathioprine was used, the dosage was maintained at 1–2 mg/kg/day for the duration of the study (3 months). The majority of patients in both C_2 (87/148 [58.8%]) and C_0 (98/158 [62.0%]) groups received triple therapy with Neoral plus steroids and azathioprine. For these patients, the rate of rejection was similar for both groups: 19/87 (21.8%) in the C_2 group vs. 26/98 (26.5%) in the C_0 group, P=0.496 (Fisher's exact test). For patients on dual therapy with Neoral and steroids, (C_2 group 61/148 [41.2%] and C_0 group 60/158 (38.0%]), the rate of rejection was lower for the C_2 group with 16/61 (26.2%) vs. 24/60 (40.0%) for the C_0 group, P=0.125 (Fisher's exact test). At any given time in the first 2 weeks after transplantation, the maximum number of patients re-

TABLE 4. Incidence of rejection and histological diagnosis of biopsy-proven rejection episodes at the end of 12 weeks[a]

	C_2 (n 148)	C_0 (n 158)	*P* value
Acute	35 (23.6%)	50 (31.6%)	[b]
Biopsy-proven	32 (21.6%)	48 (30.4%)	
Histological diagnosis			
Moderate	14 (43.7%)	28 (58.3%)	
Severe	1 (3.1%)	7 (14.6%)	
Total	15 (46.8%)	35 (62.9%)	[c]

[a] The time to event (Kaplan-Meier estimate) was tested by both the log-rank test and the Wilcoxon.

[b] For rejection: Wilcoxon, *P* 0.054; log-rank, *P* 0.085.

[c] For severity of rejection both log rank and Wilcoxon, *P* 0.004.

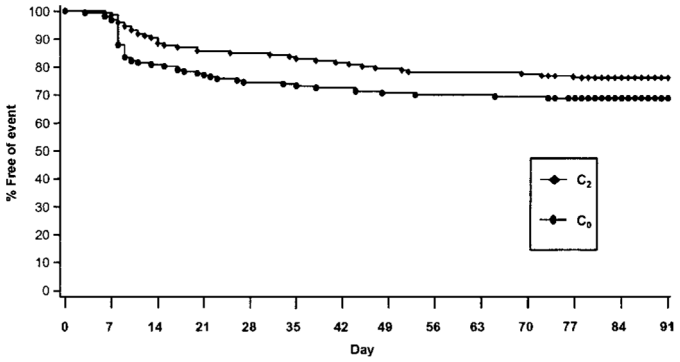

FIGURE 3. Kaplan-Meier curve showing time to acute rejection in C_0 and C_2 groups; *P* 0.085 log-rank test; *P* 0.054 Wilcoxon test.

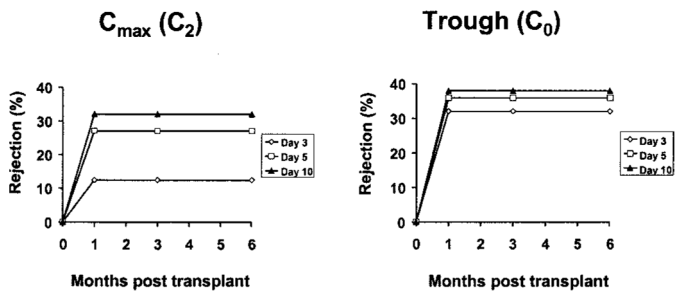

FIGURE 4. Effect of time to reach target on incidence of rejection in patients monitored by C_2 or C_0. Patients monitored by C_2 who achieved target within 3 days had a significantly lower incidence of acute cellular rejection compared with patients who only achieved target by day 10 (*P*<0.03). Time to reach target did not affect the incidence of rejection in the C_0 group (*P*>0.57).

ceiving i.v. Sandimmune was nine for the C_2 group and two for the C_0 group.

Within the C_2 group, 103/149 (69.1%) patients experienced drug-related adverse events versus 120/158 (75.9%) patients in the C_0 group. The severity of adverse events and their distribution between organ systems was similar between groups. Overall, a good tolerability profile was observed in both groups up to 3 months of Neoral treatment (Table 2). Serious adverse events were defined as any event that was life-threatening or delayed hospital discharge. The incidence of patients experiencing serious central nervous system (CNS)-related disturbances was low in both groups: 14/148 (9.4%) of patients in the C_2 group and 14/158 (8.9%) in the C_0 group. Similarly, the incidence of patients experiencing serious renal disorders was low (10/149 [6.7%] in the C_2 group and 9/158 [5.7%] in the C_0 group), with 7/149 (4.7%) C_2 and 6/158% (3.8%) C_0 of these experiencing renal failure. The total number of adverse events was 1652 (C_2) and 1822 (C_0), although most were of no clinical significance. The most common adverse events for both groups are shown in Table 5. Incidence of infections was similar between groups: 95/149 (63.8%) in the C_2 group and 114/158 (72.2%) in the C_0 group. Of these, 26 patients (17.4%) in the C_2 group and 38 patients (24.1%) in the C_0 group experienced serious infections. The most prevalent opportunistic viral infection was cytomegalovirus, which affected 29/149 (19.5%) patients in the C_2 group and 33/158 (20.9%) patients in the C_0 group. The predominant fungal infection, affecting patients in both groups, was *Candida albicans*, with 10/149 (6.7%) of C_2 patients and 9/158 (5.7%) of C_0 patients being affected.

DISCUSSION

This study was designed to investigate whether adjusting dose based on CsA levels 2 hr after Neoral administration (C_2) would result in a reduction in the incidence and severity of acute cellular rejection compared with conventional predose sampling (C_0). The sample size was chosen to provide a 80% power to detect, at a 5% significance level, and a 16% difference in acute rejection from 45% to 29%. This was derived from previous controlled studies using Neoral; however, in all of these studies intravenous CsA was co-administered in the early period posttransplantation (*4*, *12*, *16*, *17*). More recently, the negative effect of i.v. CsA on rates of rejection has been appreciated but could not be factored into the design of this study when it was initiated. The recent liver transplantation studies using C_0 monitoring showed rates of rejection of approximately 30–35%, which were con- sistent with the rate of rejection seen in the C_0 cohort in the present study. The low rate of rejection seen in the C_2 cohort was less than what we had initially predicted (25% reduction compared with the C_0 cohort) but missed statistical significance because of the lower than anticipated rate of rejection

TABLE 5. Most common adverse events occurring during the first 12 weeks after transplantation

Adverse events	C_2 (n 148)	C_0 (n 158)
Fever	27 (18.1%)	42 (26.6%)
Headache	20 (13.4%)	17 (10.8%)
Cardiac hypertension	62 (41.6%)	75 (47.5%)
Tremor	36 (24.2%)	34 (21.5%)
Abdominal pain	38 (25.5%)	39 (24.7%)
Diarrhea	26 (17.4%)	33 (20.9%)
Constipation	22 (14.8%)	28 (17.7%)
Nausea	26 (17.4%)	31 (19.6%)
Vomiting	17 (11.4%)	19 (12.0%)
Abnormal hepatic function	22 (14.8%)	28 (17.7%)
Hyperglycemia	41 (27.5%)	43 (27.2%)
Peripheral edema	24 (16.1%)	22 (13.9%)
Back pain	23 (15.4%)	21 (13.3%)
Thrombocytopenia	18 (12.1%)	27 (17.1%)
Insomnia	19 (12.8%)	18 (11.4%)
Anemia	37 (24.8%)	46 (29.1%)
Pleural effusion	29 (19.5%)	20 (12.7%)
Acute renal failure	31 (20.8%)	41 (25.9%)

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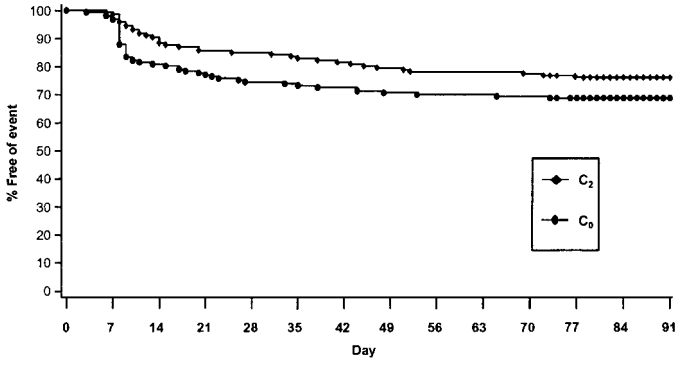

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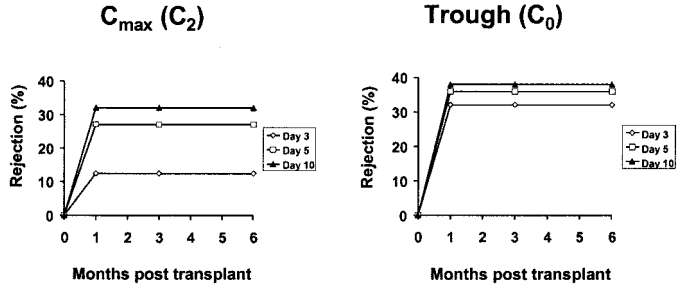

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DISCUSSION

This study was designed to investigate whether adjusting dose based on CsA levels 2 hr after Neoral administration (C_2) would result in a reduction in the incidence and severity of acute cellular rejection compared with conventional predose sampling (C_0). The sample size was chosen to provide a 80% power to detect, at a 5% significance level, and a 16% difference in acute rejection from 45% to 29%. This was derived from previous controlled studies using Neoral; however, in all of these studies intravenous CsA was co-administered in the early period posttransplantation (*4*, *12*, *16*, *17*). More recently, the negative effect of i.v. CsA on rates of rejection has been appreciated but could not be factored into the design of this study when it was initiated. The recent liver transplantation studies using C_0 monitoring showed rates of rejection of approximately 30–35%, which were consistent with the rate of rejection seen in the C_0 cohort in the present study. The low rate of rejection seen in the C_2 cohort was less than what we had initially predicted (25% reduction compared with the C_0 cohort) but missed statistical significance because of the lower than anticipated rate of rejection

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Fever	27 (18.1%)	42 (26.6%)
Headache	20 (13.4%)	17 (10.8%)
Cardiac hypertension	62 (41.6%)	75 (47.5%)
Tremor	36 (24.2%)	34 (21.5%)
Abdominal pain	38 (25.5%)	39 (24.7%)
Diarrhea	26 (17.4%)	33 (20.9%)
Constipation	22 (14.8%)	28 (17.7%)
Nausea	26 (17.4%)	31 (19.6%)
Vomiting	17 (11.4%)	19 (12.0%)
Abnormal hepatic function	22 (14.8%)	28 (17.7%)
Hyperglycemia	41 (27.5%)	43 (27.2%)
Peripheral edema	24 (16.1%)	22 (13.9%)
Back pain	23 (15.4%)	21 (13.3%)
Thrombocytopenia	18 (12.1%)	27 (17.1%)
Insomnia	19 (12.8%)	18 (11.4%)
Anemia	37 (24.8%)	46 (29.1%)
Pleural effusion	29 (19.5%)	20 (12.7%)
Acute renal failure	31 (20.8%)	41 (25.9%)

observed in the C_0 arm. The early oral dosing, instead of continuous CsA infusion, almost invariably resulted in early absorption peaks (approximated by C_2), which may explain why a lower than anticipated rate of rejection was observed in the C_0 group. However, no study using C_0 monitoring with a similar immunosuppressive regimen, even at high Neoral doses, have reported the markedly low incidence of rejection seen here in the C_2 arm. Furthermore, using C_0 monitoring strategy, one does not appreciate the high C_2 levels that may occur in many patients and that may result in over-immunosuppression and account for increased toxicity.

The time to reach the minimum of the CsA target ranges was significantly longer for C_2 patients than for C_0 patients. This is partially due to the fact that the study did not mandate investigators to achieve target levels within a specific time. However, despite the fact that during this time period many patients were maintained below the minimum of the ranges on day 3, a low rate of rejection was seen early posttransplant (Fig. 2). Furthermore, for the small group of patients in the C_2 group (n 16), who reached target range within 3 days posttransplant, a 12.5% rejection rate was observed, whereas the rejection rate for patients in the C_0 group (n 89), who reached target range within 3 days, was 31.5% and remained the same irrespective of the time to reach target levels. This suggests that aiming for, and reaching, the target C_2 levels at an early stage posttransplant is necessary if a C_2 monitoring strategy is to be adopted.

A Cox Regression Model (shown in Table 6) was used to determine the influence of dose, C_0 and C_2 (entered in the model as time-dependent co-variance), on the risk of treatment failure and rejection. The model controlled for the effects of age, sex, assay type (EMIT/non-EMIT) and baseline hepatitis C status (positive/negative). Missing values were imputed by carrying forward time-dependent co-variants or by replacing missing values with the median (numeric variables) and mode categorical variables. Dose and C_0 were not significant (P 0.1616 and P 0.6731) predictors of risk, whereas C_2 was highly significant (P 0.0001) with a Haz- zard Ratio of 0.906 per 100 g/L difference in C_2 values. Assay type did not influence outcome as determined by the Cox analysis. However, at C_0, concentration of metabolites is highest and it is known that metabolites can cross-react with the monoclonal antibodies used in the assays for CsA (*18*). This is in contrast to measurement of C_2, a time point at which metabolites are low. This is a strong argument for adopting C_2. Recent data has shown that in pairs of C_0 and C_2 samples collected over time in liver transplant recipients and analyzed by different assays, C_2 are relatively assay-independent in contrast to C_0. Because rejection is the most important single outcome, the predictors of rejection were further investigated using the same Cox model used for treatment failure. Due to the reduced number of events (78 rejections vs. 134 treatment failures) and the correlation between C_0 and C_2, neither C_0 (P 0.5051) nor C_2 (P 0.1048) were significant when the model included both measures. However, when C_2 was excluded, C_0 was not significant (P 0.1670); excluding C_0, C_2 was highly significant (P 0.04). Dose was not a significant predictor (P 0.15) in all models.

The histological diagnosis for acute rejection was significantly more severe in the C_0 group than the C_2 group (P 0.01). This is an important finding because there is good evidence that the histological severity of rejection is an important negative prognostic factor for long-term graft survival: time to death or retransplantation is shorter and use of antilymphocytes is higher in patients with moderate-to-severe rejection (*19*, *20*). The observation that the hepatitis C-negative patients at entry had a statistically significant lower (P 0.05) rate of biopsy-proven rejections in the C_2 group than in the C_0 group, whereas there was no difference between groups for the hepatitis C-positive recipients, points to the difficulty of differentiating the diagnosis of histological rejection from recurrent disease in the latter patient population whether monitoring by C_2 or C_0. Whether all of the histologic abnormalities seen in the HCV-positive patients were rejection or recurrent, HCV continues to be a problem of all studies in which HCV patients are included. In some instances, parameters of rejection including both endothelialitis and the presence of eosinophils in the portal areas make the diagnosis of rejection more likely, whereas for other histological abnormalities, the features are indistinguishable from recurrent HCV disease.

Within the study duration, most rejection episodes were successfully treated with steroids with only two patients in the C_0 group requiring treatment with antibodies. The incidence of severe rejection was low for both treatment arms, supporting the results of another recent study (*21*) that Neoral is associated with a low incidence of histologically severe rejection in patients undergoing primary liver transplantation.

TABLE 6. Effect of predictor variables on primary outcome as analyzed by the Cox regression model

Variable	Coefficient	SE (coef)[a]	z[b]	P
Age	0.011744	0.008131	1.444	0.1486
Gender: female	0.368047	0.180969	2.034	0.0420
Assay type: not emit	0.253656	0.207041	1.225	0.2205
Group: C_{max}	0.027733	0.180018	0.154	0.8776
Hepatitis C-positive	0.073249	0.188512	0.389	0.6976
DWC.C2[c]	0.003144	0.001574	1.997	0.0458
Dose (mg/kg)	0.046409	0.033154	1.400	0.1616
C_0 (g/L)[d]	0.000212	0.000503	0.422	0.6731
C_2 (g/L)[e]	0.000985	0.000247	3.985	0.0001

[a] SE (coef), standard error (coefficient).
[b] z, coefficient/SE (coef).
[c] DWC.C2, Relative absorption, i.e., dose- and weight-corrected C_2 (g/L/mg/kg) measured on day 5.
[d] C_0, trough CsA concentration.
[e] C_2, CsA concentration at 2 hr post-dose.

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