How Common Is Delayed Cyclosporine Absorption Following Liver Transplantation?

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The mean time to peak absorption of cyclosporine (CsA) in liver transplant patients is approximately 2 hours, but in some patients the peak occurs later. The goal of this study was, therefore, to investigate the incidence of delayed absorption in 27 *de novo* liver transplant recipients receiving CsA ≥10 mg/kg/day (C_2 monitoring) and in 15 maintenance patients. Patients were categorized as 'normal' absorbers (C_2 exceeding C_4 and C_6) or 'delayed' absorbers (C_4 or C_6 exceeding C_2), and as 'good' (>800 ng/mL at C_0, C_2, C_4, or C_6) or 'poor' absorbers (C_0, C_2, C_4 and C_6 <800 ng/mL) on the day of study. Among *de novo* patients, 15 (56%) had 'normal' CsA absorption and 12 (44%) 'delayed' absorption. Good CsA absorption occurred in 16 patients (59%) and poor absorption in 11 (41%). The proportion of poor absorbers was similar in patients with normal (6 / 15, 40%) or delayed (5 / 12, 42%) absorption. Among the 12 delayed absorbers, 11 had peak CsA concentration at C_4. Mean C_0 level was significantly higher in delayed absorbers (282 ± 96 ng/mL) than in normal absorbers (185 ± 88ng/mL; P = .01). Delayed absorbers reverted to normal absorption ($C_2 > C_4$) after a median of 6 days from the day of study, and no cases of delayed absorption were found among maintenance patients. In conclusion, almost 50% of the patients had delayed CsA absorption early posttransplant; around half of these exhibited normal CsA exposure. Measurement of C_4 in addition to C_2 differentiates effectively between delayed and poor absorbers of CsA such that over- or underimmunosuppression can be avoided. (*Liver Transpl 2005;11:167–173.*)

Pharmacokinetic and clinical data have demonstrated that cyclosporine (CsA) level at 2 hours postdose (C_2) is a superior marker for drug exposure and clinical events compared to trough drug blood level (predose) (C_0) in both adult[1,2] and pediatric[3] *de novo* liver transplant patients, and in maintenance[4,5] liver transplant recipients. In contrast to C_0, C_2 correlates closely to CsA exposure during the 1st 6 hours postdose (area under the concentration curve $[AUC]_{0-6}$) (r = .93), which in turn is highly predictive for risk of acute cellular rejection (ACR).[1] The greater predictive value of C_2 for CsA exposure is maintained even when CsA is administered intravenously using 4-hour infusions,[6] and regardless of which adjunctive immunosuppressive agents are used.[7] Moreover, mean time to peak concentration is approximately 2 hours in liver transplant patients,[1] such that C_2 can be regarded as a surrogate marker for peak concentration in patients with a normal absorption pattern; peak concentration also correlates with risk of ACR.[1] A randomized prospective study has demonstrated that the incidence and severity of ACR during the 1st 3 months posttransplant are reduced when the dose of CsA microemulsion (CsA-ME, Neoral) is adjusted based on C_2 level instead of C_0,[2] with continued benefits at 12 months.[8] Based on these findings, C_2 monitoring of CsA-ME is increasingly being adopted by liver transplant centers in preference to conventional C_0 monitoring.[9]

Clinical outcomes with C_2 monitoring of CsA-ME in liver transplantation are excellent. A 3-month ACR rate of 26% has been reported recently using C_2 monitoring and steroids, with azathioprine given to less than 50% of the patients.[10] This was comparable to the ACR rate seen in patients randomized to tacrolimus therapy within the same study (24%; P = not significant),[10] and graft outcomes remained similar between the 2 treatments at 12 months.[11] Previously, however, widely differing rates of rejection have been reported using C_2 monitoring depending on how quickly patients achieved C_2 target,[2] suggesting that individual absorption patterns should be taken into account to maximize efficacy. Liver transplant recipients are exposed to a range of factors that can affect absorption of CsA, especially during the 1st postoperative days, including

Abbreviations: ACR, acute cellular rejection; AUC, area under the concentration curve; CsA, cyclosporine; CsA-ME, cyclosporine microemulsion (Neoral); C_0, trough drug blood level (predose); C_2, blood concentration at 2 hours postdose; C_4, blood concentration at 4 hours postdose; C_6, blood concentration at 6 hours postdose; MMF, mycophenolate mofetil.

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occurrence of paralytic ileus, external biliary drainage clamping, improvement in graft function and cholestasis, and returning to oral feeding and a normal diet. Although the mean time to peak concentration is between 2 and 3 hours postdose during the 1st 2 weeks posttransplant,[1] a proportion of patients show atypical CsA absorption patterns, with peak concentration delayed such that the peak occurs at a later time point.[1,6] There is a paucity of data on this topic in the literature and it is not known how many patients experience delayed absorption. Neither is it known if or when delayed absorption normalizes over time. The time to peak concentration for CsA in liver transplant patients, regardless of absorption pattern, is relatively stable during the 1st weeks posttransplant but has been shown to fall from approximately 2.8 hours during the 1st week posttransplant to <2 hours at week 16.[1] Whether this change is due to the delayed absorbers of CsA achieving peak concentration earlier as gut function normalizes has not been assessed to date.

Determining whether a low C_2 value in the immediate posttransplant period is due to poor absorption of CsA, or results from delayed but adequate absorption, is critical if the CsA-ME dose is to be adjusted appropriately.[12] If the dose is increased in an attempt to achieve the C_2 target in a patient who has a late peak concentration but is absorbing therapeutic levels of CsA, the risk of toxicity increases markedly. Additionally, therefore, it would be valuable to assess what time point after C_2 is the most relevant for identifying delayed absorbers as distinct from poor absorbers of CsA.

Here we report the findings of a prospective study that investigated the incidence of delayed and poor CsA absorption among *de novo* and maintenance adult liver transplant recipients.

Patients and Methods

De Novo Patients

Patients who received a primary liver transplant from a deceased or living donor during the period March 2003 to March 2004 were eligible for inclusion in the study. CsA-ME was initiated as soon as possible after transplantation based on clinical status and renal function. The dose of CsA was adjusted to target a C_2 range of 800–1,200 ng/mL. All patients received intravenous methylprednisolone, 1 gm during surgery and 200 mg on day 1, declining to 40 mg/day at day 5 when oral prednisolone was initiated (20 mg/day). Steroids were progressively tapered thereafter according to the clinical course of each individual patient and discontinued by protocol at 1 year, except for patients grafted for autoimmune liver diseases. Mycophenolate mofetil (MMF) was added to the dual immunosuppressive regimen in patients in whom full doses of CsA were not tolerated due to renal dysfunction or other complications. Induction therapy with basiliximab (20 mg administered during surgery and on postoperative day 4) was used in patients transplanted with hepatorenal syndrome.

CsA levels were obtained at C_2, blood concentration at 4 hours postdose (C_4), blood concentration at 6 hours postdose (C_6), and C_0 on the 1st day on which the dose of CsA-ME was ≥10 mg/kg/day. Follow-up measurements of C_4 and C_6 were obtained in patients found to be delayed absorbers of CsA (see definition of delayed absorption below). CsA concentration was measured in whole blood using radioimmunoassay (CYCLO-TRAC-SP; Diasorin, Stillwater, MN). To derive the full 12-hour AUC, it was assumed that the 12-hour concentration was identical to the measured C_0 concentration. Standard noncompartmental pharmacokinetic parameters were derived in WinNonlin (version 4.0; Pharsight, Mountain View, CA). AUC metrics were determined by trapezoidal summation.

"Normal" absorption of CsA was defined as C_2 value exceeding C_4 and C_6; "delayed" absorption was defined as C_4 or C_6 exceeding C_2; "good" absorption was defined as a level greater than the target of 800 ng/mL at C_0, C_2, C_4, or C_6; "poor" absorption was defined as a level below 800 ng/mL at all time points (C_0, C_2, C_4, or C_6) on the day of the study. Accordingly, patients were categorized as "normal-good," "normal-poor," "delayed-good," or "delayed-poor" absorbers of CsA.

Graft function was assessed by total bilirubin, aspartate aminotransferase, and prothrombin time on the day of the pharmacokinetic study

Maintenance Patients

In maintenance patients, measurements of C_2, C_4, and C_6 were recorded in the outpatient clinic; measurement of C_0 was considered too impractical for patients. The C_2 target in this group of patients was 600 ng/mL. A total of 6 patients received MMF due to adverse effects of CsA, mostly nephrotoxicity.

Statistical Analysis

The significance between mean values was assessed by 2-sided *t*-test. Chi square and Fisher's exact tests were used to compare categorical variables, and linear regression analysis was used to calculate the correlation between C_2 levels and CsA exposure.

Results

CsA Absorption in *De Novo* Patients

A total of 27 *de novo* patients were included in the study. Patient demographics, donor type, indication for liver transplantation, and type of biliary anastomosis are shown in Table 1.

Table 1. Patient Characteristics

	De novo patients (n = 27)	Maintenance patients (n = 15)
Median age (years) [range]	46 [20–68]	58 [35–66]
Male gender	12 (44%)	7 (47%)
Indication for transplant		
Cirrhosis	23 (85%)*	13 (87%)†
Fulminant hepatic failure	4 (15%)	2 (13%)
Donor type		
Deceased	16 (59%)	11 (73%)
Living (right lobe)	11 (41%)	4 (27%)
Type of biliary anastomosis		
Duct-to-duct	21 (78%)	13 (87%)
Hepaticojejunostomy	6 (22%)	2 (13%)

*Comprising hepatitis C (7), autoimmune hepatitis (6), primary biliary cirrhosis (3), hepatitis B (2), alcoholic (1), other (4); 7 / 23 patients with cirrhosis had hepatocellular carcinoma.
†Comprising hepatitis C (3), autoimmune hepatitis (2), primary biliary cirrhosis (2), hepatitis B (2), alcoholic (2), other (2).

A total of 16 patients (59%) received dual therapy with CsA and steroids, 9 patients (33%) triple therapy (MMF 8, basiliximab 1), and 2 patients received quadruple therapy. CsA-ME was initiated on day 1 in 19 patients (70%), on day 2 in 6 patients (22%), on day 3 in 1 patient, and on day 5 in 1 patient. The 2 patients in whom CsA-ME was initiated on days 3 and 5, respectively, also received basiliximab.

The starting dose of CsA-ME was 5.8 ± 1.6 mg/kg/day. Across all patients, the median time to CsA-ME dose ≥10 mg/kg/day was 5 days after transplantation (range 3-12 days), at which time C_2, C_4, C_6, and C_0 were recorded. Among patients with dual therapy, median time to CsA-ME dose was 4 days (range 3-6 days), whereas for those receiving basiliximab and / or MMF, the median time was 7 days (4-12 days). The most frequent reason for delayed time to reach a dose ≥10 mg/day was renal impairment in the early postoperative period. Mean CsA-ME dose on the day of the study, at the time of measuring drug levels, was 11.1 ± 1.1 mg/kg/day. Incomplete data were available for 7 patients (C_6 was not recorded in 4 and C_0 was not recorded in 5).

A total of 15 patients (56%) had normal absorption and 12 patients (44%) had delayed absorption of CsA.

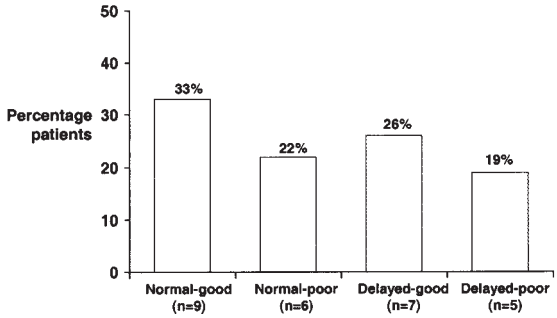

Figure 1. Incidence of normal / delayed and good / poor absorption of CsA among *de novo* liver transplant recipients (n = 27).

Among delayed absorbers, 11 (92%) had C_4 values higher than C_6 and only 1 had a C_6 value greater than C_4. Good absorption of CsA occurred in 16 patients (59%) and poor absorption in 11 (41%). Combinations of normal / delayed and good / poor absorption are shown in Figure 1. The proportion of poor absorbers was not significantly different ($P = 1.0$) in patients with normal (6 / 15; 40%) or delayed (5 / 12; 42%) pattern of CsA absorption. Mean CsA concentration at each time point on the day of the study is shown in Figure 2. As would be expected, there was no marked peak in the poor absorbers, regardless of whether they showed delayed or normal absorption of CsA. For delayed absorbers with either good or poor absorption, the highest CsA concentration was seen at C_4.

Mean C_0 level was significantly higher in delayed absorbers than in normal absorbers (Table 2). C_0 values were higher in delayed-good absorbers than normal-good absorbers, although the difference was nonsignificant (mean C_0 302 ± 85 vs. 224 ± 86 ng/mL, respectively; $P = .07$); the difference was significant between delayed-poor and normal-poor absorbers (275 ± 115

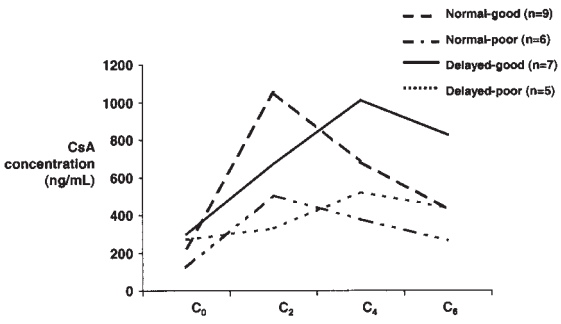

Figure 2. Mean CsA concentrations according to pattern of absorption.

Table 2. Recipient, Donor, Transplant, and Immunosuppression Variables According to Type of CsA Absorption in *De Novo* Liver Transplant Recipients

	Normal (n = 15)	Delayed (n = 12)	*P* value
Mean age (years)	39 ± 14	48 ± 14	.2
Gender			
Males (n = 12)	8 (53%)	4 (33%)	.51
Females (n = 15)	7 (47%)	8 (67%)	
Indication for transplant			
Cirrhosis (n = 23)	11 (48%)	12 (52%)	.87
Fulminant hepatic failure (n = 4)	4 (100%)	0	
Donor type			
Deceased (n = 16)	10 (63%)	6 (37%)	.45*
Living (n = 11)	5 (45%)	6 (55%)	
Type of biliary anastomosis			
Duct-to-duct (n = 21)	11 (52%)	10 (48%)	.24
Roux-en-Y (n = 6)	4 (67%)	2 (33%)	
Graft function			
Mean bilirubin (mg%)	12 ± 9.2	12.5 ± 8.5	.45
Mean AST (IU)	89 ± 49	127 ± 99	.2
Mean prothrombin (%)	68 ± 17	61 ± 20	.36
Mean CsA dose (mg/kg/day)	11.0 ± 1.0	10.7 ± 1.1	.3
Mean CsA levels (ng/mL)			
C_2	834 ± 357	531 ± 277	.02
C_{max}	834 ± 357	835 ± 296	.98
C_0	185 ± 88	282 ± 96	.01
MMF			
Yes (n = 10)	5 (50%)	5 (50%)	.7
No (n = 17)	10 (59%)	7 (41%)	
Acute cellular rejection (n = 9)	4 (27%)	5 (42%)	.68

*Fisher's exact test.

vs. 123 ± 51 ng/mL, respectively; $P = .02$). Normal absorbers of CsA had a higher mean C_2 level and a lower mean C_0 level than delayed absorbers, but almost identical mean peak concentration (Table 2). In good absorbers, mean C_2 level was approximately twice as high as in poor absorbers (Table 3).

CsA levels at 0, 2, 4, and 6 hours postdose were available for 20 patients, of whom 12 were normal absorbers and 8 delayed absorbers; the CsA levels were used to calculate AUC values. Mean AUC_{0-4} was similar in both the normal and delayed-absorption groups (2,278 ± 1,501 vs. 2,377 ± 976 ng/hour/mL; $P = .83$), but mean AUC_{0-12} was significantly higher in delayed absorbers (4,700 ± 2,139 vs. 7,045 ± 2,292 ng/hour/mL; $P = .03$). The differences in AUC_{0-12} remained significant between the delayed-good (8,009 ± 1,688 ng/hour/mL) and normal-good subgroups (5,954 ± 1,501 ng/hour/mL; $P = .04$). In both the normal and delayed absorbers, C_2 was a good predictor of AUC_{0-4} (normal absorbers $r^2 = .97$; delayed absorbers $r^2 = .98$).

Tables 2 and 3 summarize characteristics of patients with normal or delayed absorption, and good or poor absorption. Age, gender, donor type, indication for liver transplantation, type of biliary anastomosis, and graft function did not differ significantly between patients with normal or delayed CsA absorption (Table 2), or between those with good or poor absorption (Table 3). Comparison of good and poor absorbers showed significant differences in the proportion of patients receiving MMF, and in serum bilirubin levels (Table 3). The overall incidence of ACR was 33% (9 / 27) and did not differ significantly between study groups (Tables 2 and 3). Follow-up C_4 and C_6 levels were obtained in 7 of the 12 delayed absorbers. Reversion to a normal pattern of absorption ($C_2 > C_4$) occurred in all patients after a median of 6 days after the day on which the study was conducted (range 2-12 days). Representative cases are shown in Figure 3; while the course of both C_2 and C_4 values was erratic, all patients had peak CsA concentration at the C_2 time point within the 1st 3 weeks posttransplant.

Table 3. Recipient, Donor, Transplant, and Immunosuppression Variables According to Extent of CsA Absorption in *De Novo* Liver Transplant Recipients

	Good (n = 16)	Poor (n = 11)	*P* value
Mean age (years)	43 ± 16	48 ± 11	.57
Gender			
Males (n = 12)	7 (44%)	5 (45%)	1.0
Females (n = 15)	9 (56%)	6 (56%)	
Indication for transplant			
Cirrhosis (n = 23)	15 (65%)	8 (35%)	.33
Fulminant hepatic failure (n = 4)	1 (25%)	3 (75%)	
Donor type			
Deceased (n = 16)	10 (62.5%)	6 (38%)	.71*
Living (n = 11)	6 (55%)	5 (45%)	
Type of biliary anastomosis			
Duct-to-duct (n = 21)	11 (52%)	10 (48%)	.14
Roux-en-Y (n = 6)	5 (83%)	1 (17%)	
Graft function			
Mean bilirubin (mg%)	8.6 ± 6.2	16.4 ± 5.3	.02
Mean AST (IU)	121 ± 94	83 ± 29	.2
Mean prothrombin (%)	65 ± 20	64 ± 17	.94
Mean CsA dose (mg/kg/day)	10.8 ± 0.9	10.9 ± 1.2	.4
Mean CsA levels (ng/mL)			
C_2	887 ± 295	427 ± 225	.0001
C_0	254 ± 91	182 ± 106	.1
MMF			
Yes (n = 10)	3 (30%)	7 (70%)	.04*
No (n = 17)	13 (76%)	4 (24%)	
Acute cellular rejection (n = 9)	6 (37.5%)	3 (27%)	.88

*Fisher's exact test.

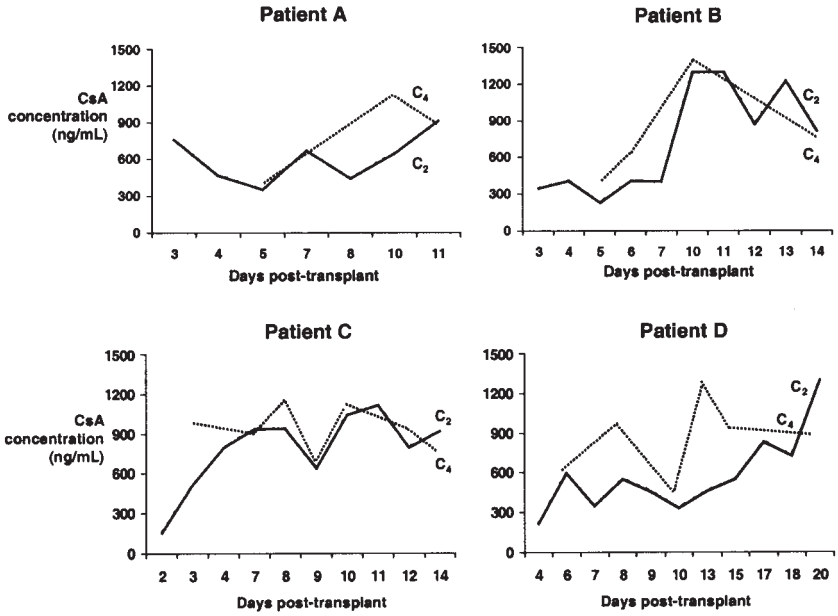

Figure 3. CsA C_2 and C_4 values in 4 individual patients showing reversion from delayed to normal absorption of CsA over time posttransplant.

CsA Absorption in Maintenance Patients

A total of 15 maintenance patients were included in the study; characteristics are shown in Table 1. The median time since liver transplantation was 32 months (range 8-96 months). At the time that CsA concentrations were recorded, the mean dose of CsA-ME was 2.5 ± 1.4 mg/kg/day. A total of 7 patients (47%) were receiving steroids and 6 (40%) were receiving MMF (mean dose 1.9 ± .5 gm/day)

All maintenance patients showed a normal pattern of CsA absorption, i.e., there were no delayed absorbers. Mean C_2 value was 621 ± 314 ng/mL, mean C_4 value was 281 ± 220 ng/mL, and mean C_6 value was 120 ± 50 ng/mL. A total of 6 patients (40%) had a C_2 value above the target of 600 ng/mL and 11 (73%) above 500 ng/mL.

Discussion

Differentiating between delayed and poor absorbers of CsA is important to avoid inadvertent overimmunosuppression, but the literature contains virtually no data on the proportion of patients who display these characteristics or the time course of delayed CsA absorption. This is the 1st study to investigate the incidence and duration of delayed CsA absorption in liver transplant recipients.

Delayed absorption of CsA was frequent in our *de novo* population (12 / 27; 44%), but reverted to a normal pattern of absorption within a few days in the majority of patients. Over 50% of the delayed absorbers achieved a peak concentration within the target range of 800–1,200 ng/mL despite having a C_2 value below target. Moreover, early CsA exposure (AUC_{0-4}), which has been shown to be highly predictive of rejection[1,13] and risk of nephrotoxicity,[13] was similar in normal and delayed absorbers (both approximately 2,300 ng/hour/mL). However, total exposure (AUC_{0-12}) was almost twice as high in delayed absorbers as in normal absorbers, and markedly higher in delayed-good absorbers vs. normal-good absorbers, differences that were statistically significant. This increase in exposure was possibly partly due to the fact that the sparse-sampling technique used may tend to overestimate the latter part of the absorption profile and thereby inflate the AUC_{0-12} estimate, but this is unlikely to account for more than a small proportion of the difference. It is clearly important to measure a subsequent concentration point (C_4 or C_6) in patients with a low C_2 value in order to avoid the risk of increasing the CsA-ME dose inappropriately in patients with delayed absorption, thus risking overimmunosuppression. In the current study, CsA dose was based on peak CsA concentration (C_4) in patients with low C_2 values, and there was no evidence of CsA-related toxicity. Combined use of both C_2 and C_4 or C_6 during the 1st few days posttransplant could be expected to effectively identify delayed absorbers. Based on our results, C_4 would appear to be the best time point to detect a late peak concentration in patients with delayed CsA absorption, since 11 out of 12 delayed absorbers showed a peak at C_4. Our findings also suggest a possible role for C_0 as an indicator of delayed absorption, since mean C_0 was significantly higher in delayed absorbers vs. normal absorbers regardless of the extent of absorption. It would appear that patients with a C_2 level below target who have a C_0 close to target (in this study, 300 ng/mL) are likely to be delayed absorbers, indicating the need for measurement of a later CsA concentration (e.g., C_4) to confirm the presence or absence of delayed absorption.

There were no significant indicators to differentiate delayed from normal absorbers in *de novo* patients other than higher C_0 values. Variables were similar between the good or poor absorbers, other than 2-fold higher bilirubin levels in the poor absorbers. An unexpected finding was the significantly higher incidence of poor CsA absorption among patients receiving MMF. However, since the study was not designed to evaluate CsA absorption with or without MMF, this finding should be interpreted with caution; for example, it may have resulted from selection of patients for MMF therapy. While there are reports in the literature of CsA affecting MMF absorption,[14,15] to our knowledge no data showing a converse effect have been published.

Delayed absorption of CsA-ME was a transient phenomenon. All patients with delayed absorption reverted to a normal pattern of absorption within the 1st 3 weeks posttransplant. There were no delayed absorbers within the maintenance group of patients.

In conclusion, C_2 monitoring of CsA-ME offers a sensitive technique to optimize CsA exposure following liver transplantation. However, approximately 25% of patients exhibit delayed but adequate absorption of CsA, and identifying these patients is important to minimize the risk of overimmunosuppression and toxicity. A relatively high C_0 value with a low C_2 value may help to indicate the presence of delayed absorption, but our results suggest that measurement of C_4 is the best single time point to use in addition to C_2 in order to differentiate effectively between delayed-good absorbers and normal-poor absorbers.

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