

Does Tacrolimus Offer Virtual Freedom From Chronic Rejection After Primary Liver Transplantation? Risk and Prognostic Factors in 1,048 Liver Transplantations With a Mean Follow-Up of 6 Years

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Tacrolimus has proven to be a potent immunosuppressive agent in liver transplantation (LT). Its introduction has led to significantly less frequent and severe acute rejection. Little is known about the rate of chronic rejection (CR) in primary LT using tacrolimus therapy. The aim of the present study is to examine the long-term incidence of CR, risk factors, prognostic factors, and outcome after CR. The present study evaluated the development of CR in 1,048 consecutive adult primary liver allograft recipients initiated and mostly maintained on tacrolimus-based immunosuppressive therapy. They were evaluated with a mean follow-up of 77.3 ± 14.7 months (range, 50.7 to 100.1 months). To assess the impact of primary diagnosis on the rate and outcome of CR, the population was divided into 3 groups. Group I included patients with hepatitis C virus (HCV)- or hepatitis B virus (HBV)-induced cirrhosis ($n = 312$); group II included patients diagnosed with primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), or autoimmune hepatitis (AIH; $n = 217$); and group III included patients with all other diagnoses ($n = 519$). Overall, 32 of 1,048 patients (3.1%) developed CR. This represented 13 (4.1%), 12 (5.5%), and 7 patients (1.3%) in groups I, II, and III, respectively. The relative risk for developing CR was 3.2 times greater for group I and 4.3 times greater for group II compared with group III. This difference was statistically significant ($P = .004$). The incidence of acute rejection and total number of acute rejection episodes were significantly greater in patients who developed CR compared with those who did not ($P < .0001$). Similarly, the mean donor age for CR was significantly older than for patients without CR (43.0 v 36.2 years; $P = .02$). Thirteen of the 32 patients (40.6%) who developed CR retained their original grafts for a mean period of 54 ± 25 months after diagnosis. Seven patients (21.9%) underwent re-LT, and 12 patients (38.3%) died. Serum bilirubin levels and the presence of arteriopathy, arterial loss, and duct loss on liver biopsy at the time of diagnosis of CR were significantly greater among the 3 groups of patients. In addition, patient and graft survival for group I were significantly worse compared with groups II and III. We conclude that CR occurred rarely among patients maintained long term on tacrolimus-based immunosuppressive therapy. When steroid use is controlled, the incidence of acute rejection, mean donor age, HBV- and/or HCV-induced cirrhosis, or a diagnosis of PBC, PSC, or AIH were found to be predictors of CR. Greater values for serum bilirubin level, duct loss, arteriopathy, arteriolar loss, and presence of HCV or HBV were found to be poor prognostic factors for the 3 groups; greater total serum bilirubin value ($P = .05$) was

the only factor found to be significant between patients who had graft loss versus those who recovered. (*Liver Transpl* 2001;7:623-630.)

The results of liver transplantation (LT) have improved significantly over the past 2 decades with improvements in surgical techniques and postoperative management and the introduction of cyclosporine (CsA).^{1,2} However, chronic rejection (CR) occurs in 2% to 20% of successful liver transplant recipients treated with CsA, and re-LT may be necessary.¹⁻⁶ Even after re-LT, the recurrence rate of CR is as high as 90%.⁷ The introduction of tacrolimus into primary LT has resulted in a significant reduction in the rate of acute rejection.⁸⁻¹² Additional studies report that up to 70% of cases of CR occurring with CsA-based therapy may be halted or reversed after the initiation of tacrolimus therapy.¹³⁻¹⁷ Although there are reports suggesting a reduction in the rate of CR among tacrolimus-treated patients compared with those maintained on CsA therapy,^{8,18} relatively little is known about the rate of CR with long-term tacrolimus therapy. The aim of the

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present study is to examine the incidence and risk factors for the development of CR among patients on long-term tacrolimus therapy and study the clinical outcome with prognostic factors.

Methods

Patients

Between April 1990 and June 1994, a total of 1,048 adults (aged > 18 years) underwent primary LT on tacrolimus-based immunosuppressive therapy. The tacrolimus treatment protocol has been described in detail elsewhere.^{10,19-23} Briefly, patients who underwent LT up to August 1991 were administered intravenous tacrolimus at a dose of 0.1 mg/kg/d; patients who underwent LT after that date were administered a dose of 0.05 mg/kg/d. Oral tacrolimus therapy was commenced when bowel function returned, usually after 2 to 5 days. The first 118 patients (11.2%) were administered only baseline steroid therapy at 20 mg/d, the next 718 patients (68.5%) were administered intravenously 1 g methylprednisone, and the remaining 212 patients (20.2%) were administered 1 g of intravenous methylprednisolone at the time of LT and an additional total dose of 600 mg of methylprednisolone, tapered over the next 5 days.

All patients were followed up until death or July 1998. Mean follow-up was 77.3 ± 14.7 months (range, 50.7 to 100.1 months). Indications for LT are listed in Table 1. Donor characteristics, transplant recipient hepatitis serological test results, and clinical courses (including changes in immunosuppression and survival) were evaluated retrospectively. The baseline steroid dose was reduced according to history of rejection, and in 70% of the patients, steroid therapy was discontinued at the end of the first year. All patients were maintained on tacrolimus therapy. Less than 5% of patients were converted to CsA therapy for neurotoxicity, and approximately 80% were converted back to tacrolimus therapy in the event of subsequent mild acute cellular rejection. Liver biopsies were performed for an increase in biochemical parameters indicative of hepatic dysfunction. Protocol liver biopsies were not performed. Patients who underwent LT for hepatitis B virus (HBV) infection were administered hyperimmune HBV globulin prophylaxis for 6 months, whereas patients with hepatitis C virus (HCV) were not administered prophylaxis.

Pathological Evaluation

During the follow-up period, 5,252 post-LT biopsy specimens were available for evaluation. Pathologists who had no knowledge of the patients' clinical courses carefully coded all specimens for CR using the International Banff criteria with the National Institute of Diabetes and Digestive and Kidney Diseases guidelines.²⁴⁻²⁶ Details of histopathologic findings in most of these patients are presented elsewhere.¹⁸

Table 1. Indications for LTs

Diagnosis	No. of Patients (%)
Group I	
HCV	221 (21)
HBV	91 (8.6)
Group I total	312 (29.8)
Group II	
PBC	98 (9.3)
PSC	81 (7.7)
AIH	38 (3.6)
Group II total	217 (20.9)
Group III	
Alcohol abuse	174 (16)
Cryptogenic cirrhosis	164 (15.6)
Hepatic neoplasm	
benign/malignant	51 (4.8)
Hemochromatosis	20 (1.9)
α_1 -Antitrypsin deficiency	18 (1.7)
Acute hepatitis	16 (1.5)
Secondary biliary cirrhosis	12 (1.1)
Budd-Chiari	7 (0.7)
Other	46 (4.4)
Unknown	11 (1.0)
Group III total	519 (49.5)
Total	1,048

Statistical Analysis

Statistical analysis included basic descriptive statistics, Chi-squared and nonparametric tests, and modeling techniques, including logistic and Cox proportional hazard regression. Models were derived using stepwise regression, with a significance of .05 for a feature to remain in the model. Time-dependant covariates were used when appropriate. Analyses were performed using Statistical Analysis System for Windows, version 8.0 (SAS Institute, Cary, NC).

Results

Incidence of CR

Biopsy specimens from 32 patients (21 men, 11 women; 3.1% of the study population) showed evidence of CR. The mean time from LT to the first histological sign of CR was 15.6 ± 19.2 months (median, 6.75 months; range, 0.6 to 83.22 months).

Clinical Outcome

Thirteen patients recovered without re-LT with relatively stable allograft function at a mean follow-up of 54 ± 25 months (median, 74 months; range, 11.3 to 93.3 months) after the diagnosis of CR. Mean bilirubin

bin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, and γ -glutamyltransferase (GGT) values at intervals after the diagnosis of CR are listed in Table 2. CR was managed by augmenting baseline immunosuppression; changes are listed in Table 2. The dose of tacrolimus or corticosteroids was increased in 8 of 13 and 6 of 13 patients, respectively. Three patients were administered mycophenolate mofetil, and the dose of azathioprine was doubled in 1 patient. Seven patients underwent re-LT at a mean interval of 2.2 ± 2.7 months (median, 0.8 months; range, 0.6 to 4.0 months) after the diagnosis of CR. Twelve patients not considered for re-LT died. Causes of death included sepsis ($n = 3$), Kaposi's sarcoma ($n = 2$), fungal infection ($n = 2$), infection with cytomegalovirus (CMV; $n = 1$), HIV ($n = 1$), replicating HBV ($n = 1$), recurrent hepatitis C with renal failure ($n = 1$), and a history of noncompliance ($n = 1$). The mean time to death was 17.04 ± 23.0 months (median, 4.8 months; range, 0.46 to 77.1 months) after the diagnosis of CR.

Risk Factors for the Development of CR

Patients who developed CR were compared with those who did not with respect to primary diagnosis, occurrence of acute rejection, HLA mismatching, CMV infection, and donor age. Changes in baseline immunosuppression were also evaluated among patients who developed CR.

Primary diagnosis. To examine the impact of primary diagnosis, the study population was divided into 3 groups. Group I consisted of patients infected with either HCV or HBV. Patients in group II had been diagnosed with primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), or autoimmune hepatitis (AIH), and group III included patients with other diagnoses at the time of LT (Table 1). The incidence of CR was significantly greater among patients in group I ($n = 13$; 4.2%) or group II ($n = 12$; 5.5%) compared with those in group III ($n = 7$; 1.3%; $P = .004$). Relative risks were 3.2 times greater (confidence interval, 1.2 to 9.5) for group I and 4.3 times greater (confidence interval, 1.5 to 13) for group II compared with group III (Table 3).

Acute rejection. There was a significant difference in freedom from acute rejection among patients who did ($n = 32$) or did not ($n = 1,016$) develop CR (12.5% v 50.0%, respectively; $P = .0001$; Table 4. Also, greater than 3 episodes of rejection was documented in 25% of patients with CR compared with 4.2% in the non-CR group ($P = .001$). The 7 patients who required re-LT

Table 2. Graft Recovery Without Re-LT, Liver Function, and Immunosuppression

	Before CR												3 Months Post-CR												1 Year Post-CR												2 Years Post-CR												3 Years Post-CR											
	Bili (mg/dL)				AST (U/L)				ALT (U/L)				ALKP (U/L)				GGT (mg/dL)				Bili (mg/dL)				AST (U/L)				ALT (U/L)				ALKP (U/L)				GGT (mg/dL)				Bili (mg/dL)				AST (U/L)				ALT (U/L)				ALKP (U/L)				GGT (mg/dL)			
	Mean	SD	Median	Dose (mg/d)	Mean	SD	Median	Level (mg/mL)	Pred (mg/d)	Aza (mg/d)	MMP (mg/d)	Dose (mg/d)	Mean	SD	Median	Level (mg/mL)	Pred (mg/d)	Aza (mg/d)	MMP (mg/d)	Dose (mg/d)	Mean	SD	Median	Level (mg/mL)	Pred (mg/d)	Aza (mg/d)	MMP (mg/d)	Dose (mg/d)	Mean	SD	Median	Level (mg/mL)	Pred (mg/d)	Aza (mg/d)	MMP (mg/d)	Dose (mg/d)	Mean	SD	Median	Level (mg/mL)	Pred (mg/d)	Aza (mg/d)	MMP (mg/d)	Dose (mg/d)																
Baseline maintenance immunosuppression	6.7	5.4	7.7	50.0	0.0	8.5	4.9	8.0	100.0	1000	8.5	5.1	5.5	100.0	1000	1000	0	6.3	5.5	4.7	100.0	1333	6.1	4.4	5.8	100.0	1333	6.7	5.4	7.7	50.0	0.0	4.5	4.7	8.0	100.0	1000	4.8	5.4	5.1	5.5	100.0	1000	0	6.3	5.5	4.7	100.0	1333	6.1	4.4	5.8	100.0	1333						
Mean	4.2	6.5	6.7	0.0	0.0	6.0	4.7	4.7	100.0	1000	8.0	5.1	5.0	100.0	1000	1000	0	5.0	6.1	4.7	100.0	1000	6.0	5.4	5.6	100.0	1000	6.0	6.5	6.7	0.0	0.0	6.0	4.7	4.7	100.0	1000	4.8	5.4	5.1	5.5	100.0	1000	0	6.3	5.5	4.7	100.0	1333	6.1	4.4	5.8	100.0	1333						
SD	6.0	1.4	10.0	50.0	0.0	6.0	1.6	7.5	100.0	1000	8.0	1.2	5.0	100.0	1000	1000	0	5.0	6.1	4.7	100.0	1000	6.0	5.4	5.6	100.0	1000	6.0	6.5	6.7	0.0	0.0	6.0	4.7	4.7	100.0	1000	4.8	5.4	5.1	5.5	100.0	1000	0	6.3	5.5	4.7	100.0	1333	6.1	4.4	5.8	100.0	1333						
Median	6.0	1.4	10.0	50.0	0.0	6.0	1.6	7.5	100.0	1000	8.0	1.2	5.0	100.0	1000	1000	0	5.0	6.1	4.7	100.0	1000	6.0	5.4	5.6	100.0	1000	6.0	6.5	6.7	0.0	0.0	6.0	4.7	4.7	100.0	1000	4.8	5.4	5.1	5.5	100.0	1000	0	6.3	5.5	4.7	100.0	1333	6.1	4.4	5.8	100.0	1333						

NOTE: N = 13 for graft recovery without re-LT; N = 1 for Aza therapy; N = 3 for mycophenolate mofetil therapy. Abbreviations: Bili, total bilirubin; ALKP, alkaline phosphatase; GGT, γ -glutamyl transferase; FK, tacrolimus; Pred, prednisone; Aza, azathioprine; MMP, mycophenolate mofetil.

Table 3. Rate of CR in Relation to Cause and Clinical Outcome

Presence of Disease	Rate of CR*	P	Relative Risk	95% Confidence Interval	Re-LT	Died Without Re-LT	Recovered Without Re-LT	Patient Survival Post-CR at 5 Years	Graft Survival Post-CR at 5 Years
Group I (n = 312) HCV + HBV	13 (4.2)	.004	3.17	1.2-9.5	4	6	3	30.7	23
Group II (n = 217) PBC + PSC + AIH	12 (5.5)	.004	4.28	1.5-13	3	3	6	72.7	54.5
Group III (n = 519) Other than HCV, HBV, PBC, PSC, AIH	7 (1.3)	.004	Reference group		0	3	4	85.6	85.6
Total (n = 1,048)	32 (3.1)				7	12	13		

* Values expressed as number (percent) unless otherwise noted.

had experienced at least 1 episode of acute rejection; 2 patients had experienced 3 or more episodes.

Donor age. There was a significant difference in mean donor age among patients who did or did not develop CR (43 ± 14.7 v 36.2 ± 15.9 years; $P = .02$). Mean donor ages among patients who recovered, underwent re-LT, or died after the diagnosis of CR were 38.7 ± 14.7 , 54.0 ± 14.2 , and 41.0 ± 12.5 years, respectively.

Steroid induction dose. A steroid induction dose of either 20 mg, 1,000 mg, or 1,000 mg plus a 600-mg taper made no difference in the rate of CR ($P = .07$).

HLA matching and/or mismatching. The mean number of HLA antigen mismatches at class I (2.93 v 2.97) or matching on class II loci (0.33 v 0.31) was not significantly different between the respective groups ($P = .79$ and $P = .89$, respectively).

CMV hepatitis. A diagnosis of CMV hepatitis was made on liver biopsy by the presence of cytomegaloviral

inclusions by immunoperoxidase staining. The overall rate of CMV hepatitis was 4.3% (46 patients). However, in patients who developed CR, the rate was 12.5% (4 of 32 patients) versus 4.1% (42 of 1,016 patients) in patients who did not develop CR. This was found to be significant ($P = .02$) on univariate analysis.

Using multivariable analysis and controlling for steroid induction, the number of acute rejection episodes ($P = .0001$), donor age ($P = .005$), HCV- and/or HBV-induced cirrhosis ($P = .03$), and diagnosis of PBC, PSC, or AIH ($P = .008$) were found to be predictors of CR.

Maintenance of immunosuppression. Reduction or discontinuation of baseline maintenance immunosuppression occurred in 14 of 32 patients (43.8%), who subsequently developed CR. Four patients were noncompliant with the immunosuppressive regimen. Immunosuppression was reduced or discontinued in the remaining 10 patients because of malignancy ($n = 4$),

Table 4. Episodes of Acute Rejection in Relation to Rate of CR and Clinical Outcome

	No CR (n = 1,016)	CR (n = 32)	Clinical Outcome Post-CR		
			Re-LT (n = 7)	Died Without Re-LT (n = 12)	Recovered Without Re-LT (n = 13)
Frequency of acute rejection					
0	508 (50)	4 (12.5)	0	2 (16.7)	2 (15.4)
1	373 (36.7)	12 (37.5)	4 (57.2)	4 (33.3)	4 (30.8)
2	93 (9.2)	8 (25)	1 (14.3)	4 (33.3)	3 (23.1)
≥3	42 (4.2)	8 (25.0)	2 (28.6)	2 (16.7)	4 (30.8)
Mean rejection per patient	0.69 ± 0.86	1.8 ± 1.3	2.1 ± 1.9	1.5 ± 1.0	1.7 ± 1.1
P		<.0001		.72	

NOTE. Values expressed as number (percent).

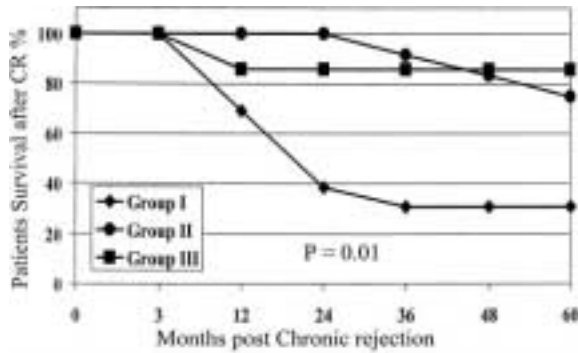


Figure 1. Patient survival for groups I, II, and III after CR.

infection ($n = 5$), or neurotoxicity ($n = 1$). Six of these 14 patients are currently alive with a functioning graft, 3 patients underwent re-LT, and 5 patients died. All 7 patients in group III who experienced reduction or discontinuation of immunosuppression developed CR. Conversely, 3 of the 13 patients in group I (23%) and 4 of the 12 patients in group II (33%) developed CR ($P = .003$).

Prognostic Factors

Patient and graft survival were significantly better among patients in groups II and III compared with those in group I ($P = .01$; Figs. 1 and 2). Biochemical profiles and histopathologic findings were evaluated at the time of diagnosis of CR to predict risk factors for graft loss, defined as re-LT or death.

Liver function profile. Mean serum bilirubin level was significantly greater among patients with CR who required re-LT or died compared with those who retained the graft ($P = .05$). Mean differences in AST, ALT, alkaline phosphatase, and GGT levels among the groups did not achieve statistical significance.

Histopathologic evaluation. Detailed histopathologic findings in most of these patients have been previously reported.¹⁸ Duct damage and duct atrophy were seen in the majority of patients who developed CR. The rate of duct loss was significantly greater for patients who required re-LT ($P = .011$).

Arteriopathy was noted in biopsy samples retrieved from only 3 of 32 patients with CR, all of whom required re-LT. Conversely, arteriopathy was not evident among 25 patients who either recovered or died after the development of CR. Arteriolar loss occurred in 5 of 32 patients; 2 of these patients recovered and the remaining 3 patients received a second liver allograft. Data suggest that arteriopathy with duct loss was associated with a poorer outcome than duct damage or duct

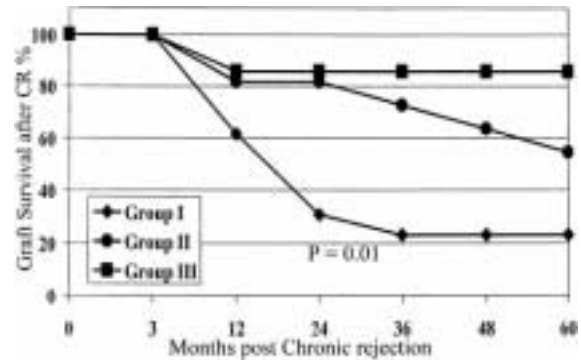


Figure 2. Graft survival for groups I, II, and III after CR.

atrophy alone. Arteriopathy and duct loss were noted in 12 patients, and arteriopathy alone was noted in 3 patients.

Discussion

The reported incidence of CR is between 2.4% and 16.8% among recipients of liver allografts maintained on CsA-based immunosuppressive therapy.^{3-5,26,27} The use of azathioprine is associated with a significantly lower incidence of CR.⁷ The results from our center and multicenter trials have suggested that CR can be successfully treated by conversion to tacrolimus-based immunosuppression.¹³⁻¹⁷

In our present series, CR occurred in 3.1% of the total patient population (32 of 1,048 patients). This condition improved significantly in 40.6% of patients (13 of 32 patients), mostly through optimization of the immunosuppressive regimen. Conversely, 19 patients (59.4%) experienced graft loss. Seven patients (21.9%) received a second allograft, and 12 patients (37.5%) died. A primary diagnosis of PBC, PSC, or AIH; previous history of acute rejection episodes and/or CMV hepatitis; and older donor age were among the identified risk factors for the development of CR. Patients infected with HBV or HCV were also at greater risk. Lower serum bilirubin levels ($P = .05$) at the time of diagnosis and absence of HBV or HCV infection were among the factors favorable to a recovery from CR.

Associations between primary diagnosis and the incidence of CR appear in the literature.²⁸ An increased incidence of CR among patients infected with HBV, HCV, or CMV has been reported previously by our center²⁹⁻³¹ and others.³²⁻³⁵

The incidence of CR also has been reported to be greater among patients with PBC, PSC, or AIH. In 1988, we reported a greater incidence of CR among patients with PBC maintained on a CsA-based immu-

nosuppressive regimen.³⁶ Candinas et al³⁷ and others³⁸ reported that AIH and PBC are risk factors for the development of CR. In another blinded study, Hubscher et al³⁹ reported histopathologic features of recurrence of PBC in 16% of patients, including duct damage, ductopenia, and portal fibrosis. Some of these findings are at least suggestive of a diagnosis of liver allograft CR.

Similarly, histopathologic characteristics of PSC recurrence can be similar to CR.^{40,41} In the present series, we observed the development of CR in 3.7% of patients (3 of 81 patients) with a primary diagnosis of PSC.

The present data confirm the association between primary diagnosis and the development of CR in a larger patient population followed up long term. Of the 32 patients in our series who developed CR, 25 patients (78.1%) had a primary diagnosis of HCV, HBV, PBC, PSC, or AIH. Specifically, 4.2% of patients (13 of 312 patients) diagnosed with HBV or HCV developed CR, whereas 5.5% of patients (12 of 217 patients) with PBC, PSC, or AIH were diagnosed with CR. Relative risk for developing CR for patients with HBV and HCV infection was 3.2 times greater, and for patients with PBC, PSC, and AIH, 4.3 times greater compared with patients who did not have HBV, HCV, PBC, PSC, or AIH. In children, in whom the incidence of HBV, HCV, PSC, PBC, and AIH is lower than that in adults, graft loss from CR is also less frequent and occurs mostly when immunosuppression is discontinued to control posttransplant lymphoproliferative disorder or in noncompliant teenagers.^{12,42,43}

Rapid reduction or discontinuation of baseline immunosuppression for a compelling clinical condition or patient noncompliance imparts a significant risk for the development of CR.^{43,44} The 7 patients in group III had a reduction in baseline immunosuppression, which was found to be the single most important cause of CR in 44% of patients who developed this condition.

A direct link between acute rejection and CR was reported by Hyashi et al,⁴⁵ who observed that a greater incidence of acute cellular rejection could lead to the development of CR among patients with autoimmune liver disease. Similarly, Kemnitz et al^{46,47} reported that irreversible bile duct injury might occur in patients with recurrent AIH who experience acute rejection episodes. In a recent review, Wiesner et al²⁶ identified a previous history of acute rejection episodes as a risk factor for the development of CR.

Our data support a relationship between acute rejection and CR: 50% of patients with CR had experienced 2 or more episodes of acute rejection. This is signifi-

cantly greater than that observed in patients who did not develop CR. In addition, mean donor age was older among patients with CR, corroborating previous findings of overall poor outcome among recipients of older organ allografts.^{48,49} It is possible that older donors have preexisting arteriopathy in the allograft and the biliary epithelium is more susceptible to immunologic insult.⁵⁰

Several reports have found a relationship between HLA antigen matching and/or mismatching, positive lymphocytotoxic cross-matching, and the development of liver allograft CR.^{30,31,51-55} In this study, these factors did not predispose to the development of CR. In summary, the incidence of CR is low among patients maintained long term on tacrolimus-based therapy. For those patients who develop CR, a combination of risk factors appears to be involved, including a primary diagnosis of PBC, PSC, or AIH; history of acute rejection episodes; CMV hepatitis; and older donor age. Lower serum bilirubin levels, absence of HCV and/or HBV infection, and lack of arteriopathy or duct loss on liver allograft biopsy proved to be useful parameters to distinguish between patients who could potentially recover from CR and those likely to require re-LT.

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