Risk Factors for Recurrence of Primary Biliary Cholangitis After Liver Transplantation in Female Patients: A Japanese Multicenter Retrospective Study

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Primary biliary cholangitis (PBC) is diagnosed mainly in female individuals, and risk factors for PBC recurrence (rPBC) after liver transplantation (LT) from cadaveric donors have been reported. We conducted a retrospective multicenter study of rPBC in female patients after living-donor LT (LDLT). A total of 388 female patients undergoing LDLT for end-stage PBC were enrolled, and the effects of preoperative and operative factors were evaluated. Postoperative factors were evaluated in 312 patients who survived for more than 1 year post-LDLT. rPBC was defined as abnormal hepatic enzyme levels with typical histological findings in liver biopsies. Fifty-eight patients (14.9%) developed rPBC with a median of 4.6 (0.8-14.5) years post-LT. Cox hazard analysis ($P < 0.05$) showed that younger recipient age (hazard ratio, 0.95; 95% confidence interval, 0.920-0.982), shorter operative time (1.00; 0.995-0.999), higher serum immunoglobulin M level (1.00; 1.001-1.002), donor sex mismatch (2.45; 1.268-4.736), human leukocyte antigen B60 (2.56; 1.336-4.921) and DR8 (1.98; 1.134-3.448), and initial treatment with cyclosporine A (3.14; 1.602-6.138) were significantly associated with rPBC. The frequencies of Child-Turcotte-Pugh class C (0.46; 0.274-0.775), the model of end-stage liver disease score (0.96; 0.914-0.998), and updated Mayo risk score (1.02; 1.005-1.033) were significantly lower in rPBC. Posttransplantation use of steroids decreased and that of antimetabolites increased the frequency of rPBC. *Conclusion*: The timing of LT, recipient conditions, donor characteristics, and immunosuppressive medications may be associated with rPBC in LT recipients. (*Hepatology Communications* 2017;1:394-405)

Introduction

Primary biliary cholangitis or cirrhosis (PBC) is a chronic cholestatic liver disease with female individuals composing 90% of cases. The clinical outcome is good following treatment using ursodeoxycholic acid (UDCA).[1] The term primary biliary "cirrhosis" was recently changed to "cholangitis"[2]; however, when end-stage disease develops, patients with PBC require liver transplantation (LT).[3] Recurrent PBC (rPBC) after LT was first reported in 1982.[4] Of patients who receive LT, 9%-35% experience recurrence at a mean of 3 years to 5.5 years post-LT,[5-7] although a recent study reported a mean time

Abbreviations: AMA, antimitochondrial antibody; CI, confidence interval; CTP score, Child-Turcotte-Pugh score; CyA, cyclosporine A; DDLT, deceased-donor liver transplantation; HLA, human leukocyte antigen; HR, hazard ratio; IgM, immunoglobulin M; LDLT, living-donor liver transplantation; LT, liver transplantation; MELD, model of end-stage liver disease; MIZO, mizoribine; MMF, mycophenolate mofetil; PBC, primary biliary cholangitis; rPBC, primary biliary cholangitis recurrence; TAC, tacrolimus; UDCA, ursodeoxycholic acid.

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to recurrence of 1.6 years.[8] rPBC is characterized by granulomatous cholangitis or florid duct lesions in the liver,[6,9,10] but as these features are frequently absent, rPBC is difficult to distinguish from chronic rejection.[11]

The effects of recipient and donor factors on the risk of recurrence have been evaluated. Recipient age,[5] persistence of serum antimitochondrial antibody (AMA),[12,13] immunosuppression,[10,11] various human leukocyte antigen (HLA) types and HLA mismatches,[14-18] and donor sex mismatch[19,20] have been reported to increase the frequency of recurrence. Calcineurin inhibitors have been associated with the risk of rPBC[10,11] as has tacrolimus (TAC); however, cyclosporine A (CyA) protected against rPBC.[21] Moreover, TAC and CyA had no significant effects on the frequency of rPBC,[22] but we found in our previous study in Japanese patients[20] that CyA for initial immunosuppression was a significant risk factor for rPBC. Interestingly, a switch from TAC to CyA within 1 year significantly reduced the risk of rPBC.[20] This analysis included patients who died within 1 year, and because 20% of patients had died at 1 year after LT, we could not evaluate the risk factors for rPBC.

Here, we evaluated rPBC in female patients who survived for at least 1 year. We excluded male patients because these patients develop hepatocellular carcinoma more frequently[23,24] and exhibit different rates of AMA positivity[25] compared with female patients. In addition, the pathologic condition of rPBC may differ between male and female individuals. This study assessed the risk factors for rPBC in female patients after living-donor LT (LDLT), taking into consideration the pathogenesis of rPBC, the influence of perioperative treatment for PBC, immunosuppressive medications, and patient and donor factors. Moreover, we evaluated the postoperative medications taken by patients surviving 1 year post-LT.

Patients and Methods

PATIENTS AND STUDY DESIGN

This was a retrospective multicenter study approved (January 19, 2012; protocol No. 2418) by the Human Ethics Review Board of Tokyo Women's Medical University (the site of data collection and analysis) according to the Declaration of Helsinki.

DATA COLLECTION

The registry of the Japanese Liver Transplant Society showed that 5,722 patients at 46 centers underwent LT between 1994 and 2010; 516 patients received living-donor or deceased-donor transplants for treatment of PBC. We obtained data from 451 patients who underwent primary LT for PBC at 28 centers. Male patients (n = 50), patients who received deceased-donor LT (DDLT) (n = 3), and patients with no available information regarding rPBC (n = 10) were excluded. Therefore, the risk factors for rPBC were examined in 388 female recipients of LDLT due to PBC. The following demographic data were collected: recipient age, past history, blood type, donor characteristics and recipient compatibility, preoperative laboratory data, operative data, preoperative and postoperative treatments for PBC, and initial and postoperative immunosuppressive medications administered.

DIAGNOSTIC CRITERIA FOR rPBC AND HISTOLOGICAL FINDINGS OF ACUTE AND CHRONIC REJECTION

Liver biopsy was performed if elevated liver enzyme levels were detected. Acute and chronic cellular

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rejection was diagnosed according to the Banff criteria.[26,27] The LT patients were diagnosed with PBC based on clinical, serological, and pathologic findings. Liver pathology was characterized as granulomatous cholangitis or florid duct lesions.[6,9,10] The presence of dense lymphoid aggregations in the portal tracts not associated with perivenular inflammation or endotheliitis was assessed.[28,29] The Scheuer classification was used for histological staging of rPBC.[30]

ESTIMATION OF THE TRANSPLANTED LIVER GRAFT SIZE AND DEFINITION OF ISCHEMIC TIME

The size of the liver graft was estimated by calculating the graft/recipient weight ratio and standard liver volume using computed tomography images. The standard liver volume was calculated according to the formula[31] liver volume (mL) = 706.2 × body surface area (m^2) + 2.4. The body surface area was derived from body weight and height as described by DuBois et al.[32]

During surgery, the time between chilling the liver after its blood supply had been removed and its removal from ice was defined as the cold ischemic time.[33] The warm ischemic time was defined as the period until initiation of portal blood flow after put-in.[33]

STATISTICAL ANALYSIS

We compared the clinical features of rPBC and non-rPBC after LT in female patients. Data are presented as medians with minimum and maximum values for continuous variables and as proportions for categorical variables. Statistical significance was considered at $P < 0.05$. Univariate and multivariate Cox regression analyses were used to evaluate the effect of patient characteristics on the time to PBC recurrence and to estimate the hazard ratio (HR) and 95% confidence intervals (CI). Cut-off sensitivity and specificity values from receiver operating characteristic analysis were established by constructing receiver operating characteristic curves, with the area under the curve indicating the accuracy of recurrence. Postoperative factors, including postoperative complications and medications for PBC and immunosuppression, were evaluated in patients who had survived for >1 year post-LT (n = 312) to exclude those who had died of operative complications. rPBC and non-rPBC were compared with regard to the medications prescribed from the time of initiation of the study by using landmark analysis according to Anderson et al.[34] The landmark method was selected to correct for the bias inherent in an analysis of time-to-event outcomes between groups. For adjustment according to preoperative parameters, a landmark analysis was performed. The Statistical Package for the Social Sciences software version 23 (IBM, New York, NY) was used for the statistical analyses.

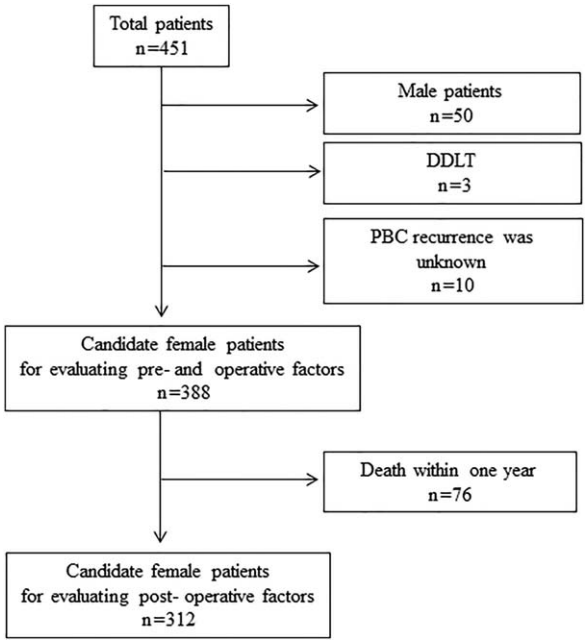

FIG. 1. Flowchart of the selection of the study group patients. We obtained data from 451 patients who underwent primary LT for PBC at 28 centers. Male patients (n = 50), patients who underwent DDLT (n = 3), and those in whom PBC recurrence was unknown (n = 10) were excluded. The effects of preoperative and operative factors were evaluated in 388 patients. Postoperative factors were evaluated in patients who had survived for >1 year post-LT (n = 312) to exclude those who had died from operative complications.

Results

BASELINE CHARACTERISTICS

A flowchart showing selection of the study group patients is illustrated Fig. 1, and the baseline characteristics of 330 patients without rPBC and 58 patients with rPBC (n = 388) are shown in Table 1. We observed autoimmune hepatitis overlapping before LT in a total of 10.3% of the patients (3.7% in rPBC, 11.4% of non-rPBC cases); there was no significant difference between rPBC and non-rPBC. Regarding

TABLE 1. BASELINE CHARACTERISTICS OF FEMALE PATIENTS ACCORDING TO PBC RECURRENCE AFTER LDLT

Characteristic	Total (n = 388)	Female Patients (n = 388) No Recurrence (n = 330)	Recurrence (n = 58)	HR	95% CI	*P* Value
Age (years)	51 (28-70)	53 (28-70)	48 (34-65)	0.95	0.920-0.982	<0.01
History of pregnancy (%)	94.6	94.6	94.2	0.96	0.298-3.074	n.s
Complication of AIH (%)	10.3	11.4	3.7	0.38	0.093-1.569	n.s
History of bone fracture (%)	9.4	10.8	1.8	0.15	0.020-1.067	0.06
HLA B60 positive (%)	14.9	12.5	29.5	2.56	1.336-4.921	<0.01
HLA DQ3 positive (%)	55.3	51.8	65.0	1.38	0.548-3.472	n.s
HLA DR8 positive (%)	37.7	35.4	50.0	1.98	1.134-3.448	<0.05
Laboratory data						
Albumin (g/dL)	2.8 (1.5-4.7)	2.8 (1.5-4.7)	3.0 (2.1-4.4)	1.53	0.920-2.543	n.s
Total bilirubin (mg/dL)	13.6 (0.5-71.8)	13.8 (0.5-63.1)	13.3 (0.9-71.8)	1.01	0.984-1.030	n.s
Aspartate aminotransferase (U/L)	117 (25-1,225)	115 (25-1,225)	135 (45-681)	1.00	1.000-1.005	0.08
Alanine transaminase (U/L)	59 (10-356)	58 (10-302)	68 (27-356)	1.00	0.999-1.009	n.s
Alkaline phosphatase (U/L)	516 (26-3,302)	516 (31-3,302)	554 (26-2,753)	1.00	1.000-1.001	n.s
Gamma-glutamyltransferase (U/L)	78 (8-1,197)	78 (8-1,197)	110 (27-1,134)	1.00	0.999-1.002	n.s
Child-Turcotte-Pugh scores	10 (6-15)	10 (6-15)	10 (6-13)	0.74	0.636-0.854	<0.01
Class C (%)	68.7%	71.6%	52.6%	0.46	0.274-0.775	<0.01
MELD scores	20 (2-57)	20 (3-57)	19 (2-39)	0.96	0.914-0.998	<0.05
Updated Mayo risk scores	10.3 (4.2-96.7)	10.3 (4.2-85.2)	10.1 (6.2-96.7)	1.02	1.005-1.033	<0.01
IgG (mg/dL)	2,125 (299-5,620)	2,116 (299-5,620)	2,284 (884-4,519)	1.00	1.000-1.001	n.s
IgM (mg/dL)	365 (25-2,024)	350 (25-2,024)	505 (164-1,850)	1.00	1.001-1.002	<0.01
Antinuclear antibodies	160	160	320	1.00	0.999-1.000	n.s
positive (%)	(20-10,240) 78.9%	(20-10,240) 79.9%	(40-1,280) 71.9%	0.81	0.373-1.754	n.s
Antimitochondrial antibody	80	80	160	1.00	0.998-1.001	n.s
positive (%)	(20-2,560) 88.2%	(20-2,560) 86.6%	(20-640) 97.5%	6.64	0.906-48.617	0.06
Antimitochondrial M2 antibody	146	138	162	1.00	1.000-1.000	n.s
positive (%)	(1-2,000) 88.2%	(1-2,000) 87.4%	(4-2,000) 93.8%	2.47	0.585-10.408	n.s
Donor						
Age	35 (18-66)	35 (18-66)	36 (19-65)	1.00	0.981-1.025	n.s
Husband (%)	88 (22.7)	74 (22.4)	14 (24.1)	1.50	0.824-2.746	n.s
Sex mismatch positive (%)	267 (68.8)	220 (66.7)	47 (81.0)	2.45	1.268-4.736	<0.01
ABO blood type compatibility (%)	21.1	20.6	24.1	1.00	-	n.s
compatible identical incompatible	67.8	67.3	70.7	0.84	0.457-1.541	
	11.1	12.1	5.2	0.50	0.144-1.745	
Cross match test positive(%)	11.2	11.8	7.7	0.67	0.207-2.182	n.s
HLA A-B-DR mismatches (≥3) (%)	62.3	61.0	69.4	1.78	0.968-3.273	0.06
Operative data						
GRWR	0.95 (0.45-2.24)	0.94 (0.46-1.88)	0.95 (0.45-2.24)	1.19	0.505-2.802	n.s
SLV	46 (24-104)	46 (24-88)	49 (24-104)	1.01	0.987-1.025	n.s
Operative time (min)	781 (335-1,990)	785 (335-1,990)	700 (370-1,150)	1.00	0.995-0.999	<0.01
Cold ischemic time (min)	71 (10-314)	73 (10-314)	61 (22-297)	1.00	0.993-1.005	n.s

TABLE 1. *CONTINUED*

Characteristic	Total (n = 388)	Female Patients (n = 388) No Recurrence (n = 330)	Recurrence (n = 58)	HR	95% CI	*P* Value
Warm ischemic time (min)	45 (21-211)	45 (21-211)	42 (22-91)	0.98	0.963-1.003	n.s
Blood loss (mL)	4,083 (130-51,216)	4,090 (130-51,216)	3,955 (480-9,700)	1.00	1.000-1.000	n.s

Abbreviations: AIH, autoimmune hepatitis; GRWR, graft/recipient weight ratio; IgG, immunoglobulin G; n.s, not significant; SLV, standard liver volume.

the pathologic stage, 89.4% of patients with non-rPBC (n = 177) and 90% of those with rPBC (n = 37) showed Scheuer stage 4, and 94.1% of those with non-rPBC (n = 112) and 100% of those with rPBC (n = 17) were Ludwig stage 4; the difference was not significant. Patients with rPBC were significantly younger than those without rPBC (48 versus 53 years; HR, 0.95; 95% CI, 0.920-0.982; $P < 0.01$). The serum immunoglobulin M (IgM) level was significantly higher in patients with rPBC compared with those without rPBC (505 versus 350 mg/dL; HR, 1.00; 95% CI, 1.001-1.002; $P < 0.01$). The frequencies of HLA B60 and DR8 were significantly higher in patients with rPBC (HR, 2.56; 95% CI, 1.336-4.921; $P < 0.01$ and HR, 1.98; 95% CI, 1.134-3.448; $P < 0.05$, respectively). The frequencies of Child-Turcotte-Pugh (CTP) class C (52.6% versus 71.6%; HR, 0.46; 95% CI, 0.274-0.775; $P < 0.01$), model of end-stage liver disease (MELD) score (19 versus 20; HR, 0.96; 95% CI, 0.914-0.998; $P < 0.05$), and updated Mayo risk score (10.1 versus 10.3; HR, 1.02; 95% CI, 1.005-1.033; $P < 0.01$) were significantly lower in patients with rPBC. Donor–recipient sex mismatch was significantly more frequent in patients with rPBC (81.0% versus 66.7%; HR, 2.45; 95% CI, 1.268-4.736; $P < 0.01$). The frequency of the husband being the donor was not significantly different between the two groups (24.1% versus 22.4%).

The implanted liver volume did not differ between patients with rPBC and with non-rPBC. However, the operative time was significantly shorter (700 versus 785 minutes; HR, 1.00; 95% CI, 0.995-0.999; $P < 0.01$) in patients with rPBC compared to those with non-rPBC.

PREOPERATIVE AND INICIATE-POSTOPERATIVE MEDICATIONS

The frequencies of use of UDCA, bezafibrate, colchicine, and steroids were similar between patients with rPBC and with non-rPBC (Table 2). Ritsuximab, basiliximab (Simulect), mouse monoclonal CD3 antibody, and hepatitis B immune globulin were used. The frequencies of TAC and steroid use were significantly greater in patients with non-rPBC than in those with rPBC (HR, 0.36; 95% CI, 0.183-0.699; $P < 0.01$ and HR, 0.19; 95% CI, 0.046-0.780; $P < 0.05$, respectively), while CyA was used less frequently in patients with non-rPBC (HR, 3.14; 95% CI, 1.602-6.138; $P < 0.05$). The frequencies of usage of UDCA and bezafibrate were similar between the groups, but usage of mizoribine (MIZO) was significantly higher in patients with rPBC compared to those with non-rPBC (25.0 versus 6.0%; HR, 3.79; 95% CI, 1.374-10.450; $P < 0.05$).

MULTIVARIATE ANALYSIS OF RISK FACTORS FOR PBC RECURRENCE

Recipient age < 52 years, positivity for HLA B60 and HLA DR8, serum IgM ≥ 355 mg/dL, CTP score-< 10, MELD score < 12, updated Mayo risk score <10, donor–recipient sex mismatch, operative time, and initiation of TAC, CyA, steroid, and MIZO treatment were evaluated by multivariate analysis using the Cox hazard model. Recipient age < 52 years (HR, 3.71; 95% CI, 1.763-7.822; $P < 0.01$), HLA B60 positivity (HR, 2.19; 95% CI, 1.033-4.624; $P < 0.05$), MELD score-< 12 (HR, 3.22; 95% CI, 1.558-6.659; $P < 0.01$), initiation of CyA (HR, 4.63; 95% CI, 2.189-9.806; $P < 0.01$), donor–recipient sex mismatch (HR, 2.61; 95% CI, 1.277-5.320; $P < 0.01$), and shorter operative time (HR, 1.00; 95% CI, 0.995-0.999; $P < 0.01$) were identified as risk factors for rPBC (Fig. 2A).

POSTOPERATIVE RISK FACTORS FOR rPBC

Postoperative risk factors for rPBC were evaluated after excluding patients who died within 1 year post-

TABLE 2. PRE-LT AND POST-LT MEDICATIONS

Characteristic (%)	Total (n = 388)	Female Patients (n = 388)		HR	95% CI	*P* Value
		No Recurrence (n = 330)	Recurrence (n = 58)			
Preoperative medications for PBC						
UDCA	94.2	94.2	94.6	1.58	0.491-5.051	n.s
Bezafibrate	32.6	34.3	22.6	0.78	0.406-1.487	n.s
Colchicine	3.6	3.9	2.0	0.57	0.079-4.156	n.s
Steroid (Pre-LT)	16.2	16.3	15.7	0.83	0.389-1.773	n.s
Immunosuppression immediately after transplantation						
Antibody treatment	7.5	8.5	1.7	0.33	0.046-2.411	n.s
TAC	88.7	90.0	81.0	0.36	0.183-0.699	<0.01
CyA	9.8	8.2	19.0	3.14	1.602-6.138	<0.01
Steroid	99.0	99.4	96.6	0.19	0.046-0.780	<0.05
Antimetabolite	39.7	39.9	38.5	1.15	0.657-2.019	n.s
AZA	19.7	17.9	30.0	1.73	0.634-4.710	n.s
MMF	71.5	75.2	50.0	0.42	0.170-1.048	n.s
MIZO	8.8	6.0	25.0	3.79	1.374-10.450	<0.05

Abbreviation: n.s, not significant.

LT (n = 312) because their death was not a result of recurrence. The incidences of vascular complications and bacterial infection were slightly higher in patients with non-rPBC, while that of chronic rejection was higher in patients with rPBC, albeit not significantly (Table 3).

The medications administered for PBC prevention and immunosuppression 1 year after LT are shown in Table 4. Few patients underwent MIZO treatment; therefore, these patients were not included in the analysis. The rates of posttreatment usage of UDCA and bezafibrate were not significantly different between patients with rPBC and with non-rPBC. The incidence of rPBC was significantly lower in patients who received steroids (HR, 0.50; 95% CI, 0.283-0.882; $P < 0.05$) but higher in patients who underwent colchicine and antimetabolite treatments (HR, 60.70; 95% CI, 7.091-519.532; $P < 0.01$ and HR, 2.02; 95% CI, 1.135-3.605; $P < 0.05$, respectively). However, few patients underwent colchicine treatment.

In the multivariate analysis using the landmark method, the preoperative risk factors were similar to those identified by Cox analysis, i.e., younger recipient age, HLA B60 positivity, lower MELD score, CyA treatment, donor sex mismatch, and shorter operative time (Fig. 2B; Table 5). The multivariate analysis showed that steroid treatment 1 year post-LT was not significantly associated with the risk of PBC (HR, 0.65; 95% CI, 0.340-1.239; $P = 0.19$). In contrast, antimetabolite treatment was associated with a risk of PBC (HR, 1.82; 95% CI, 0.938-3.538; $P = 0.08$).

Discussion

We evaluated the risk factors for rPBC in a large retrospective study of Japanese female patients. Younger recipient age, HLA B60 positivity, lower MELD score, initial treatment with CyA, donor sex mismatch, and shorter operative time were significantly associated with rPBC after LDLT by multivariate analysis. Steroid administration at 1 year after LT did not influence the frequency of rPBC; in contrast, use of antimetabolites increased the frequency of rPBC. The timing of LT, patient conditions, donor characteristics, and medications may be associated with rPBC in LDLT recipients.

PBC recurrence in DDLT recipients has been reported[4,10,21,35]; however, few studies of PBC recurrence in LDLT have been performed.[15,16,36] The difference between LDLT and DDLT may be associated with graft volume, timing of LT, and HLA background. Compared to DDLT, the graft volume of LDLT was smaller, but there was no difference in the rate of rPBC by graft volume in our study. LDLT may be performed in a planned manner after thorough preparation and little waiting time. The LDLT donors were blood relatives with close HLA matches. Hashimoto et al. [37] reported a high rate of rPBC (33% in 2 years) after LDLT, although the number of patients was limited. A recent study demonstrated that the rPBC rate after a first-degree LDLT was 17%, which was slightly higher compared to other combinations; however, there was no significant difference between LDLT and DDLT (17% for a first-degree LDLT,

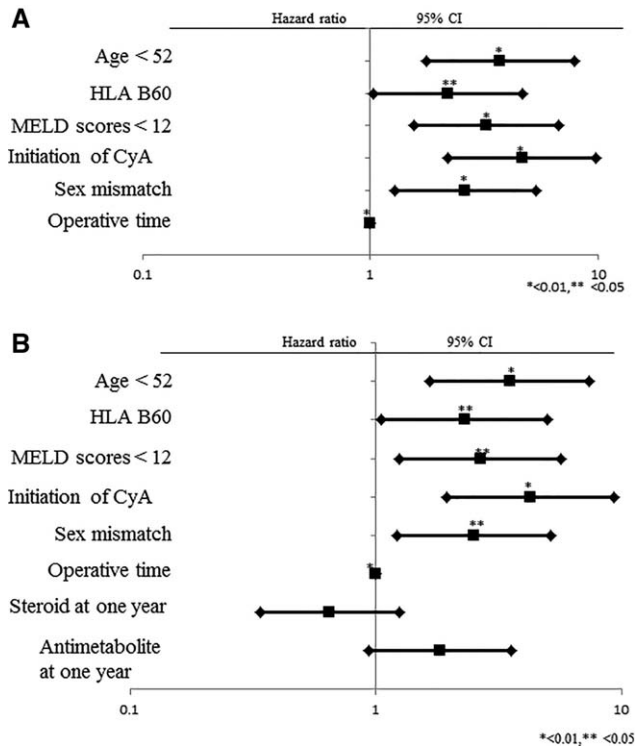

FIG. 2. Risk of PBC recurrence as determined by Cox hazard and landmark analyses. Recipient age < 52 years, HLA B60 and HLA DR8 positivity, serum IgM level ≥355 mg/dL, CTP score < 10, MELD score < 12, updated Mayo risk score <10, donor–recipient sex mismatch, and initiation of tacrolimus, CyA, steroids, and MIZO were evaluated by multivariate analysis. (A) Cox hazard model for pretransplant and immediate postoperative data and (B) landmark analysis of pretransplant, immediate postoperative, and 1-year postoperative data. (A) In the Cox hazard analysis of the risk of rPBC, recipient age <52 years (HR, 3.71; 95% CI, 1.763-7.822; $P < 0.01$), HLA B60 positivity (HR, 2.19; 95% CI, 1.033-4.624; $P < 0.05$), MELD score < 12 (HR, 3.22; 95% CI, 1.558-6.659; $P < 0.01$), initial treatment with CyA (HR, 4.63; 95% CI, 2.189-9.806; $P < 0.01$), donor sex mismatch (HR, 2.61; 95% CI, 1.277-5.320; $P < 0.01$), and operative time (HR, 1.00; 95% CI, 0.995-0.999; $P < 0.01$) were associated with the risk of rPBC (* $P < 0.01$, ** $P < 0.05$). (B) The risk factors identified by Cox hazard analysis were also identified by landmark analysis. In addition, steroid treatment after LT reduced the frequency of rPBC, albeit not significantly (HR, 0.65; 95% CI, 0.340-1.239; $P = 0.19$), whereas antimetabolite treatment was associated with an increased frequency of rPBC (HR, 1.82; 95% CI, 0.938-3.538; $P = 0.08$) (* $P < 0.01$, ** $P < 0.05$).

13% for a distant/unrelated LDLT, and 10% for DDLT; $P = 0.77$).[38] Because HLA B60 is associated with rPBC, further genetic analysis is required.

PBC is diagnosed mainly in female individuals,[1,39] who exhibit slower disease progression.[40] Male patients with PBC are reported to have more complications, including hepatocellular carcinoma,[23,24] and exhibit different AMA-positive rates compared to female patients.[25] The pathologic condition of rPBC may be different between male and female patients.[23-25] Moreover, there is a sex difference in drug metabolism,[41] and female patients are highly responsive to UDCA treatment;[42] therefore, male and female patients should be evaluated separately. However, the risk factors of rPBC in male patients could not be analyzed due to the small number available. We plan to evaluate the risk of rPBC in male patients after the accumulation of a larger number. In the present study, we evaluated PBC in female recipients of LDLT to exclude sex differences. Postoperative parameters were evaluated after excluding patients who died within 1 year due to operative complications or infections. The survival rate at 1 year was 80.4%, which was lower than expected because the grafted liver was a small-for-size graft due to LDLT. In addition, this was a multicenter study that included some centers that conducted few LT procedures. We reported previously that rPBC did not increase the retransplantation or mortality rate.[20]

rPBC is diagnosed based on the combination of consistent physical (e.g., fatigue and pruritus), serological, and pathologic findings. Serum IgM is used to diagnose rPBC after LT and is elevated in 80% of cases.[43] In our series, the serum IgM level was significantly higher in patients with rPBC than in those without rPBC. An increased serum IgM level may be associated with rPBC. Although Polson et al.[43] reported both AMA positivity and an elevated AMA titer in patients with rPBC, neither was significantly elevated in the rPBC patients of our series. Other reports also found that the AMA titer did not predict histological recurrence.[37,44,45] Difficulties in diagnosing rPBC may be one reason for such inconsistencies. Pathologic findings include granulomatous cholangitis or florid duct lesions in the liver. Graft-versus-host disease and liver allograft rejection, particularly chronic rejection, are sometimes difficult to distinguish from rPBC pathologically,[46,47] and thus markers for a diagnosis based on pathologic features of rPBC are required. Donor sex mismatch also increased the frequency of rPBC (HR 2.45; 95% CI, 1.268-4.736; $P < 0.01$), as reported.[20]

The role of the HLA type in rPBC has been elucidated, and several donor and recipient HLA types and mismatches have been demonstrated as risk factors for rPBC.[14-18] In our study, recipients with HLA B60

TABLE 3. POSTOPERATIVE COMPLICATIONS

Characteristic (%)	Total (n = 312)	Female Patients (n = 312)		HR	95% CI	*P* Value
		No Recurrence (n = 254)	Recurrence (n = 58)			
Briary complication	28.8	28.3	31.0	1.01	0.578-1.763	n.s
Vascular complication	9.9	11.4	3.4	0.32	0.078-1.311	n.s
Acute rejection	40.2	38.3	48.3	1.38	0.823-2.310	n.s
Chronic rejection	2.6	2.0	5.3	1.93	0.601-6.199	n.s
Bacterial infection	18.6	20.8	10.4	0.51	0.203-1.292	n.s
Fungal infection	5.8	6.8	1.7	0.27	0.038-1.970	n.s
CMV infection	36.1	37.3	30.4	0.83	0.470-1.476	n.s

Abbreviations: CMV, cytomegalovirus; n.s, not significant.

TABLE 4. MEDICATIONS USED 1 YEAR POSTTRANSPLANTATION

Medication (%)	Total (n = 312)	Female (n = 312)		HR	95% CI	*P* Value
		No Recurrence (n = 254)	Recurrence (n = 58)			
Medications for PBC						
UDCA	82.7	81.1	89.5	2.03	0.865-4.755	n.s
Bezafibrate	2.6	2.8	1.8	0.45	0.062-3.288	n.s
Colchicine	0.3	0.0	1.8	60.70	7.091-519.532	<0.01
Immunosuppression						
Steroid	73.3	75.3	64.2	0.50	0.283-0.882	<0.05
TAC	80.7	80.4	82.1	1.02	0.513-2.021	n.s
CyA	19.3	19.6	17.9	0.98	0.495-1.950	n.s
Antimetabolite	30.4	28.1	40.8	2.02	1.135-3.605	<0.05
AZA	7.8	6.5	13.0	1.20	0.340-4.257	n.s
MMF	58.3	58.7	56.5	1.32	0.566-3.085	n.s

Abbreviation: n.s, not significant.

and DR8 had a higher frequency of rPBC, whereas HLA DR8 but not B60 has been reported to be associated with an increased frequency of PBC.[48] The host immune response could be linked to the severity of rPBC after LT.[49] PBC begins with a loss of tolerance to mitochondrial self-antigens, in particular the

TABLE 5. RISK FACTORS FOR RPBC BY MULTIVARIATE ANALYSIS

		Number of Patients	Recurrence	HR	95% CI	*P* Value
Preoperative						
Recipient age < 52 years	(−)	146	9	1.00	-	
	(+)	151	40	3.49	1.658-7.355	<0.01
HLA B60	(−)	200	28	1.00	-	
	(+)	38	10	2.29	1.056-4.948	<0.05
MELD score < 12	(−)	252	30	1.00	-	
	(+)	33	1	2.66	1.250-5.645	<0.05
Initial treatment with CyA	(−)	268	39	1.00	-	
	(+)	29	10	4.25	1.948-9.275	<0.01
Sex mismatch	(−)	99	10	1.00	-	
	(+)	198	39	2.49	1.213-5.112	<0.05
Operation time		297	49	1.00	0.995-0.999	<0.01
1 year after LT						
Steroid treatment	(−)	77	17	1.00	-	
	(+)	216	31	0.65	0.340-1.239	n.s
Antimetabolite treatment	(−)	186	22	1.00	-	
	(+)	84	20	1.82	0.938-3.538	0.08

Abbreviations: n.s, not significant; (−), negative or not matching criteria; (+), positive or matching criteria.

E2 subunit of the pyruvate dehydrogenase complex. Invariant natural killer T cells accelerate disease progression in a manner dependent on AMA production and increase $CD8^+$ T-cell biliary infiltration, portal inflammation, granuloma formation, bile duct damage, and fibrosis. These mechanisms are modulated by innate immunity in the pathogenesis of PBC and rPBC after LT.[49] Innate and adaptive immune dysfunction has been reported in advanced cirrhosis.[50] Márquez et al.[50] reported that in patients with decompensated cirrhosis an increase in CD25-positive effector $CD4^+$ T cells was accompanied by an increase in $CD4^+$ $CD25^{high}$ $Foxp3^+$ regulatory T cells, which may suppress T-cell responses. In our study, recipients who were younger or had preserved preoperative liver function (in terms of low CTP, MELD, and updated Mayo risk scores) were at an increased risk of rPBC. We speculated that these conditions may induce rPBC by maintaining the immune response. In contrast, patients who underwent steroid treatment 1 year after LT had a relatively low rate of recurrence. These findings suggest that immunosuppressive medications may reduce the incidence or progression of PBC.

We found that long-term steroid use reduced the risk of rPBC in a univariate analysis; however, this was not significant in the multivariate analysis. Mycophenolate mofetil (MMF) and azathioprine are antimetabolites that inhibit B lymphocyte proliferation. Few studies have evaluated the effect of antimetabolites in rPBC patients. MMF was reported to increase the risk of rPBC[14,21] and azathioprine to reduce the risk[10,14,18,21]; however, Talwalkar et al.[51] failed to show any effect of MMF on rPBC. The use of steroids in LT patients is not recommended because of the risk of osteoporosis; antimetabolites are used instead. In our study, although MIZO treatment was associated with an increased frequency of rPBC (HR 3.79; 95% CI, 1.374-10.450; $P < 0.05$), the frequency of initiation of antimetabolite therapy did not differ significantly in patients with or without rPBC. In contrast, univariate analysis showed that postoperative long-term use of antimetabolites increased the frequency of rPBC (HR, 2.02; 95% CI, 1.135-3.605; $P < 0.05$). If a P value < 0.1 was taken to indicate a statistically significant tendency, long-term use of antimetabolites also increased the frequency of rPBC in the multivariate analysis. Thus, steroids and antimetabolites may exert opposite effects on rPBC. We speculated that antimetabolites, especially MMF, suppress the function of B lymphocytes, whereas steroids strongly suppress both B and T lymphocytes.[52] Therefore, the T-cell response may be associated with the pathogenesis of rPBC. Although both CyA and TAC are calcineurin inhibitors, their pharmacological action differs, for instance, the activation of transforming growth factor beta.[53] For this reason, the difference in the roles of CyA and TAC in the pathogenesis of rPBC was evaluated, leading to our opinion that the direct effect of antimetabolites on rPBC should be further studied. In addition, a shorter operative time was associated with a risk of rPBC. This may be associated (albeit not significantly) with transfusion of a lower blood volume and less blood loss. Persistence of cytokines or immune cells may accelerate the progression of PBC; however, our results were insufficient to explain the mechanism of pathogenesis of rPBC. Moreover, it is possible that disease recurrence and immunosuppressants are not simply correlated. Carbone et al.[54] showed an association of rPBC with the interleukin 12A locus of the recipient and that use of TAC in patients with the rs62270414 genotype AG or GG was associated with an increased risk of rPBC whereas the use of CyA in patients with the rs62270414 genotype AA was related to a lower risk of rPBC. Therefore, further studies that include medications and genetic factors are warranted.

Several medications are used to treat rPBC; however, there is no consensus on the most appropriate regimen. The use of UDCA for PBC has a beneficial effect on cholestasis and pruritus.[35] UDCA decreases levels of toxic hydrophobic bile acids, stabilizes cell membranes, and exerts immunomodulatory effects. More than 50% of patients treated with UDCA compared with 22% of untreated patients showed normalization of biliary enzyme levels over a 36-month period.[7,35] Conversely, UDCA treatment after LT did not affect the frequency of rPBC.

Bezafibrate improves biliary enzyme levels in patients with PBC.[55,56] The combination of bezafibrate and UDCA was superior to UDCA monotherapy in a randomized crossover study.[57] In our study, although preoperative use of bezafibrate was infrequent, there was no significant difference in the numbers of patients with rPBC and those with non-rPBC prescribed the drug.

No consensus therapy preventing rPBC progression has been described. Among patients who received UDCA posttransplantation, 32% developed rPBC over a 13-year period.[7] UDCA does not influence

histological progression[35] and was not associated with survival after 1 year.[58] Thus, further study is needed to determine the associations between such medications and the risk of rPBC, treatment effectiveness, and protection against rPBC. To this end, we plan to perform a further large prospective study.

The limitations of the current study were its retrospective nature, the lack of a standard biopsy protocol, and the difficulty with diagnosing rPBC in patients with normal liver function tests; therefore, the frequency of rPBC could be underestimated. There may have been a physician-based or institution-based bias in the diagnosis of recurrence in patients with PBC. The effects of drug combinations on rPBC should be evaluated because together these treatments may exert additive or synergistic effects on rPBC. We could not evaluate drug combinations due to the lack of precise data for each treatment.

In conclusion, the immune response may be associated with rPBC, and an immunosuppressed condition due to older age, a higher CTP score, sex-matched donor, steroid administration, and minimal use of antimetabolites after LT may reduce the incidence of rPBC in LDLT recipients. Further study of the effects of antimetabolites on rPBC is warranted.

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