ORIGINAL ARTICLE

Donor age (≥45 years) and reduced immunosuppression are associated with the recurrent primary sclerosing cholangitis after liver transplantation – a multicenter retrospective study

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ABSTRACT

The present study investigated the possible risk factors, including relationship/HLA matching between donor and recipient, and immunosuppressive therapies on the recurrence of primary sclerosing cholangitis (PSC) after liver transplantation (LT). Subjects were 197 recipients of LT for PSC, among whom 180 surviving more than 1 year after LT were further analyzed for risk factors of recurrence. The 5- and 10-year patient- and graft survival rates were 83% and 68%, and 71% and 62%, respectively. The overall PSC recurrence rate was 25% with a 5- and 10-year graft survival rate of 34% and 18%, which was significantly lower than the survival rate of those without recurrence ($P < 0.001$). Univariate analysis identified the following as risk factors for recurrence: donor age ($P < 0.001$), cyclosporine use ($P = 0.012$), mono or no immunosuppressive agent ($P < 0.001$), postoperative biliary complication ($P < 0.001$), and active intestinal bowel disease after LT ($P < 0.001$). Among these factors, donor age ≥45 years [hazard ratio (HR), 1.65; 95% confidence interval (CI), 1.21–2.69; $P = 0.003$] and mono or no immunosuppressive agent 1-year after LT (HR, 2.38; 95% CI, 1.23–3.45; $P = 0.011$) were identified as independent risk factors in the final multivariate Cox regression model. The results were similar in sub-analysis for ABO-identical/compatible adult living donor LT cases.

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Key words
disease recurrence, living donor liver transplantation, primary sclerosing cholangitis, risk factor

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Introduction

Primary sclerosing cholangitis (PSC) is a chronic, immune-mediated cholestatic liver disease characterized by progressive inflammation and fibrosis of the intrahepatic and extrahepatic bile ducts, which lead to the formation of multifocal biliary strictures, biliary cirrhosis, and portal hypertension. PSC is often complicated by inflammatory bowel disease (IBD), which increases the risk of developing cholangiocarcinoma and colorectal carcinoma [1,2]. The etiology of PSC is still unknown, and no specific treatment has yet been shown to attenuate the progressive course of the disease. Liver transplantation (LT) is currently the only life-saving therapy [3].

The long-term outcome of LT for PSC in Western countries is reportedly good [4–7], but disease recurrence after LT is approximately 15–20% [8]. In Japan, where deceased donor livers are scarce and end-stage PSC patients depend mainly on living donor liver transplantation (LDLT), the risk of recurrent PSC is increased, resulting in an impaired long-term outcome [9,10]. Our previous Japanese nationwide study revealed a recurrence rate of 27%, with a graft loss rate of 69% among those with recurring disease [11].

Because recurrent PSC definitely worsens the prognosis of LT recipients, it is important to determine the factors associated with disease recurrence. Possible risk factors reported in previous studies include active IBD after LT [12–15], higher model for end-stage liver disease (MELD) score [11,16], younger recipient age [15,17], male sex [12], sex mismatch [18], episode of acute cellular rejection [17,19], cytomegalovirus (CMV) infection [11,17], related donor [11], use of extended-donor criteria graft [20], and the presence of cholangiocarcinoma before transplantation [21], but the results are conflicting. One of the most controversial and important risk factors for recurrent PSC is the use of living-related donors in LDLT [6,11,16], especially in Asia where the number of deceased donors is limited, which was the major concern in the present study.

In the present study, we investigated the influence of genetic factors, including relationship/ HLA matching between the donor and recipient, and immunosuppressive therapies on the recurrence of PSC after LT among a Japanese multicenter cohort.

Patients and methods

Patients

Datasets of LT recipients for PSC based on questionnaires were collected from each Japanese LDLT center in December 2017. The study population comprised 197 patients who had undergone LT for PSC at 20 centers in Japan between 1998 and 2016. The questionnaires addressed pre-LT patient characteristics, treatments, and posttransplant clinical courses. Patient characteristics included age, sex, donor and recipient blood type and HLA, MELD score, the new Mayo score for PSC, the Child–Pugh–Turcotte (CPT) score, presence of IBD or cholangiocarcinoma, and relationship to the donor. Treatment data included graft type, ABO-blood-type compatibility, manner of biliary reconstruction, immunosuppressants in the initiation phase and maintenance phase, and steroid pulse treatment for acute cellular rejection (ACR). Clinical course data included hepatic arterial/portal venous complications, CMV antigenemia including CMV diseases, episodes of ACR and steroid-resistant ACR, biliary anastomotic complications, post-LT active IBD, development of colorectal and cholangiocarcinoma, PSC recurrence, graft loss, and patient survival. Data on mortality and causes of death were also collected. Detailed immunosuppression data, such as drugs used for induction (including rituximab), type of calcineurin inhibitor (CNI), use of mycophenolate mofetil (MMF) and steroids, and the CNI trough level in both the early posttransplant period and maintenance phase, were collected. In addition, regarding maintenance phase, in cases who underwent further immunosuppressive regimen modification during maintenance phase or those with recurrent PSC, the latest regimen and CNI trough level at these events were collected and recorded. HLA typing for HLA-A, HLA-B, and HLA-DR for class I and II loci was performed in patients and donors at each institution or commercial laboratories.

Operative procedure, postoperative management, and follow-up

The operative procedure, including selection of the graft in the LDLT setting, depended on each center following standard LT procedures. The immunosuppression regimen was started with a conventional double or triple regimen, including CNIs (cyclosporine or tacrolimus) and steroids with or without MMF in every center, and was tapered along the course, the details of which depended on the protocol for each center. Dose reduction or withdrawal of immunosuppressants during the maintenance phase was also center-determined. Inhibitors of mammalian target of rapamycin (mTOR) were not used in the present cohort, as it was not approved for liver transplant recipients in Japan until April 2018.

Induction was not used in this cohort except for rituximab in cases of LDLT with ABO-blood-type incompatibility. The maintenance immunosuppressive regimen was categorized as mono therapy (CNI only), double therapy (CNI and steroid/MMF), and triple therapy (CNI, steroid, and MMF). Mono therapy was done with CNI (cyclosporine or tacrolimus) in all cases. There were two pediatric cases who had been free from immunosuppression at maintenance phase: one was on the trial of immune tolerance and the other was due to drug withdrawal. Pathologic diagnosis of ACR or chronic rejection was made according to the Banff criteria [22]. When ACR was confirmed, patients were initially treated with a high-dose corticosteroid, and lymphocyte antibodies were indicated for those with steroid-resistant rejection.

Definition of PSC recurrence

In cases suspected of PSC recurrence on the basis of abnormal liver tests, including increases in cholestatic biochemical data, further investigations were carried out with biopsies, magnetic resonance cholangiography (MRC), and/or endoscopic retrograde cholangiography (ERC). PSC recurrence was strictly defined using both positive and negative criteria according to Graziadei [23] in all centers participating in this study. Criteria included a confirmed diagnosis of PSC before transplantation and intrahepatic multiple biliary strictures confirmed by cholangiography occurring more than 90 days after transplantation, or biopsy findings showing fibrous cholangitis and/or fibro-obliterative lesions in the absence of hepatic artery thrombosis/stenosis, chronic ductopenic rejection, isolated anastomotic strictures, and nonanastomotic strictures prior to posttransplant day 90. Biliary complications were defined as anastomotic stricture, bile leak, or biliary casts after transplantation. According to recent advances in the management of ABO-incompatible LDLT with rituximab [24] and the possible efficacy of rituximab for the prevention of PSC recurrence [25], ABO-incompatible cases were not excluded in this study provided that these cases were free from chronic ductopenic rejection. All data needed to define recurrent PSC were captured from each center using a standardized questionnaire form, with the case definition being adjudicated by each institution's primary investigator.

Statistical analysis

Data were collected into a standard Excel Database (Microsoft Corporation, Redmond, WA, USA) and further analyzed using SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, USA). Continuous variables are presented as mean ± standard deviation and were compared using the Mann–Whitney *U* test. Categorical parameters are expressed as *n* (%) and were compared using the chi-square test. Patient and graft survival were determined by Kaplan–Meier survival analysis. The log-rank test was used to evaluate the effects of characteristics on PSC recurrence, graft survival, and patient survival. To specifically examine PSC recurrence and risk factors associated with recurrence, we estimated cause-specific outcomes, censoring at retransplant and death. All tests were 2-sided, and a *P* value of 0.05 or less was considered statistically significant. For analysis of the risk factors for recurrent PSC, hazard ratios (HRs) were estimated using Cox proportional hazards regression applied to the Kaplan–Meier curves. A receiver-operating characteristics curve analysis and Youden's index were used to define the ideal cutoff values for each continuous variable. Univariate and multivariate analyses were performed by Cox regression analysis, and the final multivariate model was selected using a backward stepwise method in order to only keep significant variables.

This study was conducted in collaboration with the Japanese Liver Transplantation Society and the intractable hepatobiliary diseases study group in Japan. The study protocol was approved as project number 11116 by the Research Ethics Committee/Institutional Review Board of the Graduate School of Medicine and Faculty of Medicine, University of Tokyo. All efforts were made to protect patient privacy and anonymity during the preparation of this manuscript.

Results

Patient demographics

Patients' inclusion and exclusion in this study were shown in Fig. 1. A total of 197 patients (age at LT: 10–68 years, median: 35 years; 181 adult cases, 16 pediatric cases; 111 (56%) male) from 20 centers in Japan were enrolled in the study. The MELD score ranged from 5 to 37 with a median score of 18. The new Mayo score for PSC and CPT score ranged from −0.7411 to 5.122, with a median score of 2.546, and from 6 to 14 with a median score of 10, respectively. Co-existence of IBD was observed in 70 patients (36%). The donors were living in 174 cases and deceased in 23 cases, with ages ranging from19 to 65 years (median, 44 years). Parents were the most frequent donors (n = 55, 28%), followed by siblings (n = 49, 25%), spouses (n = 33, 13%),

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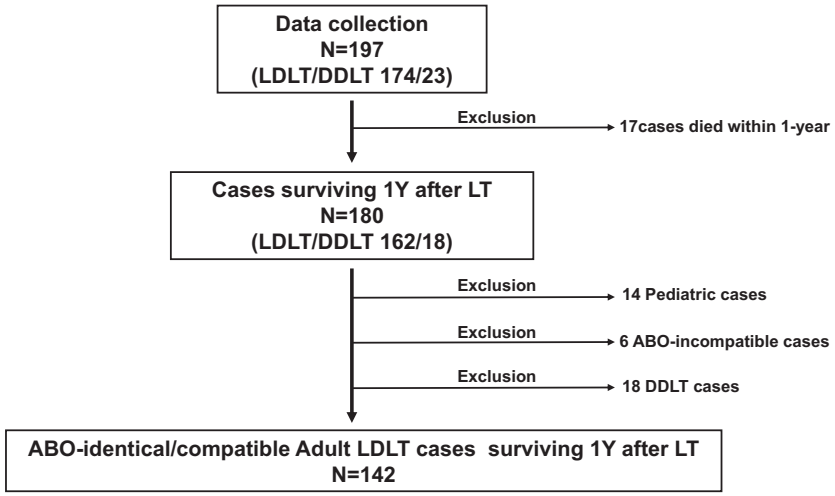

Figure 1 Patients' inclusion and exclusion. DDLT, deceased donor liver transplantation; LDLT, living donor liver transplantation; LT, liver transplantation.

children (n = 31, 16%), deceased donors (n = 23, 12%), domino-living donors (n = 3, 6%), and other related donors (n = 3, 6%). There were 19 ABO-blood-type incompatible LDLTs (10%). The graft type was the right liver in 96 cases, the left liver or lateral segment in 69, the whole liver in 22, the right lateral sector graft in 5, and other type or unknown in five cases. The manner of biliary reconstruction was hepaticojejunostomy in 181 cases and duct-to-duct in 16 cases. The follow-up period ranged from 5 to 270 months, with a median length of 72 months (83 $\pm$ 69 months).

Overall outcome of the total cohort of primary liver transplantation for PSC

The 5- and 10-year patient survival and primary graft survival rates of the 197 patients were 83% and 68%, and 71% and 62%, respectively. The patient survival rate and the recurrence rate were shown in Fig. 2. A total of 49 patients died after LT; the leading cause was graft failure due to PSC recurrence in 30 cases, followed by infection in 11, graft failure due to acute and chronic rejection in 4, malignancies (including cholangiocarcinoma) in 3, and unknown cause in 1. Recurrence of PSC after primary LT was observed in 51 cases (25%) at 35 (5–235) months after LT, among which 23 underwent retransplantation for graft failure due to recurrent PSC. Patient and graft survival stratified by the presence of PSC recurrence are shown in Fig. 3. Although patient survival did not differ significantly between those with and without PSC recurrence, the graft survival rate was significantly worse among those with recurrence ($P < 0.001$). The 5-, 10-, and 15-year primary graft survival after LT among those with PSC recurrence was 34%, 18%, and 7%, respectively. Cholangiocarcinoma developed in five patients (3%), including two cases diagnosed at the time of LT. Colorectal cancer developed in 4 (2%), and active IBD after LT occurred in 49 recipients (25%).

Factors associated with PSC recurrence

In this analysis, to elucidate the factors associated with disease recurrence in the graft, including not only preoperative and donor characteristics but also postoperative factors such as immunosuppressants and complications, we included only those surviving at least >1 year after LT. Consequently, 17 patients who died within 1 year after LT were excluded. Among 17 patients, there was one patient who had been diagnosed with recurrent PSC five months after LT and died due to subsequent sepsis at six months. The most frequent cause of death among 17 patients was infection (n = 11), followed by rejection (n = 4), cholangiocarcinoma (n = 1), and unknown (n = 1). Finally, 180 recipients were the subjects of this investigation, and their demographics are shown in Table 1. Univariate Cox model of preoperative and donor characteristics revealed a significant association of PSC recurrence with donor age ($P < 0.001$), and no significant relationship between PSC recurrence and first-degree donors ($P = 0.152$). Operative and postoperative factors, such as cyclosporine use (CNI, $P = 0.012$), mono or no immunosuppressive agent ($P < 0.001$), postoperative biliary complications ($P < 0.001$), and active IBD after LT ($P < 0.001$), were significantly associated with PSC recurrence. In the final multivariate Cox regression model, donor age ≥45 years [HR, 1.65; 95% confidence interval (CI), 1.21–2.69; $P = 0.003$] and mono or no immunosuppressive agent 1-year after LT [HR, 2.38;

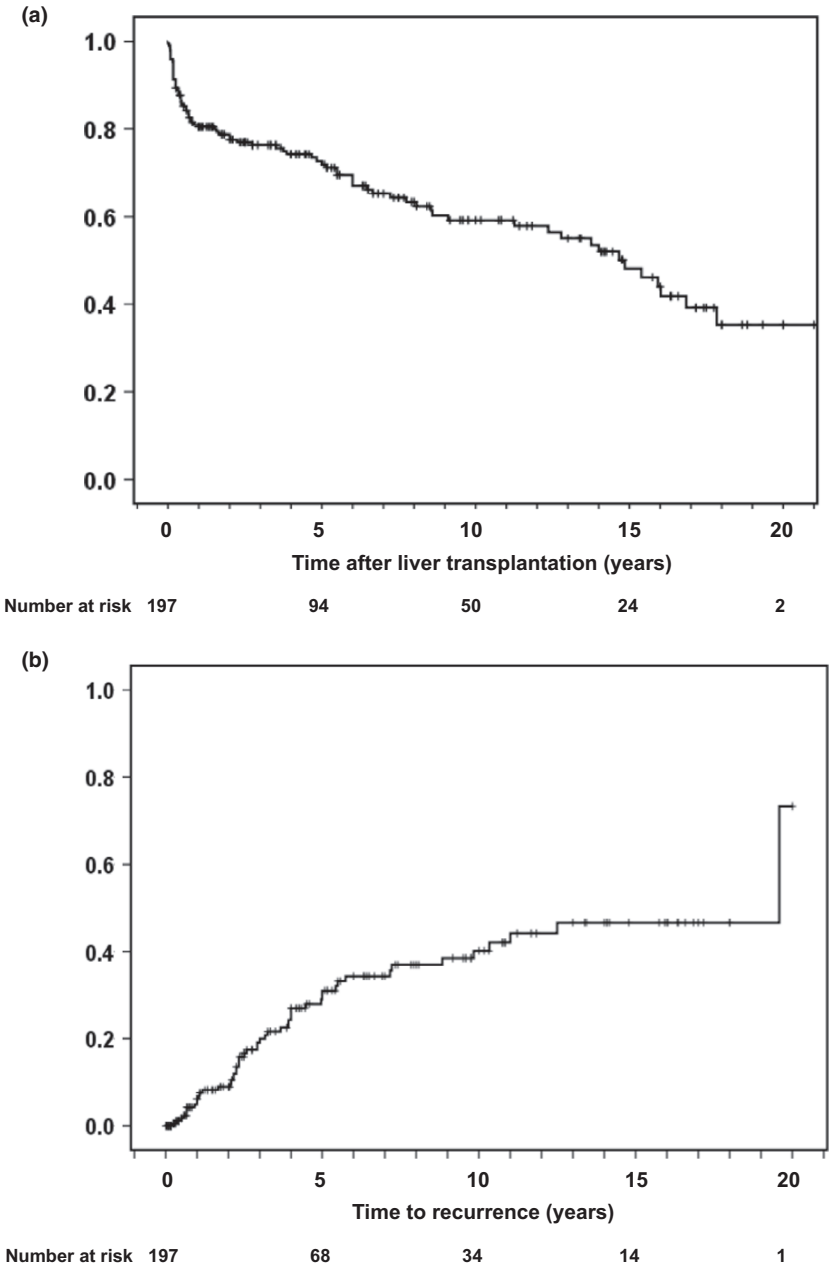

Figure 2 Overall patient survival rate (a) and recurrence rate (b) in 197 patients.

95% CI, 1.23–3.45; $P = 0.011$] were independent factors for PSC recurrence (Table 2). Recurrence-free graft survival stratified by these two factors is shown in Fig. 4.

Factors associated with PSC recurrence among ABO-identical or -compatible adult LDLT patients

To exclude the heterogeneity and the possible bias, the same analysis to elucidate the factors associated with disease recurrence in the graft was done among ABO-identical or -compatible adult LDLT patients. Finally, 142 recipients were the subjects in this sub-analysis, and their demographics are shown in Table 3. The results were similar with those presented above. Univariate analysis of preoperative and donor characteristics revealed a significant association of PSC recurrence with donor age ($P < 0.001$) and HLA matched number ≥4 loci ($P = 0.021$). Operative and postoperative factors, such as mono or no immunosuppressive agent ($P < 0.001$), postoperative biliary complications ($P < 0.001$), and active IBD after LT ($P < 0.001$), were significantly associated with PSC recurrence. In the final multivariate Cox regression model, donor age ≥45 years (HR, 1.71; 95% CI, 1.22–2.91; $P = 0.002$) and mono or no immunosuppressive agent 1-year after LT (HR, 2.35; 95% CI, 1.61–3.74; $P = 0.003$) were independent factors

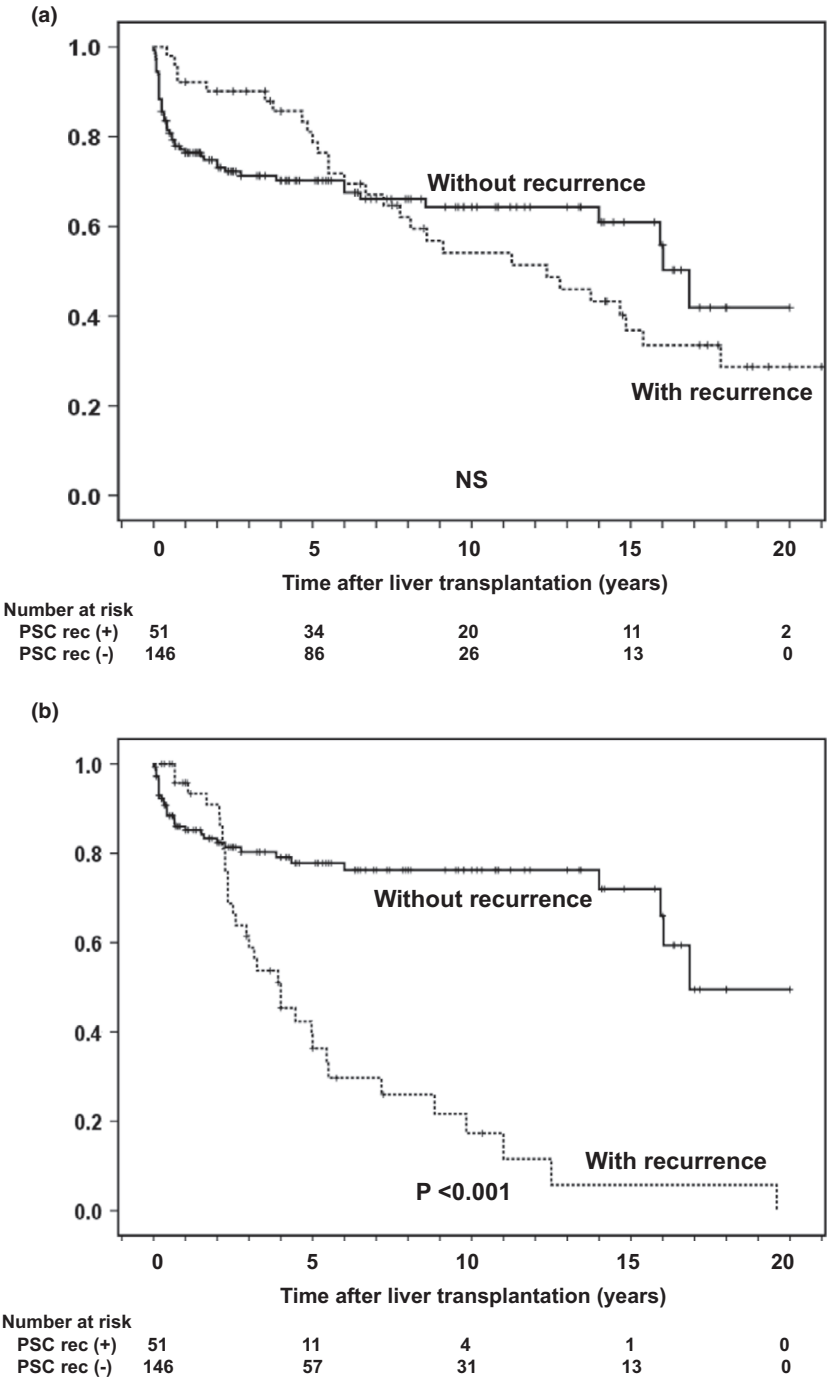

Figure 3 Patient survival (a) and graft survival (b) stratified by the development of PSC recurrence in 197 patients.

for PSC recurrence (Table 4). In addition, considering the confounding bias between first-degree donors and HLA matched number ≥4 loci, additional analysis was done excluding first-degree donors factor, which led to similar results (Table S1).

Discussion

This study of 197 consecutive LT recipients for PSC from a Japanese multicenter cohort revealed a PSC recurrence rate of 25%. The present results confirmed poor graft survival among those with recurrent PSC. Independent factors associated with recurrent disease were older donors (age ≥45 years) and maintenance with decreased immunosuppression (mono or no immunosuppressive agent 1-year after LT).

The incidence of recurrent PSC varies widely among transplant centers, which may reflect differences in the diagnostic criteria, length and type of follow-up, and inclusion of protocol biopsies. Recent systematic reviews

Table 1. Patient demographics.

	All patients (n = 180)	Recurrence (n = 46)	No-recurrence (n = 134)	P value
Recipient factors				
Age	36.4 ± 15.5, 35 (10–68)	31.3 ± 14.7	38.4 ± 15.3	0.417
Adult/pediatric	166/14	42/4	124/10	0.561
Gender (male/female)	102/78	26/20	76/58	0.982
MELD score	18.0 ± 6.7, 18 (5–37)	16.9 ± 7.3	18.3 ± 6.4	0.205
Child–Pugh–Turcotte score	9.8 ± 1.9, 10 (5–14)	9.6 ± 2.1	9.9 ± 1.8	0.361
Pre-LT IBD (yes/no)	36% (65/115)	39% (18/28)	35% (47/87)	0.621
Donor factors				
Donor gender (male/female)	100/80	28/18	72/62	0.969
Donor age	42.4 ± 12.4, 44 (19–65)	47.2 ± 10.7	40.8 ± 12.6	0.001
Related/unrelated (deceased donor or spouse)	126/54	38/18	88/46	0.031
1st degree relationship (yes/no)	48% (86/94)	61% (28/18)	43% (58/76)	0.039
ABO-incompatible (yes/no)	9 % (16/164)	4/42	12/122	0.957
HLA-A matched number of loci (0/1/2)	33/108/39	3/30/13	30/78/26	0.105
HLA-B matched number of loci (0/1/2)	50/107/23	7/33/6	43/74/17	0.079
HLA-DR matched number of loci (0/1/2)	39/110/31	9/30/7	30/80/24	0.802
Total matched number of HLA loci (≥4/<4)	24% (43/137)	35% (16/30)	24% (32/102)	0.149
Gender (match/mismatch)	82/98	22/24	60/74	0.720
Operative factors				
LDLT/DDLT	90% (162/18)	91% (42/4)	88% (120/14)	0.733
Hepaticojejunostomy/duct-to-duct	89% (166/14)	91% (42/4)	93% (124/10)	0.788
Immunosuppression				
CNI = cyclosporine (tacrolimus/cyclosporine)	17% (149/31)	24% (35/11)	15% (114/20)	0.013
Number of immunosuppresive agents at LT (2/3)	161/19	40/6	121/13	0.524
Number of immunosuppresive agents at 1-year after LT (0/1/2/3)	2/40/79/59	1/32/9/4	1/8/70/55	<0.001
Mono or none immunosuppression 1-year after LT (yes/no)	23% (42/138)	72% (33/13)	6.7% (9/125)	<0.001
Steroid 1-year after LT (yes/no)	74% (136/44)	65% (30/16)	79% (106/28)	0.059
Postoperative factors				
Biliary complication (yes/no)	25% (45/136)	48% (22/24)	17% (23/112)	<0.001
Acute rejection (yes/no)	42% (76/104)	41% (19/27)	43% (57/77)	0.884
CMV antigenemia (yes/no)	31% (56/124)	33% (15/31)	27% (36/93)	0.456
Active IBD after LT (yes/no)	26% (47/133)	52% (24/22)	17% (23/111)	<0.001

CMV, cytomegalovirus; CNI, calcineurin inhibitor; DDLT, deceased donor liver transplantation, IBD, inflammatory bowel disease; LDLT, living donor liver transplantation; LT, liver transplantation; MELD, model for end-stage liver disease.

Table 2. Cox models for time to PSC recurrence.

	Univariate model		Multivariate model	
	Hazard ratio [95% confidence interval]	*P* value	Hazard ratio [95% confidence interval]	*P* value
Recipient factors				
Age	0.88 [0.57–2.15]	0.428		
Gender	1.31 [0.92–2.58]	0.611		
MELD score	0.56 [0.32–2.02]	0.203		
Pre-LT IBD	0.33 [0.23–1.45]	0.119		
Donor factors				
Donor gender	0.58 [0.33–3.14]	0.458		
Donor age ≥ 45 years	2.83 [1.41–5.65]	<0.001	1.65 [1.21–2.69]	0.003
1st degree relationship	0.96 [0.69–1.33]	0.152		
ABO-incompatible	1.336 [0.98–3.12]	0.301		
Total matched number of HLA loci ≥ 4	0.76 [0.55–1.36]	0.122		
Gender match	0.99 [0.98–1.02]	0.203		
Operative factors				
LDLT/DDLT	1.01 [0.95–1.11]	0.889		
Biliary reconstruction	1.22 [0.78–3.24]	0.791		
Immunosuppression				
Cyclosporine as CNI	1.08 [1.02–1.21]	0.012		
Mono or none immunosuppression 1-year after LT	2.21 [1.81–3.15]	<0.001	2.38 [1.23–3.45]	0.011
Steroid 1-year after LT	0.65 [0.54–2.33]	0.665		
Postoperative factors				
Biliary complication	2.69 [1.23–5.85]	<0.001		
Acute rejection	1.06 [0.89–1.36]	0.991		
CMV antigenemia	0.65 [0.45–1.69]	0.521		
Active IBD after LT	1.87 [1.31–3.25]	<0.001		

CMV, cytomegalovirus; CNI, calcineurin inhibitor; DDLT, deceased donor liver transplantation, IBD, inflammatory bowel disease; LDLT, living donor liver transplantation; LT, liver transplantation; MELD, model for end-stage liver disease.

of 22 and 14 publications reported average recurrence rates of 18.5% (5.7–59.1%) [8] and 17.7% (10.1–27.1%) [26], respectively. Early studies reported equivalent graft and patient survival rates among LT recipients for PSC with or without recurrent disease [7,27], but more recent reports, including the present report, based on long-term data indicate that recurrent PSC significantly affects graft survival, need for retransplant, and patient survival [14,20,28]. In the present study, the patient and primary graft survival curves (Fig. 2) cross over during first five years. This can be explained by the facts that those with recurrence survive in short-term at least as long as the time to diagnosis of recurrent disease and that 45% (23/51) of those with progressive recurrent disease underwent re-LT. According to the Japanese Liver Transplant Registry (1989–2017) [29], the 5-, 10-, and 15-year patient survival rates among PSC recipients ($n = 245$) were 73%, 58%, and 48%—significantly worse than those of recipients with primary biliary cholangitis (79%, 74%, and 68%, $n = 744$) and all recipients (79%, 74%, and 69%, $n = 8572$; $P < 0.001$). Consequently, identifying potential risk factors is essential toward categorizing high-risk patients and developing interventions to reduce the incidence of recurrent disease.

The etiopathogenesis of PSC is multifactorial and includes genetics (HLA), autoimmunity, and inflammation caused by infectious agents, especially with regard to the association with IBD [2]. Accordingly, these factors have been investigated regarding recurrent PSC among LT recipients in previous studies with variable results. A recent systematic review with a meta-analysis [26] found that cholangiocarcinoma before LT, co-existence of active IBD, donor age, MELD score, and ACR were risk factors for recurrent PSC, while colectomy before LT was protective, among 2159 PSC recipients. Co-existence of IBD is a major risk factor for recurrent disease [12–14], but the incidence is significantly lower among Japanese PSC patients compared with Western populations [30,31]. Therefore, the rate of IBD was low

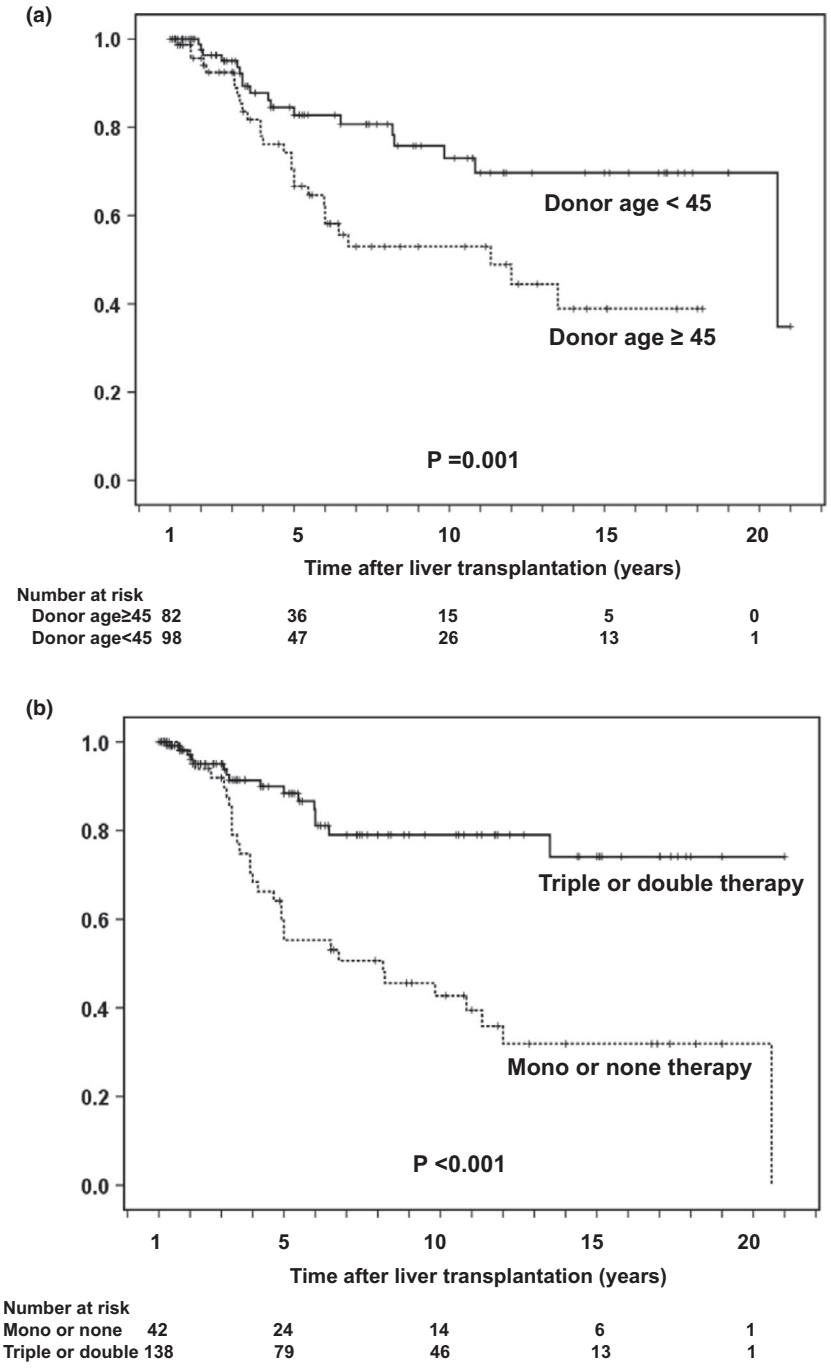

Figure 4 Recurrence-free survival stratified by the donor age (a) and the immunosuppressive regimen (b) among 180 patients who survived more than 1 year after liver transplantation.

in the present LT cohort, yet the results of the univariate analysis indicated that active IBD after LT may affect PSC recurrence. Colectomy may be protective against recurrent PSC after LT and disease progression itself [15,32], but the colectomy rate among Japanese patients in the present study was too low to confirm such an effect. Another frequent and important comorbidity of PSC and risk factor for recurrent PSC is cholangiocarcinoma [21], but the rate of pre-LT cholangiocarcinoma was zero in the present cohort. A possible association between biliary complications with PSC recurrence was demonstrated in the present study. As Hildebrand *et al.* [13] reported, however, biliary strictures, especially anastomotic strictures with or without biliary leakage, are closely linked with PSC recurrence and it is difficult to completely distinguish between them in a retrospective study despite the attempts of investigators to exclude anastomotic

Table 3. Patient demographics (ABO-identical or -compatible adult LDLT patients).

	Patients (n = 142)	Recurrence (n = 40)	No-recurrence (n = 102)	P value
Recipient factors				
Age	43.2 ± 12.8, 40 (18-68)	42.2 ± 13.1	43.1 ± 10.9	0.556
Gender (male/female)	81/61	22/18	59/43	0.758
MELD score	16.9 ± 7.2, 18(6-37)	16.5 ± 7.9	17.5 ± 6.9	0.339
Child–Pugh–Turcotte score	10.7 ± 1.5, 10(5-14)	10.4 ± 2.9	10.9 ± 3.1	0.776
Pre-LT IBD (yes/no)	38% (54/88)	48% (18/22)	34% (36/66)	0.284
Donor factors				
Donor gender (male/female)	82/60	22/18	60/42	0.135
Donor age	39.5 ± 9.2, 39(20-65)	44.2 ± 11.2	38.4 ± 9.3	<0.001
Related/unrelated (spouse)	106/36	29/11	67/35	0.435
1st degree relationship (yes/no)	49% (70/72)	60% (24/16)	45% (46/56)	0.110
HLA-A matched number of loci (0/1/2)	20/92/30	3/27/11	17/65/19	0.337
HLA-B matched number of loci (0/1/2)	30/98/14	6/29/5	24/69/9	0.477
HLA-DR matched number of loci (0/1/2)	17/100/25	6/25/9	11/75/16	0.432
Total matched number of HLA loci (≥4/<4)	24% (34/108)	38% (15/25)	19% (19/83)	0.001
Gender (match/mismatch)	65/77	20/20	45/57	0.598
Operative factors				
Hepaticojejunostomy/duct-to-duct	99% (141/1)	100% (40/0)	99% (101/1)	0.530
Immunosuppression				
CNI = cyclosporine (tacrolimus/cyclosporine)	12% (125/17)	25% (30/10)	6.8% (95/7)	0.002
Number of immunosuppresive agents at LT (2/3)	125/17	35/5	90/12	0.903
Number of immunosuppresive agents at 1-year after LT (0/1/2/3)	0/37/63/42	0/31/6/3	0/6/57/39	<0.001
Mono or none immunosuppression 1-year after LT(yes/no)	26% (37/105)	78% (31/9)	5.9% (6/96)	<0.001
Steroid 1-year after LT (yes/no)	84% (119/23)	70% (28/12)	89% (91/11)	0.051
Postoperative factors				
Biliary complication (yes/no)	27% (38/104)	45% (18/22)	20% (20/82)	0.002
Acute rejection (yes/no)	43% (61/81)	40% (16/24)	44% (45/57)	0.656
CMV antigenemia (yes/no)	35% (50/92)	33% (13/27)	26% (27/65)	0.472
Active IBD after LT (yes/no)	28% (40/102)	55% (22/18)	18% (18/84)	<0.001

CMV, cytomegalovirus; CNI, calcineurin inhibitor; DDLT, deceased donor liver transplantation, IBD, inflammatory bowel disease; LDLT, living donor liver transplantation; LT, liver transplantation; MELD, model for end-stage liver disease.

Table 4. Cox models for time to PSC recurrence (ABO-identical or -compatible adult LDLT patients).

	Univariate model		Multivariate model	
	Hazard ratio [95% confidence interval]	*P* value	Hazard ratio [95% confidence interval]	*P* value
Recipient factors				
Age	0.99 [0.64–1.65]	0.611		
Gender	1.24 [0.78–1.98]	0.498		
MELD score	0.71 [0.45–2.33]	0.364		
Pre-LT IBD	0.84 [0.77–1.32]	0.121		
Donor factors				
Donor gender	0.97 [0.88–2.12]	0.332		
Donor age ≥45 years	2.58 [1.52–4.63]	<0.001	1.71 [1.22–2.91]	0.002
1st degree relationship	0.96 [0.88–3.21]	0.082		
Total matched number of HLA loci ≥4	1.14 [1.03–1.45]	0.021		
Gender match	0.88 [0.78–1.78]	0.203		
Operative factors				
Biliary reconstruction	0.99 [0.91–1.09]	0.995		
Immunosuppression				
Cyclosporine as CNI	1.21 [0.99–1.45]	0.052		
Mono or none immunosuppression 1-year after LT	2.31 [1.61–4.05]	<0.001	2.31 [1.61–3.74]	0.003
Steroid 1-year after LT	1.45 [0.23–2.88]	0.154		
Postoperative factors				
Biliary complication	2.71 [1.31–6.12]	<0.001		
Acute rejection	1.34 [0.74–1.65]	0.821		
CMV antigenemia	0.98 [0.85–1.71]	0.659		
Active IBD after LT	1.61 [1.13–3.21]	<0.001		

CMV, cytomegalovirus; CNI, calcineurin inhibitor; DDLT, deceased donor liver transplantation, IBD, inflammatory bowel disease; LDLT, living donor liver transplantation; LT, liver transplantation; MELD, model for end-stage liver disease.

strictures from the recurrent disease. Other risk factors identified in previous studies, such as high MELD score, episodes of ACR and its treatments, and CMV infection, were not confirmed to be associated with disease recurrence in the present study. Finally, as for donor factors, donor age was found to be a significant risk factor for recurrent PSC in accordance with previous reports [16,26].

Bergquist *et al*. [33] reported that first-degree relatives and siblings are associated with an 11-fold higher PSC prevalence compared with those from nonrelatives, which might indicate a shared genetic susceptibility factors for PSC. In genome-wide association studies, several genome-wide significant risk loci have been identified, which positions autoimmune processes central to the pathogenesis of PSC [2,34]. The predominant genetic findings localize within the HLA complex on chromosome 6, suggesting that adaptive immune responses are involved [35]. The HLA class I and/or class II genes are most likely responsible for the findings in PSC. In this regard, we collected data of HLA-A, HLA-B, and HLA-DR from both donors and recipients, and investigated HLA matching in each case. Neither related donor nor first-degree relative donor was significantly related to recurrent disease in the present study, in contrast to our previous survey, although a weak relation was still detected with first-degree relative donors. The effects of living-related donors, especially first-degree donors, on outcomes of LT recipients for PSC are still controversial with conflicting results [6,11,16,36]. Most recently, Indian study demonstrated excellent short-term outcome in LDLT [37]. Regarding HLA, while the matching number in each HLA was not associated with the disease recurrence, total number of matching loci (4 and more) was associated with PSC recurrence in the univariate model among LDLT recipients, but did not reach significance in multivariate analysis.

The most important issue in this study was the hypothesis that immunosuppressive modification, namely enhancement of immunosuppression during the maintenance phase, may be protective against recurrent PSC. Reports of an association between immunosuppression and recurrent PSC are scarce [17,19,32].

Consistent with other autoimmune liver diseases, disease recurrence as well as rejection is more common after LT for PSC [8]; hence, immunosuppression combining long-term steroids, CNI, with or without a third agent (MMF, mTOR inhibitor, or azathioprine) is used in many transplant programs. The continuation of steroid was once reported to be not protective in the previous report [38]. Evidence-based guidelines regarding the optimal immunosuppressive regimen in PSC are lacking, however, and according to previous reports on LT for PSC, current practice ranges widely from mono to triple immunosuppression over the long-term. Since recognizing the possible increased risk for recurrent PSC among LDLT recipients with grafts from closely related donors [11], Japanese transplant clinicians have tended to use a triple regimen (CNI, steroid, and MMF) or to increase the maintenance dose of CNI and steroid among PSC recipients. Actually, double or triple therapy accounted for 91% of PSC during the maintenance phase among recent cases, in contrast to the high proportion of those receiving single treatment (56%) among previous cases in the present cohort (data not shown). In this study, cyclosporine use as a CNI, and mono or no immunosuppressive regimen during the maintenance phase, with mono or no immunosuppressive regimen being an independent risk factor for recurrent disease. The present study demonstrated the possible protective effect of immunosuppressive regimen modulation on the development of recurrent PSC after LT.

The main limitation of the study is the retrospective multicenter design. The lack of routine MRC, ERC, and/or protocol liver biopsies is another major limitation of our study as well as most previous studies on LT for PSC. With regard to PSC itself, it is well known that considerable bile duct irregularities might be present for a long time before patients show symptoms or liver enzyme abnormalities. Consequently, some recurrent cases might be missed. In addition, while we noted low immunosuppression as a risk factor for disease recurrence, the total dose, the maintenance dose, and the actual serum trough level of each immunosuppressive drug were center-determined and not collected. The intensity of immunosuppression depends not only on the number of drugs used but also on the serum level of each drug. Most importantly, present study design, a multicenter retrospective study, does not allow for deriving causality between mono (or no) immunosuppressive therapy and recurrent PSC. Factors associated with the intensity of immunosuppression such as, actual trough level of CNI, the way of tapering immunosuppressants, and the timing of withdrawal or addition of some drug, were not considered in the risk analyses for recurrent PSC. A multicenter prospective register on LT for PSC might be essential for collecting these data and verifying the present results, and ideally, regular posttransplant MRCs and protocol liver biopsies should be performed in such studies.

In conclusion, in this Japanese multicenter retrospective study of 197 LT recipients for PSC, we found a PSC recurrence rate of 25% with significantly worse graft survival (18% 10-year after LT). Older donors (age ≥45 years) and maintenance with mono or no immunotherapy were found to be independent predictors of disease recurrence.

Authorship

NA, HE, HO, ST, NK, AT and HT: designed the study. NA and AY: participated in data acquisition. KH: participated in the analysis and interpretation of data. NA: wrote the manuscript. All the authors approved the final version of the manuscript.

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Conflict of interest

None to be declared.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Cox models for time to PSC recurrence (ABO-identical or -compatible adult LDLT patients).

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