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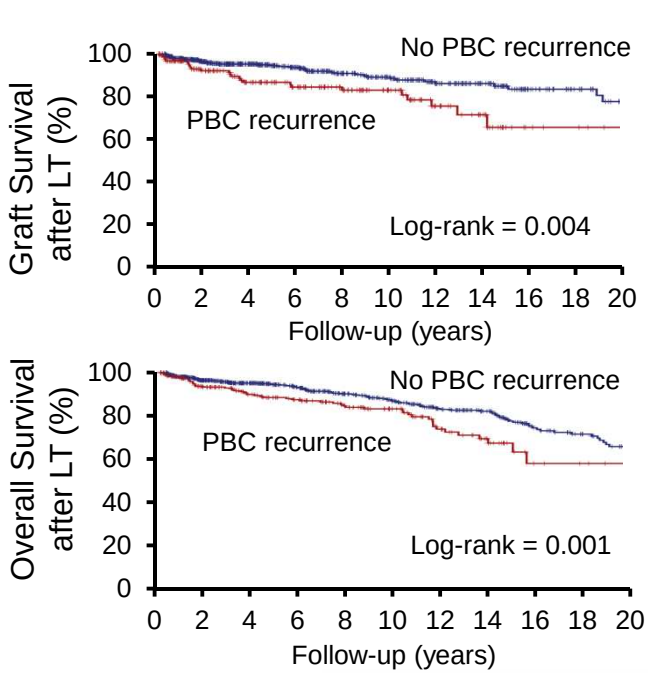
Global PBC Group

785 pts received **Liver Transplant (LT)** due to **Primary Biliary Cholangitis (PBC)**

240 pts developed **Recurrence of PBC**

Risk Factors for rPBC

- **Younger Age** at the time of diagnosis and LT
- **Tacrolimus** use
- **Biochemical Cholestasis** after LT



Gastroenterology

Factors Associated With Recurrence of Primary Biliary Cholangitis After Liver Transplantation and Effects on Graft and Patient Survival

Short Title: PBC recurrence after transplant and survival

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Abbreviations:

ALT, alanine aminotransferase

AMA, anti-mitochondrial antibodies

AST, aspartate aminotransferase

ALP, alkaline phosphatase

HR, hazard ratio

INR, international normalized ratio

MELD, model for end-stage liver disease

LT, liver transplantation

OCA, obeticholic acid

PBC, primary biliary cholangitis

UDCA, ursodeoxycholic acid

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Aldo J. Montano-Loza, study design, analyses of the data, creation of the first draft of the manuscript and final version; Bettina E. Hansen, analyses of the data, interpretation of data and writing the manuscript; Christophe Corpechot analyses of the data, interpretation of data and writing the manuscript; Davide Roccarina, interpretation of data, writing the manuscript, and critical revision of the manuscript for important intellectual content; Douglas Thorburn, assisted with the study conception and revising the manuscript; Palak Trivedi, assisted with the study conception and writing the manuscript; Gideon Hirschfield, interpretation of data, writing the manuscript, and critical revision of the manuscript for important intellectual content; Patrick McDowell, Raoul Poupon, tabulating data and writing the manuscript; Jerome Dumortier, tabulating data, interpretation of data, and writing the manuscript; Alexie Bosch, tabulating data, and writing the manuscript; Emiliano Giostria, tabulating data and writing the manuscript; Filomena Conti, tabulating data and writing the manuscript; Albert Parés, interpretation of data, writing the manuscript, and critical revision of the manuscript for important intellectual content; Anna Reig, Annarosa Floreani, Francesco Paolo Russo, assisted with the compilation of the data, revising the manuscript; Jorn Goet, tabulating data, interpretation of data, writing the manuscript, and critical revision of the manuscript for important intellectual content; Maren H. Harms, Henk van Buuren, Natalie Van den Ende, assisted with critical revision of the manuscript for important intellectual content; Frederik Nevens, assisted with the study conception and writing the manuscript; Xavier Verhelst, Maria Francesca Donato, Federica Malinverno assisted with critical revision of the manuscript for important intellectual content; Maryam Ebadi, tabulating data; organization of the database, analyses of the data, revision of the final version of the manuscript and submitting manuscript for review; Andrew Mason, study design, analyses of the data, and creation of the first draft of the manuscript and final version.

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ABSTRACT

Background and aims: Primary biliary cholangitis (PBC) frequently recurs after liver transplantation. We evaluated risk factors associated with recurrence of PBC and its effects on patient and graft survival in a multi-center, international cohort (the global PBC group).

Methods: We collected demographic and clinical data from 785 patients (89% female) with PBC who underwent liver transplantation (mean age, 54±9 years) from February 1983 through June 2016, among 13 centers in North America and Europe. Results from biochemical tests performed within 12 months of liver transplantation were analyzed to determine whether markers of cholestasis could identify patients with recurrence of PBC (based on histologic analysis). Patients were followed for a median 6.9 years (interquartile range, 6.1–7.9 years).

Results: PBC recurred in 22% of patients after 5 years and 36% after 10-years. Age at diagnosis less than 50 years (hazard ratio [HR], 1.79; 95% CI, 1.36–2.36; $P<.001$), age at liver transplantation less than 60 years (HR, 1.39; 95% CI, 1.02–1.90; $P=.04$), use of tacrolimus (HR, 2.31; 95% CI, 1.72–3.10; $P<.001$), and biochemical markers of severe cholestasis (bilirubin ≥100 μmol or alkaline phosphatase >3-fold the upper-limit of normal) at 6 months after liver transplantation (HR, 1.79; 95% CI, 1.16–2.76; $P=.008$) were associated with higher risk of PBC recurrence, whereas use of cyclosporine reduced risk of PBC recurrence (HR, 0.62; 95% CI, 0.46–0.82; $P=.001$). In multivariable Cox regression with time-dependent covariate, recurrence of PBC significantly associated with graft loss (HR, 2.01; 95% CI, 1.16–3.51; $P=.01$) and death (HR, 1.72; 95% CI, 1.11–2.65; $P=.02$).

Conclusion: Younger age at the time of diagnosis with PBC or at liver transplantation, tacrolimus use, and biochemical markers of cholestasis after liver transplantation are associated

with PBC recurrence. PBC recurrence reduces odds of graft and patient survival. Strategies are needed to prevent PBC recurrence or reduce its negative effects.

Keywords: cholestatic; recurrent disease; re-transplantation; autoimmune liver disease.

INTRODUCTION

Primary biliary cholangitis (PBC) [1-3] is a chronic cholestatic disease characterized by granulomatous destruction of intrahepatic bile ducts. Up to 10% of the patients listed for liver transplantation (LT) in North America and Europe have a diagnosis of PBC [4, 5]. The outcome after LT for patients with PBC is generally good, but recurrent PBC has been reported in a range from 17% up to 46% after LT [4, 6-11]. This divergence in frequency may be related to differences in these studies with respect to the use of protocol versus clinically indicated liver biopsies, number of patients in each series, and follow-up [4, 6-11]. Prior reports have also suggested that the development of PBC recurrence has no significant impact on long-term patient survival or need for a second LT [4, 12], and thus its clinical impact has been questioned. However, these observations may be related to inadequate follow-up and limited numbers of patients.

The specific immunosuppression regimens employed are the best reproducible factors linked with recurrence of PBC [13-15]. Tacrolimus has been associated with accelerated onset and severity of PBC recurrence, whereas cyclosporine may be more protective [4, 10, 15-17]. The relevance of other factors associated with recurrence of PBC, such as changes in the liver biochemistry shortly after LT, remain relatively unexplored in PBC, whereas biochemical evidence of early cholestasis has been associated with recurrent disease and worse outcomes in patients with chronic hepatitis C infection and primary sclerosing cholangitis [18].

Accordingly, we conducted a multicenter study in 13 LT centers to evaluate the probability and risk factors associated with recurrence of PBC and the association between recurrence of PBC and patient and graft survival. Second, to determine biomarkers that may identify patients at risk of PBC recurrence, we evaluated whether liver biochemistry tests within the first year after LT were associated with subsequent recurrent disease. It was hypothesized that biochemical

abnormalities during the first year after LT increase the risk for recurrence of PBC which in turn negatively impacts graft and patient survival.

METHODS

Study Population

Seven hundred-eighty five patients who received a LT from February 1983 until June 2016 with diagnosis of PBC from 13 centers across North America and Europe were evaluated (Supplementary Figure 1). All patients included in Global PBC group database were diagnosed according to the guidelines of European Association for the Study of the Liver (EASL)[19]. Participant LT centers were the Liver Unit, University of Alberta, Edmonton, AB, Canada (n=153), Erasmus MC, University Medical Center Rotterdam, the Netherlands (n=48), UCL Institute for Liver and Digestive Health, Royal Free Hospital, London, United Kingdom (n=158), NIHR Centre for Liver Research, University of Birmingham, United Kingdom (n=127), Reference Center for Inflammatory Biliary Diseases, Saint-Antoine Hospital, Paris, France (n=29), Liver Transplant Unit, Edouard Herriot Hospital, Hospices Civils de Lyon, Lyon, France (n=27), Hepatology and Gastroenterology Department, University Hospitals of Geneva, Geneva, Switzerland (n=20), Liver Transplant Unit, Pitié-Salpêtrière Hôpital, Paris France (n=15), Liver Unit, Hospital Clínic, University of Barcelona, IDIBAPS, CIBERehd, Barcelona Spain (n=45), Department of Surgery, Oncology and Gastroenterology, University of Padova, Padova, Italy (n=25), Division Liver and Biliopancreatic Disorders, Leuven, Belgium (n=55), Department of Gastroenterology and Hepatology, Ghent University Hospital, Ghent, Belgium (n=15), and Transplant Hepatology Unit, Division of Gastroenterology and Hepatology, Maggiore Hospital

Policlinico, Milan, Italy (n=68). Centers contributing more than 50 LT for PBC were defined as high volume centers.

Clinical and Laboratory Assessments

Data retracted from the medical records included gender, age at diagnosis of PBC and LT, time between diagnosis of PBC and LT, antimitochondrial antibodies (AMA), immunoglobulin M (IgM) levels before LT, MELD score [20]. Post-LT viral infections, rejection episodes, compatibility, and immunosuppressive regimens were also recorded. The use of potential hepatotoxic drugs were reviewed in all patients with abnormal liver biochemistry tests after LT and recurrence of PBC diagnosis. Also, the use of ursodeoxycholic acid (UDCA), either as preventive or curative treatment was collected. Treatment was classified as preventative UDCA when introduced immediately after LT (first or second postoperative week), and continued long-term as reported elsewhere (24). Treatment was classified as curative UDCA when started after histological diagnosis of recurrence of PBC, or because of abnormal liver biochemistry tests. One hundred and ninety-three patients received UDCA, 13 as preventive treatment (1.7%) and 180 as curative treatment (23%). Of the patients who received curative UDCA, 64 (36%) had histologically documented recurrence of PBC and 116 (64%) had abnormal liver biochemistry tests. In order to minimize the risk of variation of data collection, we discussed this project in our bi-annual meetings of the Global PBC group, and develop instructions and standardized the collection of variables. Clinical practice based laboratory follow-up of patients was conducted in a similar fashion where routine measurements of liver biochemistry test and immunosuppression levels were performed every month after LT within the first year and at least at two to three month intervals after the first year.

Liver Biochemistry Tests Post-Liver Transplant

Liver biochemistries including ALT, AST, ALP, GGT and bilirubin were collected at 3-, 6-, and 12-months after LT. Values, both raw and divided by the upper limit of normal (ULN), based on center-specific values were taken into account for the analyses. The ULN for ALT ranged from 32 to 50 U/L, AST from 32 to 40 U/L, ALP from 105 to 190 U/L, GGT from 35 to 61 U/L, and bilirubin 17 to 22 µmol/L between the different LT centers. Biochemical mild cholestasis after LT was defined as ALP level >2 times the ULN or a combined elevation of both bilirubin and ALP levels; whereas biochemical severe cholestasis was defined as (bilirubin ≥100 µmol [>5.9 mg/dL] or ALP >3 times the ULN as reported elsewhere [18, 21].

Histological Assessment for Recurrent Primary Biliary Cholangitis

Biopsies after LT were performed in 522 patients (67%) with mean 1.7±0.1 biopsies per patient (median 1, range 1-11). Of those, 270 patients (52%) underwent protocol liver biopsies whereas in 252 patients (48%) had clinically-driven biopsies with abnormal liver biochemistry. A diagnosis of recurrence of PBC was made histologically and defined by the presence of liver histology compatible with PBC in the absence of other biliary disease including hepatic artery thrombosis, and anastomosis stricture [22]. In addition, allograft rejection, presence of infections, and concomitant use of potential hepatotoxic drugs were ruled out. Histologic features of recurrence of PBC were the presence of florid duct lesions or destructive lymphocytic cholangitis with significant portal infiltrate in the absence of endothelialitis [22] (Supplementary Figure 1). Histological diagnosis of recurrence of PBC was made by liver pathologists in all cases. For majority of the cases (83%), pathologists were blinded to the clinical question and in 48% of the cases another pathologist was asked for the second opinion. Histological recurrence

of PBC was graded according to Ludwig and Scheuer classification [23]. Overlap syndrome with autoimmune hepatitis was ruled out in all patients with recurrence of PBC according to Paris criteria [24]. At the diagnosis of recurrence of PBC, ALT and AST were lower than five times the ULN (mean ALT 68±6 U/L; mean AST 74±9 U/L), IgG levels lower than two times the ULN (mean IgG 13.3±0.6 g/L) and the histological examination revealed lack of confluent and severe interface hepatitis with plasma cells infiltration.

Cholestasis Evaluation after Liver Transplantation

All patients with cholestasis and suspicious of recurrence of PBC after LT had an ultrasound doppler examination to rule out the presence of biliary duct dilation or stricture, and hepatic artery thrombosis as reported elsewhere [18].

Immunosuppression Regimens

The type of immunosuppression during the first year was recorded. The predominant calcineurin inhibitor, either cyclosporine or tacrolimus and other immunosuppression medications including azathioprine, mycophenolate mofetil, prednisone and sirolimus were all assessed. Changes in the main immunosuppression after the first year of LT were also recorded. Forty-four patients had changes in their main immunosuppression after the first year including cyclosporine to tacrolimus (n=20), cyclosporine to sirolimus (n=10), tacrolimus to cyclosporine (n=6), sirolimus to cyclosporine (n=3), tacrolimus to sirolimus (n=4), and sirolimus to tacrolimus (n=1).

Statistical Analyses

The Fisher exact probability test was used to compare categorical variables, and the unpaired *t* test was used to compare differences in means of continuous variables.

Prognostic factors for recurrence of PBC were analyzed by Cox regression univariate analysis [25]. Variables with P-value less than 0.1 in the univariate analysis were included in the Cox regression multivariate analysis. Age cut-offs at diagnosis and time of LT associated with higher risk of PBC recurrence were established using a receiver-operating characteristic (ROC) analysis. Model ability to differentiate between outcome groups was assessed using the area under the curve (AUC) and cut-offs with the highest Youden's Index (sensitivity + specificity − 1) were included in adjusted Cox regression multivariate analysis. Value with the highest significant P-value was considered as the optimal cut-off point. Cumulative incidence of recurrence of PBC after LT were calculated using the Kaplan-Meier method, and they were compared using the Log-Rank (Mantel-Cox) test [26].

To determine whether incidence of recurrence of PBC was significantly associated with graft loss and overall survival, recurrence of PBC impact on the hazard rate of graft loss and survival was assessed in univariate and multivariable Cox regression analyses. In these analyses, the time until patients had recurrence of PBC was modelled as a time-dependent covariate. The association of recurrence of PBC with graft loss and overall survival was analyzed as time-dependent covariate. Variables with p-value less than 0.1 in the univariate analysis and other relevant variables were included in the Cox proportional hazard regression multivariate analysis. Patients who did not develop recurrence of PBC and died and those who were lost during follow-up were censored at the time of death or at the time of their last visit. In order to analyze the clinical impact of recurrence of PBC, patients who died or lost the graft within the first three-months after LT were excluded from the survival analysis, as these outcomes were deemed

related to surgical complications. Graft loss was defined using death-censored definition of graft failure, therefore, graft loss did not include patients who died with a functioning graft, and included only deaths secondary to or associated with graft failure (i.e. recurrent disease, *de novo* disease, chronic ductopenic rejection, sepsis in patients with biliary or vascular complications, or cirrhosis development on the graft) or re-transplantation.

Cumulative probabilities of graft and overall survival after LT were calculated using semi-Markov models (so-called "clock reset" models), because each time the patient enters a new state time is reset to 0 (in this case recurrence of PBC) [27]. Data are presented as the mean ± standard error in tables and text, and median with interquartile ranges (IQR) in case data was not normally distributed.

RESULTS

Characteristics, Frequency and Probability of Primary Biliary Cholangitis Recurrence

The mean age of the study population at LT was 54±1 years (median, 54 years; IQR: 53-56 years), and 696 patients (89%) were women.

The main features of patients who received a LT for PBC are shown in Table 1. Recurrence of PBC was diagnosed in 240 of the 785 patients (31%). The median time for recurrence of PBC in the 240 patients was 4.4 years (IQR: 3.4-5.1). The probability of recurrence of PBC was 22%, 36%, 50%, and 55% at 5-, 10-, 15-, and 20-years, respectively (Figure 1a). In patients with recurrence of PBC, typical PBC symptoms were reported in 50 patients, including pruritus (59%), fatigue (29%), and jaundice (12%), and 190 patients were asymptomatic at the time of recurrence of PBC diagnosis.

The frequency of recurrence of PBC varied between 10% to 75% among centers. Yearly recurrence rate ranged from 0.4% to 6.7%. The overall incidence rate of recurrence of PBC after LT was 4.56 cases per 100 patient-years (95% CI, 3.1 to 6.02 cases per 100 patient-years with a total of 5260 patient-years). The biochemical features after LT in patients with and without recurrence of PBC are presented in Table 2.

The histological stage frequency at recurrence of PBC was one in 38%, stage two in 38%, and stage there in 24%. The only difference between centers regarding disease stage at the time of recurrence of PBC was stage one (P<0.001) with the highest frequency of 28% and the lowest of 3%. No significant differences in stage two (P=0.09) and three (P=0.15) was observed between centers.

Clinical Features Associated with Primary Biliary Cholangitis Recurrence

By univariate Cox proportional hazard regression analysis, younger age at diagnosis of PBC (HR 0.98, 95% CI 0.97-0.99, p=0.005) and at the time of LT (HR 0.98, 95% CI 0.96-0.99, p=0.001) were weakly associated with higher risk of recurrence of PBC. Patients younger than 50-year at diagnosis of PBC, and younger than 60-year at the time of LT had higher risk for recurrence of PBC (HR 1.79, 95% CI 1.36-2.36, p<0.001, and HR 1.39, 95% CI 1.02-1.90, p=0.04; respectively; Table 1 & Figure 1b-c). Heterogeneity of centers (HR 1.03, 95% CI 0.99-1.06, p=0.14) and LT center volume (HR 0.97, 95% CI 0.74-1.27, p=0.82) did not show a significant association with recurrence of PBC. The use of tacrolimus (HR 2.31, 95% CI, 1.72-3.10, p<0.001, Figure 1d), and sirolimus (HR 2.12, 95% CI, 1.05-4.30, p=0.04) were associated with higher risk of recurrence of PBC (Table 1 & Figure 1d). Use of mycophenolate mofetil (HR 1.56, 95% CI, 1.19-2.04, p=0.001) and cyclosporine (HR 0.62; 95% CI 0.46-0.82; p=0.001)

weakly associated with recurrence of PBC (Table 1 & Figure 1d). There was no significant association with other clinical features such as gender, ethnicity, living-related LT, age or gender of the donor, gender mismatch, type of bile duct anastomosis, rejection episodes, changes in the main immunosuppression after the first year of LT, BMI, presence of diabetes and the risk of recurrence of PBC (Table 1).

Biochemical Features Associated with Primary Biliary Cholangitis Recurrence

By univariate Cox proportional hazard analysis, patients who had elevation of ALP at 6- and 12 month after LT, had higher risk of recurrence of PBC (Table 2). Specifically, patients who had ALP above the ULN at 6- (HR 1.12, 95% CI 1.05-1.19, $p<0.001$), and 12-month after LT (HR 1.23, 95% CI 1.13-1.35, $p=0.001$) had higher risk to develop recurrence of PBC (Table 2) but the association was weak. Patients who had severe biochemical cholestasis at 6-month (HR 1.79, 95% CI 1.16-2.76, $p=0.008$), and those with mild and severe biochemical cholestasis at 12-month had higher risk of recurrence of PBC (HR 1.63, 95% CI 1.11-2.39, $p=0.01$, and HR 1.49, 95% CI 1.01-2.20, $p=0.04$, respectively; Table 2).

Multivariable Analyses of Features Associated with Primary Biliary Cholangitis Recurrence

For the multivariable analysis, we developed two models according to the presence of biochemical cholestasis at-6 or 12-month after LT. In the multivariable analysis for Model 1, which includes age at LT, the year of LT, use of tacrolimus, cyclosporine, mycophenolate mofetil, sirolimus and severe biochemical cholestasis at 6-month after LT, only age at LT, tacrolimus and mycophenolate use, and severe biochemical cholestasis at 6-month were

independently associated with recurrence of PBC (Table 3). The strongest associations, however, was observed between tacrolimus use and recurrence of PBC.

In model 2, age at LT, tacrolimus and mycophenolate use, and mild and severe biochemical cholestasis at 12-month were independently associated with recurrence of PBC (Model 2). Association with recurrence of PBC was stronger for tacrolimus use, and mild biochemical cholestasis at 12-month.

In addition, we did a multivariable subanalysis including only patients who had liver biopsy after LT (n=526). In these analyses (Model 1 and 2), LT year was associated with a higher risk of recurrence of PBC. Otherwise, the results were similar as the one including all patients (supplement Table 1).

Patient and Graft Survival Associated with Recurrent Disease and Biochemical Abnormalities

Overall median survival after LT was 21 (IQR: 18-24) years. The overall 5-, 10-, 15-and 20-year probability of survival was 90%, 81%, 70%, and 53%, respectively (Figure 2a). Graft median survival was 23 years (IQR: 22-24). The graft 5-, 10-, 15-, and 20-year probability of graft survival was 94%, 90%, 86%, and 77%, respectively (Figure 2b).

In Cox proportional hazard regression analysis implementing recurrence as time-dependent covariate, recurrence of PBC (HR 2.12, 95% CI 1.22-3.67, p=0.008) was associated with graft failure. Use of cyclosporine (HR 0.96, 95% CI 0.91-1.01, p=0.10) weakly associated with graft failure. However, only recurrence of PBC was independently associated with graft failure in the multivariable analysis (time dependent HR 2.01, 95% CI 1.16-3.51, p=0.01, Table 4).

Also, by univariable Cox regression analysis as time-dependent covariate, age at LT (HR 1.05, 95% CI 1.03-1.07, p<0.001), age at diagnosis (HR 1.04, 95% CI 1.02-1.06, p<0.001), and use of

UDCA (HR 0.96, 95% CI 0.92-1.00, p=0.03) were weakly associated with overall survival after LT; however, in the multivariable analysis only age at LT (1.06, 95% CI 1.02-1.10, p=0.004), and recurrence of PBC (HR 1.72, 95% CI 1.11-2.65, p=0.02) were independently, but weakly associated with overall survival after LT (Table 4).

Graft survival was significantly diminished in patients with recurrence of PBC compared to patients without recurrence of PBC (19 years (95% CI 18-21) versus 24 years (95% CI 23-25), p=0.004, Figure 3a). We have specified causes of graft lost. As expected, the majority of patients with recurrence of PBC lost their graft as a result of cirrhosis related to recurrence of PBC (93%), and the remainder were attributed to either rejection (4%) or hepatic artery thrombosis-ischemic cholangiopathy (3%). In contrast, patients without recurrence of PBC lost their allograft as a result of rejection (45%), hepatic artery thrombosis-ischemic cholangiopathy (32%), HCC (10%), unknown causes (10%), or *de novo* viral hepatitis (3%). Overall survival was lower in patients with recurrence of PBC compared to those with no recurrent disease (15 years (95% CI 14-17) versus 19 years (95% CI 18-20), p=0.001, Figure 3b). In patients with recurrence of PBC and protocol biopsies, the overall 5-, 10-, 15-and 20-year probability of survival was 75%, 64%, 48% and 0% in those with severe biochemical cholestasis at 12 months compared to 97%, 93%, 80% and 54% in patients without severe biochemical cholestasis (p=0.002).

DISCUSSION

In the largest cohort of transplanted patients with PBC to date, we are the first to demonstrate an association between disease recurrence and impaired graft survival in patients with PBC. To our knowledge, this is also the first study to show that abnormalities in liver biochemistry within the first year following LT are associated with an increased risk of recurrence of PBC, suggesting an

impetus to consider early intervention to prevent recurrence of PBC [28]. The type of immunosuppression after LT was also found to be associated with the incidence of recurrence of PBC.

Prior single center reports indicated that recurrence of PBC has no significant impact on long-term survival or need for re-transplantation [4, 12, 15]. However, a major limitation of these studies is their lack of long-term follow-up following LT, thereby limiting the probability of detecting differences in outcomes. Further, the probability of recurrence of PBC we found to exceed 50% at 20 years, even though the incidence of recurrence of PBC in this study might be an underestimated, since not all patients underwent protocol liver biopsies and the diagnosis is currently dependent on histological confirmation. In addition, younger age at LT and at the time of diagnosis are both associated with higher risk of recurrence of PBC, which in turn is a risk factor for graft loss and poor overall survival. These findings are in agreement with other studies suggesting that age of onset and LT may be associated with a more aggressive PBC phenotype [13, 14].

This study provides further support regarding the type of immunosuppression after LT and its associations with both the incidence and time of onset of recurrence of PBC. Previous studies have demonstrated that patients receiving the more potent calcineurin inhibitor, tacrolimus, have a higher risk of recurrence of PBC, whereas, the use of cyclosporine is associated with a reduced prevalence of recurrence of PBC [4, 10, 16, 17].

It is also noteworthy that the use of mycophenolate mofetil (MMF) was associated with increased risk of recurrence of PBC, whereas there was a trend for azathioprine to be protective. This observation generates different hypotheses. Some have argued for an “era effect” when the use of cyclosporine and azathioprine was more prevalent in the 1980s and 1990s, and other

factors such as cold ischemia times, shorter waiting period and less sick patients might have impacted the development of recurrence of PBC [15]. Another argument suggests that more potent immunosuppression regimens using tacrolimus and mycophenolate mofetil actually hasten the onset of recurrence of PBC [15]. The debate is important because the addition of MMF has been used to treat recurrence of PBC, whereas the conclusions of studies assessing the efficacy of mycophenolate mofetil in pre-transplant patients have been somewhat contradictory [29, 30].

We also speculate that cyclosporine may be protective against recurrence of PBC due to off target effects. The immunosuppressive activity is mediated by inhibiting cyclophilin A, which in turn prevents calcineurin from regulating cytokine gene transcription in lymphocytes. As a intracellular chaperone, cyclophilin A also plays a central role in the assembly of many viruses and as a result, cyclosporine has been shown to have broad spectrum antiviral activity against hepatitis C virus, HIV as well as a human betaretrovirus linked with PBC [31]. However, not all studies suggest a protective role for cyclosporine including the recent multicenter study in Japanese patients with living-donor LT [32]. Other factors might also impact the development of recurrence of PBC such as genetic predisposition [33]. One European candidate risk allele at the IL12A locus has been shown to be an additive risk factor for recurrence of PBC on and above tacrolimus use [34].

Another novel finding in this study is that early development of cholestasis is helpful in risk stratification of patients with regard to development of recurrence of PBC. This finding suggests that there might be factors triggering recurrence that are already present within the first six months following LT and causing pathology. In the consideration of different hypotheses proposed for the etiology of PBC, this would either suggest the early recurrence of factors mediating an autoimmune response or alternatively, an infectious disease process. A key

consideration is that more potent immunosuppression is used within the first 6 to 12 months following LT. Accordingly, the abnormal hepatic biochemistry data might be more in keeping with an infectious disease model in which immunosuppression would be more injurious than an autoimmune disease model, where immunosuppression would rather be protective.

We recognize that we were not able to conclusively distinguish whether the presence of high ALP and severe biochemical cholestasis at six months constitutes risk factors for subsequent development of recurrence of PBC or a finding of established recurrence of PBC. Indeed, the use of more potent immunosuppression within the first year may mask the characteristic histological presentation of PBC, whereas non-specific inflammatory changes are a more common finding early on in the disease process. Moreover, AMA frequently persist following LT in patients without recurrence of PBC, and therefore, cannot be used to specifically signal a definitive diagnosis as they do prior to LT. Accordingly, cholestatic changes in the first 12 months after LT can only be considered as factors linked with a higher risk of recurrence of PBC. In prior studies with protocol biopsies, established recurrence of PBC was observed after a median of three to six years after LT [12, 13, 16]. In the same line, the earlier case of PBC recurrence described was after nine months of LT [35].

Interestingly, we found that in patients with protocol biopsies, the overall probability of survival was lower in patients with severe biochemical cholestasis at 12 months, compared to patients without severe biochemical cholestasis ($p=0.002$). This result suggests that patients with recurrence of PBC and abnormal liver biochemistry test at one year after transplant could constitute a subgroup of patients with higher risk of progressive recurrence of PBC.

Our results imply that patients at higher risk of recurrence of PBC should be considered for therapeutic strategies within the first year of LT to prevent occurrence of PBC recurrence.

Ursodeoxycholic acid (UDCA) is generally employed after the diagnosis of recurrence of PBC has been established and has been associated with improvement of liver biochemistry tests in patients with recurrence of PBC. However, we lack data documenting a delay in histologic progression, or improvement of graft and patient survival [12]. Of note, observational studies suggest that long-term preventive administration of UDCA following LT impacts on recurrence of PBC [28].

As preventative UDCA commenced early following LT is associated with a decreased risk of recurrence of PBC [36]; patients with biochemical features associated with higher risk of recurrence of PBC may subsequently benefit from early intervention of UDCA treatment as well. However, the benefit should be investigated in prospective studies along with evaluation of second line therapies such as obeticholic acid [37] or bezafibrate [38] to reduce the risk of graft loss related to recurrence of PBC. Moreover, the validity of GLOBE score and the UK-PBC score for prediction of outcomes after recurrence in patients on treatment with ursodeoxycholic acid (UDCA) needs to be determined in future studies.

A large variance in frequency of recurrence of PBC was observed, varying from 10% to 75% among centers, with a yearly recurrence rate ranging from 0.4% to 6.7%. The most parsimonious explanation we can provide are related to (i) different follow-up times between centers (mean total follow-up range 38-174 months, $p<0.001$), and (ii) difference in protocol liver biopsies that were not performed in four of the 13 LT centers.

We acknowledge there are limitations in this study, while the diagnosis of recurrence of PBC in our cohort was established according to liver biopsies [23], in some centers biopsies were protocol-driven and in other centers clinically-driven. This could have led to differences in time to diagnosis of recurrence of PBC between the different centers. However, in the Cox regression

analysis, the comparison of protocol- and clinically-driven liver biopsies was not associated with recurrence of PBC (Table 1). Moreover, the median time for recurrence of PBC was not different between centers who perform protocol- and clinically-driven liver biopsies (13 years, 95% CI 11-16 vs. 13 years, 95% CI 8-19 years, P=0.99).

In conclusion, in this large cohort following patients after LT for PBC, a younger age at the time of diagnosis and LT, tacrolimus use, and severe biochemical cholestasis within the first six months after LT were independently associated with an increased risk of recurrence of PBC. The pathogenesis explaining the association between early abnormal liver biochemistries tests within one year of LT and a higher risk of recurrence of PBC needs clarification in future studies. Recurrence of PBC was associated with worse graft and overall survival after LT. The exploration of therapeutic interventions to prevent and treat recurrence of PBC are therefore warranted.

FIGURE LEGENDS

Figure 1a. Cumulative probability of recurrence of PBC. The probability of recurrence of PBC was 22%, 36%, 50%, and 55% at 5-, 10-, 15-, and 20-years, respectively.

Figure 1b. Cumulative probability of recurrence of PBC in patients younger and older than 50 years at diagnosis. The 5-year probability of recurrence of PBC was 30% and 15%, respectively ($p<0.001$, log-rank test). The 10-year probability of survival was 46% and 28% in these same groups.

Figure 1c. Cumulative probability of recurrence of PBC in patients younger and older than 60 years at liver transplantation. The 5-year probability of recurrence of PBC was 25% and 16%, respectively ($p=0.03$, log-rank test). The 10-year probability of survival was 39% and 29% in these same groups.

Figure 1d. Cumulative probability of recurrence of PBC in patients receiving tacrolimus or cyclosporine after liver transplantation. The 5-year probability of recurrence of PBC was 28% and 11%, respectively ($p<0.001$, log-rank test). The 10-year probability of survival was 45% and 23% in these same groups.

Figure 2a. Overall survival of PBC patients after liver transplantation. The 5-, 10-, 15- and 20-year probability of survival was 90%, 81%, 70% and 53%, respectively.

Figure 2b. Graft survival of PBC patients after liver transplantation. The 5-, 10-, 15-, and 20-year graft survival probability was 94%, 90%, 86% and 77%, respectively.

Figure 3a. Graft survival in patients with and without recurrence of PBC after liver transplantation using the semi-Markov models ("clock reset" model) approach (p=0.004). Patients who had no recurrence of PBC during their follow-up are in the solid line. Patients who developed recurrence of PBC are only represented in the solid line until they developed recurrence of PBC. These patients are censored and switched to a new survival curve (dotted line) once they have recurrence of PBC. The time is then reset as time 0 for their further follow-up.

Figure 3b. Overall survival in patients with and without recurrence of PBC after liver transplantation using the semi-Markov models ("clock reset" model) approach (p=0.001).

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Table 1

Clinical Features Associated with Recurrent PBC at the Time of Liver Transplantation in Univariable Cox Proportional Hazard Regression Analyses

Clinical Features	All Patients (n=785)	PBC recurrence (n=240)	No PBC recurrence (n=545)	HR	95% CI	p-value
Age at the Time of Diagnosis PBC (years)	47±1	44±1	48±1	0.98	0.97-0.99	0.005
Age at diagnosis ≤50 (years), n (%)	366 (47)	144 (60)	222 (41)	1.79	1.36-2.36	<0.001
Age at LT (years)	54±1	51±1	55±1	0.98	0.96-99	0.001
Age at LT ≤60 (years), n (%)	541 (69)	189 (79)	352 (65)	1.39	1.02-1.90	0.04
Men: women	89: 696	27: 213	62: 483	1.06	0.71-1.58	0.78
Caucasian: Non-Caucasian[¥]	605:180	188:52	417:128	0.92	0.68-1.26	0.60
Time from PBC diagnosis to LT (years)	7.6±1	6.9±1	7.9±1	1.00	0.99-1.00	0.71
Type of LT, n (%):						
- Cadaveric	757 (96)	234 (97)	523 (96)			
- Living Related	28 (4)	6 (3)	22 (4)	1.03	0.46-2.33	0.94
Bile Duct Anastomosis, n (%):						
- End-to-end	750 (95.5)	223 (93)	527 (97)			
- Roux-en-Y	35 (4.5)	17 (7)	18 (3)	0.70	0.43-1.15	0.16
Initial Immunosuppression, n (%):						
- Tacrolimus	527 (67)	171 (71)	356 (65)	2.31	1.72-3.10	<0.001
- Cyclosporine	220 (28)	74 (31)	146 (27)	0.62	0.46-0.82	0.001
- Sirolimus	631 (80)	189 (79)	442 (81)	2.12	1.05-4.30	0.04
- Prednisone	15 (2)	8 (3)	7 (1)	0.91	0.67-1.25	0.57
- Mycophenolate Mofetil	267 (34)	84 (35)	183 (34)	1.56	1.19-2.04	0.001
- Azathioprine	265 (34)	84 (35)	181 (33)	0.89	0.68-1.16	0.38
- Everolimus	1 (0.1)	0 (0)	1 (0.2)	0.05	0.0-727	0.54
Changes in immunosuppression after the first year of LT	44 (6)	28 (12)	16 (3)	1.32	0.87-1.98	0.19
Liver Biopsies after LT, n (%):						
- Protocol	252 (32)	113 (22)	139 (27)			
- Clinically-driven	270 (34)	90 (17)	180 (35)	1.00	0.76-1.32	0.99
AMA-M2	513 (65)	173 (72)	340 (62)	1.59	0.87-2.90	0.13
LT Calendar Year	2003±1	2000±1	2004±1	1.04	1.02-1.06	<0.001
LT Center Volume (High*: Low), n	224: 561	78: 162	146: 399	0.97	0.74-1.27	0.82
Donor Age (years)	40±1	39±1	41±1	1.00	0.99-1.01	0.76
Donor Gender (Men: women), n	328: 457	108: 132	220: 325	0.99	0.77-1.28	0.93
Gender Mismatch, n (%)	331 (42)	101 (42)	230 (42)	0.59	0.30-1.16	0.13
Rejections, n (%)	134 (17)	48 (20)	86 (16)	0.85	0.62-1.16	0.31
BMI (kg/m^2)	24±0.2	24±0.4	25±0.2	1.02	0.99-1.05	0.26
Diabetes	102 (13)	39 (16)	63 (12)	1.12	0.80-1.59	0.51

LT = liver transplant; PBC = primary biliary cholangitis; AMA = antimitochodrial antibodies.
*>50 LT performed for PBC.
[¥] Non-Caucasian includes 1% African, 1% Latin American, 2% Aboriginal, 3% Asian, and 16% unknown ethnicity. (Caucasian: 77%)

Table 2

Biochemical Features Associated with Recurrent PBC after Liver Transplantation in Univariable Cox Proportional Hazard Regression Analyses

Biochemical Features	**All Patients (n=785)**	**PBC recurrence (n=240)**	**No PBC recurrence (n=545)**	**HR**	**95% CI**	**p-value**
ALT U/L (3-mo)	53±5	55±8	52±6	1.001	0.99-1.003	0.32
ALT times ULN (3-mo)	1.1±0.3	1.4±0.2	1.3±0.1	0.90	0.48-1.69	0.74
AST U/L (3-mo)	41±3	36±4	43±4	0.99	0.99-1.002	0.41
AST times ULN (3-mo)	0.9±0.2	0.9±0.1	1.1±0.1	0.71	0.38-1.32	0.28
ALP (3-mo)	239±15	201±14	255±211	1.00	0.99-1.00	0.26
ALP times ULN (3-mo)	1.4±0.1	1.4±0.1	1.8±0.1	0.94	0.79-1.12	0.50
GGT (3-mo)	140±14	139±27	140±16	1.00	1.00-1.002	0.28
GGT times ULN (3-mo)	2.5±0.2	2.5±0.5	2.5±0.3	1.03	0.98-1.09	0.28
Bilirubin µmol/L (3-mo)	22±2	16±2	23±3	0.99	0.99-1.007	0.78
Bilirubin times ULN (3-mo)	0.8±0.1	0.86±0.1	1.2±0.2	0.52	0.21-1.29	0.16
*Mild Cholestasis (3-mo)	67 (9)	26 (11)	41 (8)	1.40	0.92-2.14	0.12
†Severe Cholestasis (3-mo)	68 (9)	19 (8)	49 (9)	0.95	0.59-1.54	0.84
ALT U/L (6-mo)	45±4	46±4	44±4	1.001	0.99-1.003	0.36
ALT times ULN (6-mo)	1.3±0.2	1.2±0.1	1.1±0.1	0.96	0.84-1.09	0.54
AST U/L (6-mo)	40±2	38±3	42±3	1.001	0.99-1.004	0.69
AST times ULN (6-mo)	1.0±0.9	0.9±0.1	1.10±0.1	0.92	0.76-1.12	0.42
ALP (6-mo)	211±11	233±18	199±14	1.001	1.00-1.001	0.01
ALP times ULN (6-mo)	1.5±0.1	1.6±0.1	1.4±0.1	1.12	1.05-1.19	<0.001
GGT (6-mo)	128±16	127±21	128±22	1.00	1.00-1.001	0.59
GGT times ULN (6-mo)	2.3±0.3	2.3±0.4	2.3±0.4	1.01	0.98-1.04	0.59
Bilirubin µmol/L (6-mo)	16±1	14±1	16±2	0.99	0.99-1.006	0.62
Bilirubin times ULN (6-mo)	0.6±0.1	0.8±0.1	0.8±0.1	0.69	0.24-2.02	0.50
*Mild Cholestasis (6-mo)	72 (9)	33 (14)	39 (7)	1.27	0.87-1.85	0.21
†Severe Cholestasis (6-mo)	60 (8)	24 (10)	36 (7)	1.79	1.16-2.76	0.008
ALT U/L (12-mo)	38±3	45±6	33±3	1.002	1.001-1.004	0.01
ALT times ULN (12-mo)	1.1±0.2	1.4±0.4	0.8±0.2	1.07	0.99-1.14	0.08
AST U/L (12-mo)	37±2	41±4	34±3	1.001	1.00-1.003	0.13
AST times ULN (12-mo)	1.0±0.1	1.1±0.2	0.8±0.2	1.06	0.98-1.14	0.14
ALP (12-mo)	186±9	209±14	174±12	1.001	1.00-1.001	0.001
ALP times ULN (12-mo)	1.4±0.1	1.4±0.1	1.2±0.1	1.23	1.13-1.35	<0.001
GGT (12-mo)	97±9	137±22	74±7	1.002	1.001-1.002	<0.001
GGT times ULN (12-mo)	1.8±0.2	2.5±0.4	1.3±0.1	1.09	1.05-1.13	<0.001
Bilirubin µmol/L (12-mo)	19±2	22±4	18±3	1.002	1.00-1.004	0.10
Bilirubin times ULN (12-mo)	0.9±0.2	1.1±0.4	0.7±0.1	0.90	0.72-1.13	0.36
*Mild Cholestasis (12-mo)	57 (7)	32 (13)	25 (5)	1.63	1.11-2.39	0.01
†Severe Cholestasis (12-mo)	63 (8)	30 (13)	33 (6)	1.49	1.01-2.20	0.04

PBC = primary biliary cholangitis; ULN = upper limit of normal.

*ALP level >2 times the ULN or a combined elevation of both bilirubin and ALP levels.

† Bilirubin ≥100 µmol [>5.9 mg/dL] or ALP >3 times the ULN.

Table 3

Features Associated with Primary Biliary Cholangitis Recurrence by Multivariable Cox Proportional Hazard Regression Analyses

Model 1	**HR**	**95% CI**	**p-value**
Age at LT (years)	0.98	0.97-0.99	0.002
LT Year	1.02	0.99-1.05	0.27
Tacrolimus	3.41	1.42-8.15	0.006
Cyclosporine	2.32	0.99-5.40	0.052
Mycophenolate Mofetil	1.46	1.03-2.08	0.03
Sirolimus	1.74	0.64-4.72	0.28
†Severe Cholestasis (6-mo)	1.98	1.28-3.06	0.002
Model 2	**HR**	**95% CI**	**P-value**
Age at LT (years)	0.98	0.97-0.99	<0.001
LT Year	1.02	0.99-1.05	0.32
Tacrolimus	4.22	1.75-10.16	0.001
Cyclosporine	2.20	0.93-5.20	0.07
Mycophenolate Mofetil	1.41	1.01-1.98	0.04
Sirolimus	1.43	0.62-3.27	0.40
*Mild Cholestasis (12-mo)	2.26	1.52-3.36	<0.001
†Severe Cholestasis (12-mo)	1.78	1.19-2.68	0.005

*ALP level >2 times the ULN or a combined elevation of both bilirubin and ALP levels.
† Bilirubin ≥100 µmol [>5.9 mg/dL] or ALP >3 times the ULN.
Two models were developed according to the presence cholestasis at-6 or 12-months after liver transplant (LT). Significant variables in univariate analysis (Table 2) were included in multivariate Cox analysis. Mild Cholestasis (six months) was not significant in univariate model (Table 2) and therefore excluded from Table 3.

Table 4

Features Associated with Graft and Patient Survival after Liver Transplantation

Graft Survival Analysis						
Features	**Univariate**			**Multivariable**		
	HR	**95% CI**	**P-value**	**HR**	**95% CI**	**p-value**
Gender	0.98	0.90-1.07	0.65			
Age at LT	1.02	0.99-1.05	0.20			
Age at Diagnosis	1.02	0.99-1.06	0.2			
Cyclosporine	0.96	0.91-1.01	0.10	0.70	0.41-1.21	0.20
Recurrence of PBC **	2.12	1.22-3.67	0.008	2.01	1.16-3.51	0.01
Tacrolimus	0.97	0.92-1.02	0.25			
*Cholestasis Mild	1.27	0.47-3.43	0.64			
†Cholestasis Severe	1.06	0.42-2.70	0.91			
UDCA	1.02	0.97-1.07	0.50			
Overall Survival Analysis						
Features	**Univariate**			**Multivariable**		
	HR	**95% CI**	**P-value**	**HR**	**95% CI**	**p-value**
Gender	0.98	0.93-1.03	0.35			
Age at LT	1.05	1.03-1.07	<0.001	1.06	1.02-1.10	0.004
Age at Diagnosis	1.04	1.02-1.06	<0.001	1.00	0.97-1.03	0.99
Cyclosporine	0.98	0.95-1.02	0.22			
Recurrence of PBC **	1.27	0.90-1.79	0.18	1.72	1.11-2.65	0.02
Tacrolimus	1.00	0.97-1.03	0.83			
*Cholestasis Mild	0.72	0.32-1.63	0.43			
†Cholestasis Severe	0.61	0.29-1.26	0.18			
UDCA	0.96	0.92-1.00	0.03	0.71	0.43-1.18	0.19

*ALP level >2 times the ULN or a combined elevation of both bilirubin and ALP levels.
† Bilirubin ≥100 µmol [>5.9 mg/dL] or ALP >3 times the ULN.
UDCA = preventive or curative.
** These hazard ratios were obtained by considering recurrence of PBC as a time-dependent covariate in univariable and multivariable analyses.

Figure 1a.

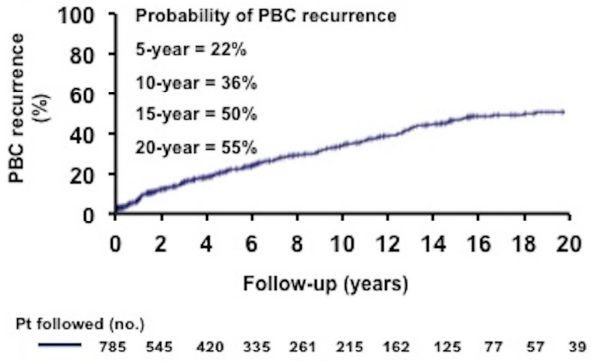


Figure 1b.

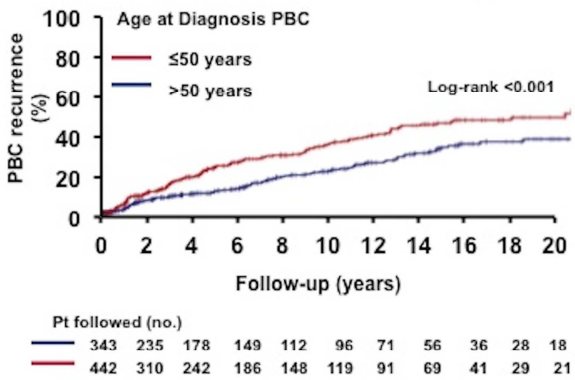


Figure 1c.

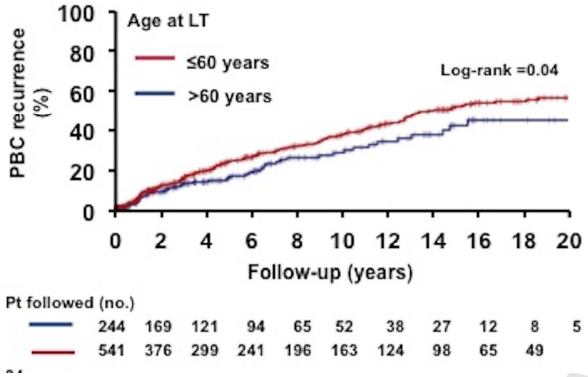


Figure 1d.

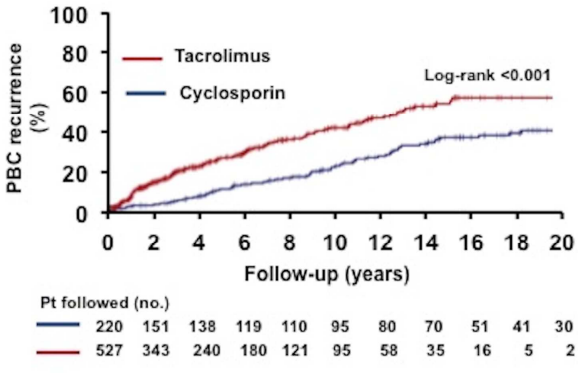


Figure 2a.

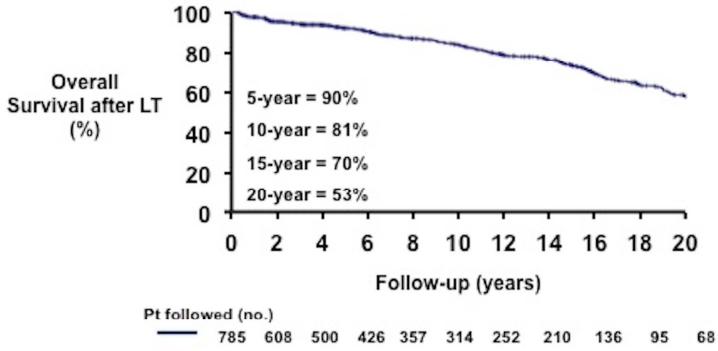


Figure 2b.

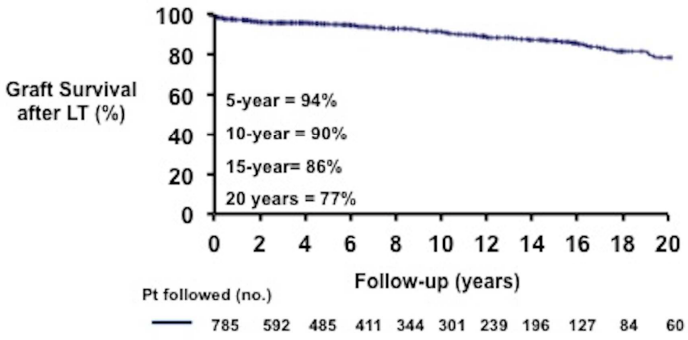


Figure 3a.

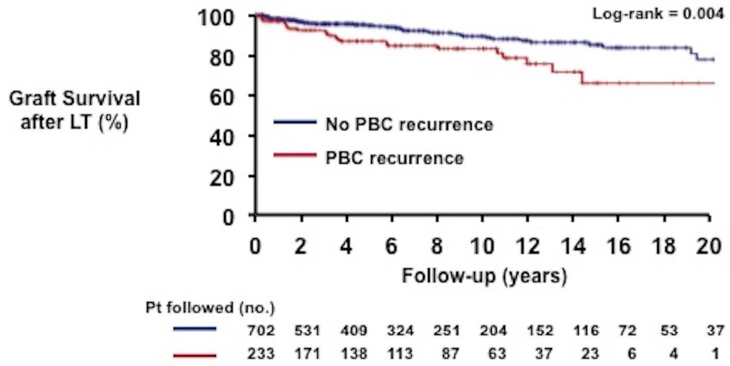
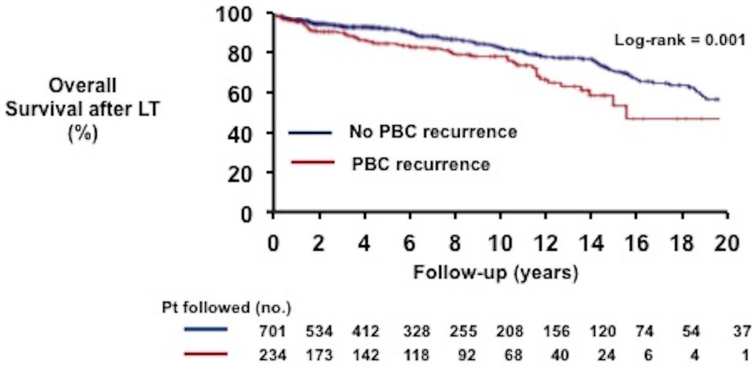


Figure 3b.



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Supplementary Table 1.

Features Associated with Primary Biliary Cholangitis Recurrence by Multivariable Cox Proportional Hazard Regression Analyses in Patients who Underwent Liver Biopsy

Model 1	**HR**	**95% CI**	**p-value**
Age at LT (years)	0.98	0.97-0.99	0.002
LT Year	1.06	1.02-1.10	0.005
Tacrolimus	6.74	2.49-18.28	<0.001
Cyclosporine	5.10	1.89-13.74	0.001
Mycophenolate Mofetil	1.15	0.78-1.70	0.48
Sirolimus	1.46	0.53-3.97	0.46
†Severe Cholestasis (6-mo)	1.72	1.05-2.81	0.03
Model 2	**HR**	**95% CI**	**P-value**
Age at LT (years)	0.98	0.96-0.99	<0.001
LT Year	1.05	1.02-1.09	0.005
Tacrolimus	7.09	2.63-19.13	<0.001
Cyclosporine	4.31	1.60-11.58	0.004
Mycophenolate Mofetil	1.08	0.75-1.57	0.68
Sirolimus	1.22	0.53-2.82	0.64
*Mild Cholestasis (12-mo)	2.26	1.47-3.45	<0.001
†Severe Cholestasis (12-mo)	1.96	1.26-3.05	0.003

*ALP level >2 times the ULN or a combined elevation of both bilirubin and ALP levels.
† Bilirubin ≥100 µmol [>5.9 mg/dL] or ALP >3 times the ULN.

Supplementary Figure 1. Flowchart of patients excluded/included for the recurrence of primary biliary cholangitis after liver transplant.