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Prognostic Significance of Bile Duct Loss in Early-Stage Primary Biliary Cholangitis: A Long-Term Observational Study

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Keywords: ALBI grade | bile duct loss | Nakanuma classification | primary biliary cholangitis

ABSTRACT

Background & Aims: Primary biliary cholangitis (PBC) is a chronic, slowly progressive, and autoimmune liver disease. This study aimed to establish the clinicopathological features that accurately predict long-term prognosis in patients with early-stage PBC.

Methods: The present long-term (8.8 years), multicenter, and retrospective investigation enrolled 274 treatment-naïve PBC patients who had undergone liver biopsy. Among them, 207 patients with albumin–bilirubin (ALBI) grade 1 were categorized as clinical early-stage, and 230 patients with Nakanuma stage 1/2 were classified as pathological early-stage. The prognostic factors related to the time to liver-related events (LRE) were statistically evaluated.

Results: Cox regression analysis identified Nakanuma bile duct loss score of ≥ 1 as a significant independent factor associated with LRE development in clinical early-stage PBC patients (hazard ratio [HR] 12.89, 95% confidence interval [95% CI] 1.60–103.96, $P = 0.016$). Kaplan–Meier testing revealed that the cumulative incidence of LRE was significantly higher in patients with bile duct loss score of ≥ 1 than in those with bile duct loss score 0 (log-rank test; $P < 0.001$). Similarly, bile duct loss score could predict LRE in pathological early-stage PBC patients, as confirmed by both multivariable Cox regression (HR 6.60, 95% CI 1.37–31.86, $P = 0.019$) and Kaplan–Meier (log-rank test; $P < 0.001$) analyses.

Conclusions: Nakanuma bile duct loss score may be a valuable prognostic indicator in the early clinical and pathological stages of PBC.

Abbreviations: 95% CI, 95% confidence interval; γ -GTP, γ -glutamyltransferase; ALBI, albumin–bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HR, hazard ratio; IgG, immunoglobulin G; IgM, immunoglobulin M; LRE, liver-related event; PBC, primary biliary cholangitis; UDCA, ursodeoxycholic acid.

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1 | Introduction

Primary biliary cholangitis (PBC) is characterized by an ongoing immunologic attack on the intralobular bile ducts, gradually leading to chronic cholestasis and eventually cirrhosis. With the wider recognition and earlier detection of PBC through disease-specific antimitochondrial antibodies, the majority of afflicted patients are asymptomatic and at an early stage at the time of diagnosis [1]. Moreover, the prognosis of PBC patients has significantly improved and is now comparable to that of healthy individuals owing to earlier diagnosis and the widespread use of ursodeoxycholic acid (UDCA) treatment. However, a subset of patients still suffer a poor clinical course, including the development of liver-related events (LRE) and liver-related death [2, 3]. Although it is well established that the stage of liver cirrhosis at PBC diagnosis is linked to disease progression, it remains difficult to precisely predict which patients will experience disease progression at the time of diagnosis, especially in early-stage cases. It has therefore become crucial to identify predictive markers at diagnosis to better manage and treat those patients.

This study investigated the clinicopathological factors associated with the long-term development of LRE in PBC patients at an early clinicopathological stage.

2 | Materials and Methods

2.1 | Patients

This retrospective study included 274 biopsy-proven patients diagnosed as having PBC based on the criteria of the Japan Society of Hepatology [4], who were treated at Shinshu University Hospital (Matsumoto, Nagano, Japan) or Hamamatsu University Hospital (Hamamatsu, Shizuoka, Japan) between March 1978 and March 2021. When recruiting cases, we retrospectively reassessed the clinical and pathological findings of all cases included in this study to ensure that they met the diagnostic criteria outlined by the Japan Society of Hepatology. The diagnostic criteria for PBC include (1) histologically confirmed CNSDC with laboratory findings compatible with PBC; (2) positivity for antimitochondrial antibodies (AMAs) with histological findings compatible with PBC but in the absence of characteristic histological findings of CNSDC; and (3) no histological findings available, but positivity for AMAs as well as clinical findings and a course indicative of typical cholestatic PBC [4]. Because all patients in this study had undergone liver biopsy, criterion (3) was not applicable.

The inclusion criteria were (1) histologically confirmed diagnosis of PBC based on liver biopsy, (2) treatment-naïve status for UDCA at the time of diagnosis, and (3) routine PBC surveillance at either institution with surveillance every 3–6 months, including ultrasound and blood tests, based on the clinical guidelines of the Japanese Society of Hepatology [4]. The exclusion criteria were (1) positivity for hepatitis B surface antigen or antibodies to hepatitis C virus or the human immunodeficiency virus, (2) any previous or ongoing LRE, and (3) incomplete data. Liver biopsy was performed at diagnosis in patients with suspected PBC, regardless of the degree of

biochemical abnormality, except in cases with contraindications such as thrombocytopenia, impaired coagulation, or patient refusal after informed consent.

Follow-up time was defined as the interval between liver biopsy and the first diagnosis of an LRE or at the most recent follow-up visit in LRE-free patients. LRE were defined as the development of (1) hepatocellular carcinoma (HCC), which was diagnosed by imaging characteristics, arterial hypervascularity, and venous or delayed phase washout by contrast-enhanced dynamic computed tomography and/or magnetic resonance imaging when a nodular lesion was detected by ultrasonography or a tumor marker was elevated; (2) hepatic encephalopathy of grade II or higher requiring hospitalization; (3) poorly controlled ascites requiring hospitalization for administration of albumin, diuretics, abdominal paracentesis, or concentrated ascites reinfusion therapy; and (4) esophagogastric varices requiring endoscopic ligation, sclerotherapy, or balloon-occluded retrograde transvenous obliteration, including varices rupture requiring hospitalization. Albumin–bilirubin (ALBI) score was calculated as follows: $\log_{10}(\text{bilirubin [mg/dL]} \times 17.1 \times 0.66) + (\text{albumin [g/dL]} \times 10 \times -0.085)$. ALBI grade was defined based on calculated scores as follows: grade 1 (≤ -2.60), grade 2 (> -2.60 to ≤ -1.39), and grade 3 (> -1.39) [5].

2.2 | Liver Samples

Tissue samples from liver biopsies were formalin-fixed and paraffin-embedded, followed by staining with hematoxylin and eosin in addition to orcein. Liver biopsy specimens were deemed adequate if they contained ≥ 10 portal tracts, in accordance with established criteria for histological evaluation in chronic liver diseases [6]. Experienced pathologist (KH) reviewed the samples while blinded to clinicopathological data and assigned grading according to the Nakanuma histologic staging and grading system [7, 8]. In the Nakanuma system, two factors (fibrosis and bile duct loss) were evaluated, whereas the necroinflammatory activity of PBC was graded using cholangitis activity (CA) and hepatitis activity (HA).

2.3 | Statistical Analysis

Statistical analysis and data visualization were carried out using StatFlex ver. 7.0.11 software (Artech Co. Ltd., Osaka, Japan). Continuous variables were compared using the Mann–Whitney *U* test. Categorical variables were evaluated by means of Pearson's chi-squared test, Fisher's exact test, or Yates' continuity correction, as appropriate. Survival curves were calculated using the log-rank test and visualized using Kaplan–Meier plots. Univariable and multivariable analyses were conducted with Cox regression models. A *p*-value of < 0.05 was considered statistically significant.

We selected the binary cut-offs for fibrosis score, bile duct loss, CA, and HA based on the point at which the Youden index was maximum among the possible scores of 1, 2, and 3. The variables were incorporated into the analysis as binary factors accordingly. Two multivariable Cox proportional hazards

models were constructed to identify factors associated with LRE. The first model included covariates that were significantly associated with the outcome in univariable analysis ($p < 0.05$).

TABLE 1 | Demographic and clinical characteristics of patients.

	All patients ($n = 274$) Median (IQR)
Age, years	57.5 (51–65)
Male, n (%)	40 (13.9%)
PLT, $\times 10^4/\mu\text{L}$	22.7 (18.4–26.8)
Alb, g/dL	4.2 (4.0–4.4)
T-bil, mg/dL	0.75 (0.60–1.00)
AST, U/L	40 (28–63)
ALT, U/L	40 (26–72)
ALP, U/L	421 (312–583)
γ -GTP, U/L	132 (73–232)
IgG, mg/dL	1545 (1320–1887)
IgM, mg/dL	249 (144–408)
ALBI grade 1/2/3, n	207/63/4
Nakanuma stage 1/2/3/4, n	97/131/41/5
Fibrosis score 0/1/2/3, n	132/105/24/13
Bile duct loss score 0/1/2/3, n	145/99/25/5
Cholangitis activity 0/1/2/3, n	117/52/30/75
Hepatitis activity 0/1/2/3, n	82/97/70/25
Second line therapy with bezafibrate	47 (17.1%)
Observation period, years	8.8 (4.4–13.3)

Abbreviations: γ -GTP, γ -glutamyltranspeptidase; Alb, albumin; ALBI, albumin-bilirubin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IgG, immunoglobulin G; IgM, immunoglobulin M; IQR, interquartile range; PLT, platelets; T-bil, total bilirubin.

The second model was based on a priori clinical knowledge from the literature and included sex, ALBI score, and fibrosis stage, regardless of their statistical significance in univariable analysis. This dual-model approach was adopted to ensure both empirical robustness and clinical relevance in the evaluation of prognostic factors.

2.4 | Ethical Statement

The protocol of this study was reviewed and approved by the Institutional Review Board of Shinshu University School of Medicine (approval number: 5797, approved on March 24, 2023). All research in this investigation was conducted in accordance with the Declaration of Helsinki (revised in 2013 by Fortaleza) and the Ethical Guidelines for Medical Research Involving Human Subjects (partially revised on February 28, 2017). An opt-out system is in place at our institution. All information on the purpose, protocol, and conduct of the study is available on the Department of Medicine of Shinshu University School of Medicine website (<http://www.shinshu-u.ac.jp/faculty/medicine/chair/i-2nai/>). Patients not wishing to participate in the research are freely able to opt-out of the study.

3 | Results

3.1 | Patient Characteristics

The characteristics of all patients histopathologically diagnosed as having PBC are summarized in Table 1. Median age was 57.5 years, and the majority of patients were female (86.1%). Based on ALBI classification, the patients were categorized as ALBI grade 1 ($n = 207$) or ALBI grade 2/3 ($n = 67$). The patients were also classified as Nakanuma stage 1/2 ($n = 230$) or Nakanuma stage 3/4 ($n = 44$) [9]. In this research, ALBI grade 1 and Nakanuma stage 1/2 were defined as clinical early-stage

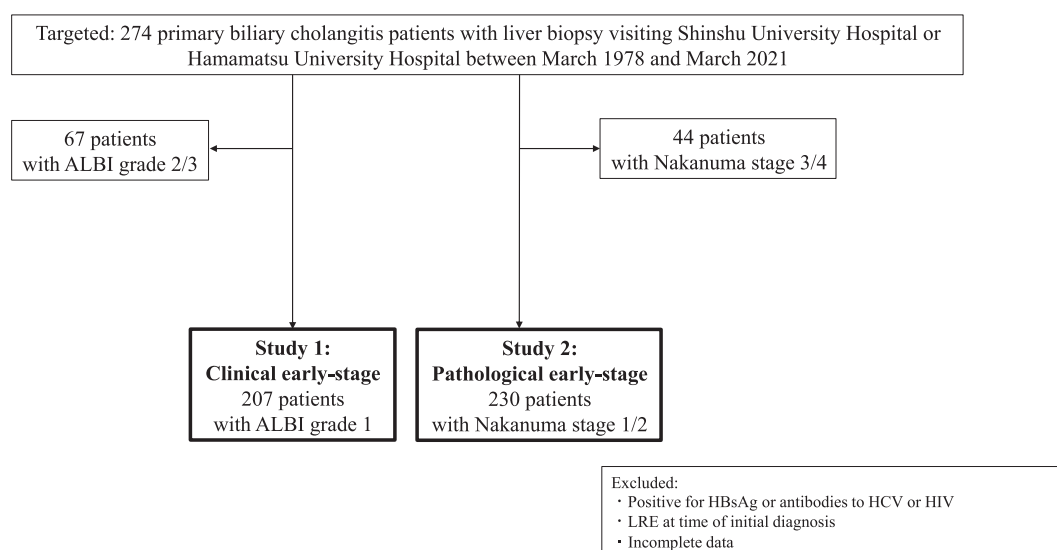


FIGURE 1 | Flowchart of this study. HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; LRE, liver-related event.

and pathological early-stage, respectively. The study of clinical early-stage PBC was termed study 1, whereas that of pathological early-stage PBC was termed study 2 (Figure 1).

Regarding the relationship between Nakanuma stage and ALBI grade, 27 patients were classified as ALBI grade 1 with Nakanuma stage 3/4, 180 patients as ALBI grade 1 with Nakanuma stage 1/2, and 50 patients as ALBI grade 2/3 with Nakanuma stage 1/2 (Supporting Information S1: Figure S1).

3.2 | Cumulative Incidences and Prognostic Factors Associated With LRE Development in All Patients

During the mean follow-up period of 8.8 years, 19 patients experienced LRE (HCC, esophagogastric varices, ascites, and hepatic encephalopathy in 3, 9, 4, and 3 patients, respectively). The cumulative incidences of LRE in all patients at 5, 10, and 15 years were 3.9%, 5.4%, and 9.9%, respectively (Supporting Information S1: Figure S2). Bivariate analysis of all patients identified higher Nakanuma stage of ≥ 3 (hazard ratio [HR]: 3.39, 95% confidence interval [95% CI]: 1.22–5.18, $P = 0.012$) and higher ALBI grade of ≥ 2 (HR: 2.52, 95% CI: 1.96–5.87,

$P < 0.001$) as significantly associated with the development of LRE (Supporting Information S1: Table S1).

3.3 | Prognostic Factors Associated With LRE Development in Clinical Early-Stage PBC Patients (Study 1)

We next performed a prognosis analysis of patients with clinical early-stage (i.e., ALBI grade 1) PBC, the characteristics of whom were summarized in Table 2. The cumulative incidences of LRE in clinical early-stage patients at 10, 20, and 30 years were 3.0%, 7.4%, and 13.6%, respectively (Figure 2a). First, we performed the univariable analysis with using age, sex, platelet, albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), ALP, γ -GTP, immunoglobulin G (IgG), immunoglobulin M (IgM), Nakanuma fibrosis score, Nakanuma bile duct loss score, cholangitis activity, hepatitis activity, and presence of second line treatment with bezafibrate. In terms of prognostic factors, the univariable analysis showed that total bilirubin (HR: 19.59, 95% CI: 2.60–147.68, $P = 0.004$), higher Nakanuma bile duct loss score of ≥ 1 (HR: 14.46, 95% CI: 1.80–115.84, $P = 0.012$), higher Nakanuma fibrosis score of ≥ 1 (HR: 13.63, 95% CI: 1.67–111.58, $P = 0.015$), and higher Nakanuma

TABLE 2 | Demographic and clinical characteristics of patients at clinical early-stage (ALBI grade 1; study 1).

	All patients (<i>n</i> = 207)	Nakanuma bile duct loss score ≥ 1 (<i>n</i> = 86)	Nakanuma bile duct loss score 0 (<i>n</i> = 121)	<i>p</i> -value
	Median (IQR)			
Age, years	57 (51–65)	57 (48–63)	57 (52–65)	0.601
Male, <i>n</i>	27 (12.7%)	7 (8.0%)	20 (16.4%)	0.071
PLT, $\times 10^4/\mu\text{L}$	23.5 (19.3–27.1)	23.0 (19.2–27.8)	23.6 (19.3–26.8)	0.901
Alb, g/dL	4.3 (4.1–4.5)	4.3 (4.1–4.4)	4.3 (4.1–4.5)	0.376
T-bil, mg/dL	0.71 (0.56–0.91)	0.72 (0.56–0.93)	0.71 (0.56–0.90)	0.607
AST, U/L	37 (27–56)	44 (30–65)	34 (24–51)	0.001
ALT, U/L	37 (27–65)	46 (27–74)	34 (24–57)	0.037
ALP, U/L	409 (307–570)	439 (349–669)	395 (271–522)	0.001
γ -GTP, U/L	120 (73–210)	153 (86–281)	112 (71–172)	0.007
IgG, mg/dL	1480 (1258–1781)	1576 (1335–1859)	1440 (1240–1717)	0.024
IgM, mg/dL	224 (129–366)	296 (195–443)	198 (109–293)	< 0.001
Nakanuma stage 1/2/3/4, <i>n</i>	83/96/26/2	0/54/23/3	83/37/1/0	< 0.001
Fibrosis score 0/1/2/3, <i>n</i>	107/81/12/7	24/47/9/6	83/34/3/1	< 0.001
Bile duct loss score 0/1/2/3, <i>n</i>	121/68/14/4	0/68/14/4	121/0/0/0	< 0.001
Cholangitis activity 0/1/2/3, <i>n</i>	90/41/20/56	21/20/13/32	69/21/7/24	< 0.001
Hepatitis activity 0/1/2/3, <i>n</i>	74/76/42/15	7/36/32/11	67/40/10/4	< 0.001
Second line therapy with bezafibrate	33 (15.9%)	17 (19.8%)	16 (13.2%)	0.205

Note: Bold entries indicate $p < 0.05$.

Abbreviations: γ -GTP, γ -glutamyltranspeptidase; Alb, albumin; ALBI, albumin-bilirubin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IgG, immunoglobulin G; IgM, immunoglobulin M; IQR, interquartile range; PLT, platelets; T-bil, total bilirubin.

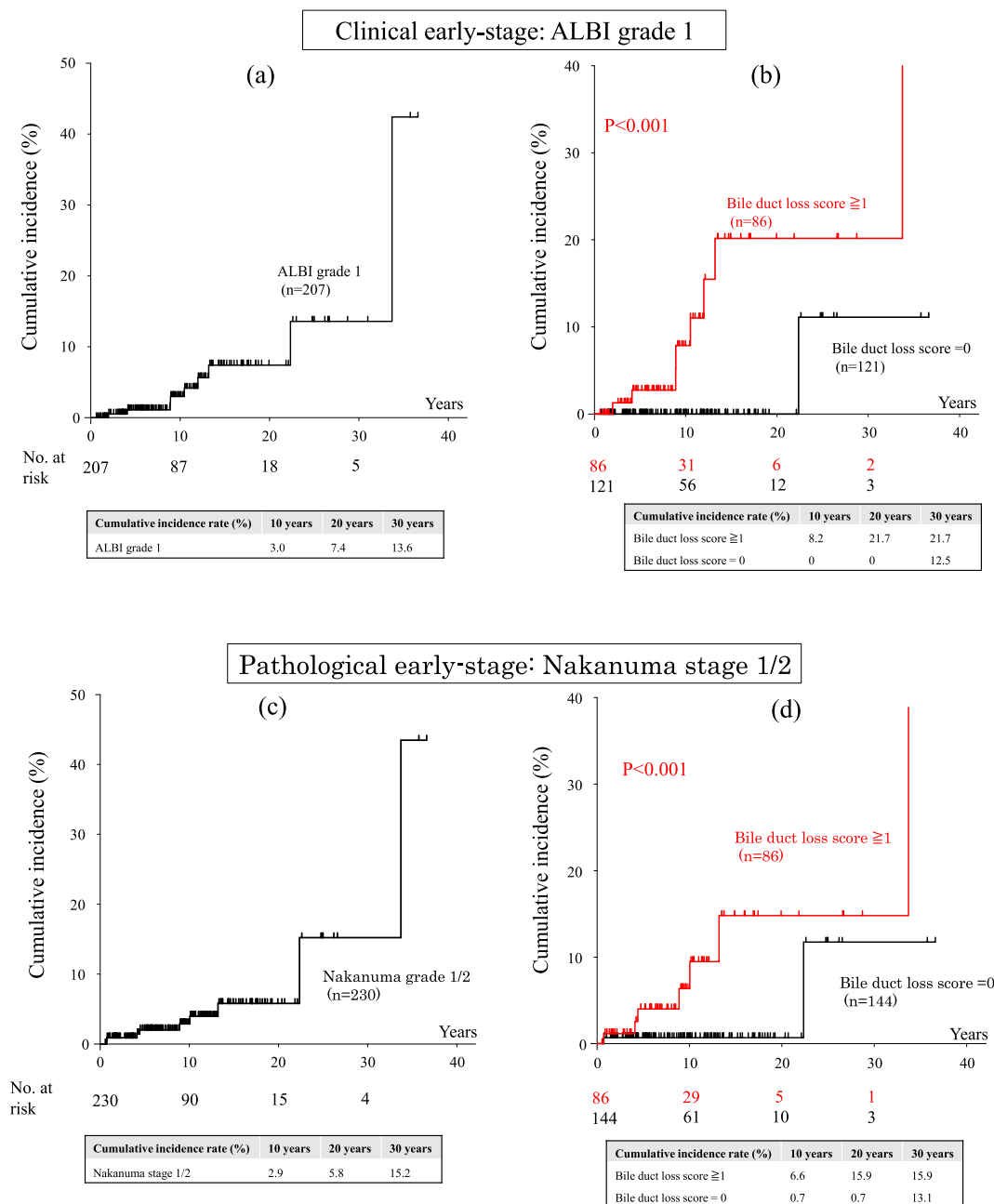


FIGURE 2 | (a) Cumulative incidence of LREs in patients with clinical early-stage (ALBI grade 1) PBC. (b) Comparison of cumulative LRE incidence rates in patients with clinical early-stage PBC between bile duct loss scores of ≥ 1 and 0. *p*-values were calculated by the log-rank test. (c) Cumulative incidence of LRE in patients with pathological early-stage (Nakanuma stage 1/2) PBC. (d) Comparison of cumulative LRE incidence rates in patients with pathological early-stage PBC between bile duct loss scores of ≥ 1 and 0. *p*-values were calculated by the log-rank test. ALBI, albumin-bilirubin; LRE, liver-related event; PBC, primary biliary cholangitis.

TABLE 3 | Univariable and multivariable Cox regression analyses of prognostic factors for observation period before LRE development in patients at clinical early-stage (ALBI grade 1; study 1).

	Univariable		Multivariable	
	HR (95% CI)	<i>p</i> -value	HR (95% CI)	<i>p</i> -value
T-bil, mg/dL	19.59 (2.60–147.68)	0.004	21.57 (1.82–255.88)	0.015
Nakanuma bile duct loss score of ≥ 1	14.46 (1.80–115.84)	0.012	12.89 (1.60–103.96)	0.016
Nakanuma fibrosis score of ≥ 1	13.63 (1.67–111.58)	0.015		
Nakanuma hepatitis score of ≥ 1	9.98 (1.18–84.27)	0.035		

Note: Bold entries indicate *p* < 0.05.

Abbreviations: 95% CI, 95% confidence interval; ALBI, albumin-bilirubin; HR, hazard ratio; LRE, liver-related event; T-bil, total bilirubin.

hepatitis score of ≥ 1 (HR: 9.98, 95% CI: 1.18–84.27, $P = 0.035$) were significantly associated with LRE development (Table 3). Among those factors, multivariable analysis confirmed higher Nakanuma bile duct loss score (HR: 12.89, 95% CI: 1.60–103.96, $P = 0.012$) and total bilirubin (HR: 21.57, 95% CI: 1.82–255.88, $P = 0.015$) as significant independent factors. To identify potential predictive factors for LRE development, we performed additional multivariable Cox proportional hazards analyses. Two additional models were constructed, each including sex and bile duct loss score, together with either pathological fibrosis score (Model 1) or ALBI score (Model 2). Among these models analyzed, bile duct loss score emerged as the strongest predictor of LRE development in Model 1 (HR: 7.18, 95% CI: 0.68–76.01, $P = 0.101$) and Model 2 (HR: 16.53, 95% CI: 2.04–134.06, $P = 0.008$), respectively (Supporting Information S1: Table S2). Additionally, the area under the receiver operating characteristic curve (AUROC) for predicting LRE was 0.753 for bile duct loss score, 0.645 for FIB-4 index, 0.501 for ALBI score, 0.634 for total bilirubin (Supporting Information S1: Figure S3a–d).

3.4 | Cumulative Incidences of LRE According to Nakanuma Bile Duct Loss Score (Study 1)

We divided the clinical early-stage PBC patients into Nakanuma bile duct loss scores of ≥ 1 or 0 based on the above multivariable analysis results. Patients with Nakanuma bile duct loss score of ≥ 1 had significantly higher levels of ALT ($P = 0.001$), AST ($P = 0.037$), ALP ($P = 0.001$), γ -GTP ($P = 0.007$), IgG ($P = 0.024$), and IgM ($P < 0.001$) compared with Nakanuma bile duct loss score 0 patients (Table 2). Regarding pathological findings, patients with Nakanuma bile duct loss score of ≥ 1 exhibited a significantly higher Nakanuma stage at PBC diagnosis ($P < 0.001$). The cumulative incidences of LRE were also significantly higher in these patients (log-rank test; $P < 0.004$) (Figure 2b). Regarding death or liver transplantation, Kaplan–Meier survival analysis revealed a significantly lower survival rate in patients with bile duct loss score of ≥ 1 than in those with score of 0 (log-rank $p = 0.014$) (Supporting Information S1: Figure S4a).

We next focused our analysis on the UDCA nonresponder group. UDCA response was assessed according to the Paris II criteria. Among clinical early-stage PBC patients, 20 were assessable for UDCA response. Bile duct loss score of ≥ 1 remained a significant predictor of LRE in UDCA nonresponders (log-rank test, $P = 0.033$; Figure 3a).

3.5 | Prognostic Factors Associated With LRE Development in Pathological Early-Stage PBC Patients (Study 2)

The characteristics of patients with pathological early-stage (i.e., Nakanuma stage 1/2) PBC are presented in Table 4. The cumulative incidences of LRE at 10, 20, and 30 years were 2.9%, 5.8%, and 15.2%, respectively (Figure 2c). First, we performed the univariable analysis with using similar variables as Study 1. Regarding prognostic factors, univariable analysis identified

higher Nakanuma bile duct loss score of ≥ 1 (HR: 6.60, 95% CI: 1.37–31.86, $P = 0.019$), higher Nakanuma hepatitis score of ≥ 1 (HR: 8.94, 95% CI: 1.04–76.80, $P = 0.046$), and IgM (HR: 1.002, 95% CI: 1.000–1.004, $P = 0.013$) as significantly associated with LRE development (Table 5). Multivariable analysis confirmed that higher Nakanuma bile duct loss score (HR: 6.60, 95% CI: 1.37–31.86, $P = 0.019$) was a significant independent factor of LRE. To identify potential predictive factors for LRE development, we performed additional multivariable Cox proportional hazards analyses. Two additional different models were constructed, each including sex, bile duct loss score, and additionally pathological fibrosis score (model 1) or ALBI score (model 2) to them. Among these analyzed models, bile duct loss score emerged as the strongest predictor of LRE development in model 1 (HR: 5.85, 95% CI: 0.99–34.52, $P = 0.051$) and model 2 (HR: 7.03, 95% CI: 1.44–34.39, $P = 0.016$), respectively (Supporting Information S1: Table S2). Additionally, AUROC in predicting LRE was 0.716 for bile duct loss score, 0.599 for FIB-4 index, 0.558 for ALBI score, 0.625 for total bilirubin (Supporting Information S1: Figure S3e–h).

3.6 | Cumulative Incidences of LRE According to Nakanuma Bile Duct Loss Score (Study 2)

We divided the pathological early-stage PBC patients into Nakanuma bile duct loss scores of ≥ 1 and 0 based on the above multivariable findings. Patients with a Nakanuma bile duct loss score of ≥ 1 had significantly higher levels of ALT ($P = 0.014$), AST ($P = 0.001$), γ -GTP ($P = 0.004$), IgG ($P < 0.001$), and IgM ($P < 0.001$) versus those with Nakanuma bile duct loss score of 0 (Table 4). Concerning pathological findings, patients with Nakanuma bile duct loss score of ≥ 1 exhibited a significantly higher Nakanuma stage at PBC diagnosis ($P < 0.001$). The cumulative incidences of LRE were significantly higher in patients with Nakanuma bile duct loss score of ≥ 1 (log-rank test; $P < 0.001$) (Figure 2d). Regarding death or liver transplantation, Kaplan–Meier survival analysis revealed a lower survival rate in bile duct loss score of ≥ 1 than in score of 0 (log-rank $p = 0.059$) (Supporting Information S1: Figure S4b).

We next focused our analysis on the UDCA nonresponder group, as in Study 1. Among pathological early-stage PBC patients, 22 were assessable for UDCA response according to the Paris II criteria. Bile duct loss score of ≥ 1 remained a significant predictor of LRE among UDCA nonresponders (log-rank test, $P = 0.046$; Figure 3b).

3.7 | Non-Invasive Predictors Associated With Pathological Bile Duct Loss in Early-Stage PBC Patients

To identify noninvasive predictors of pathological bile duct loss, we conducted multivariable logistic regression analyses in two cohorts: clinical early-stage PBC (Study 1) and pathological early-stage PBC (Study 2). In both analyses, univariable screening was performed using age, sex, platelet count, albumin,

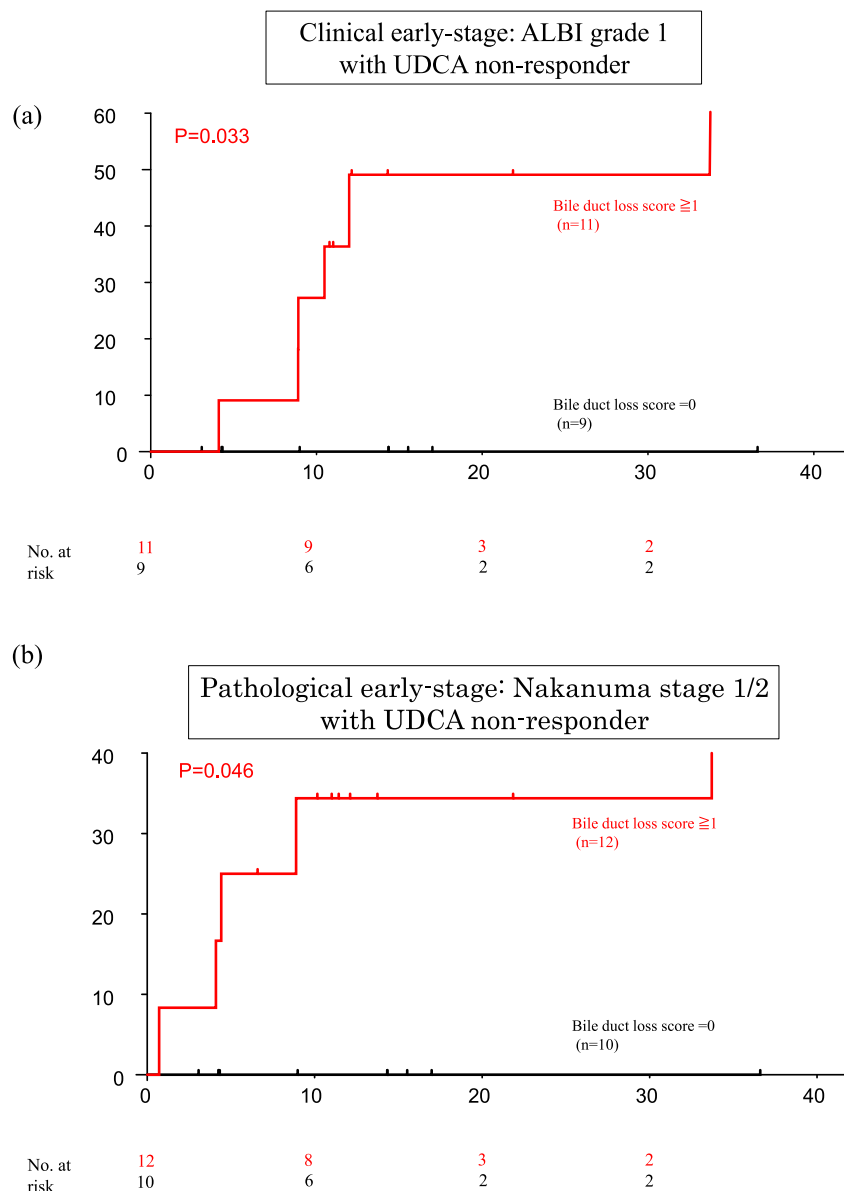


FIGURE 3 | (a) Comparison of cumulative LRE incidence rates in patients with clinical early-stage (ALBI grade 1) PBC between bile duct loss scores of ≥ 1 and 0 in UDCA non-responders. (b) Comparison of cumulative LRE incidence rates in patients with pathological early-stage (Nakanuma stage 1/2) PBC between bile duct loss scores of ≥ 1 and 0 in UDCA non-responders. p -values were calculated by the log-rank test. ALBI, albumin-bilirubin; LRE, liver-related event; PBC, primary biliary cholangitis; UDCA, ursodeoxycholic acid.

total bilirubin, AST, ALT, ALP, γ -GTP, IgG, and IgM. In clinical early-stage PBC (Study 1), ALP, γ -GTP, and IgM were significantly associated with bile duct loss in univariable analysis. Among them, ALP (HR: 1.002, 95% CI: 1.001–1.004, $P = 0.028$) and IgM (HR: 1.002, 95% CI: 1.001–1.004, $P = 0.003$) remained as significant independent predictors in multivariable analysis (Supporting Information S1: Table S3). In pathological early-stage PBC (Study 2), ALP, γ -GTP, IgM, and IgG were significant in univariable analysis, and γ -GTP (HR: 1.002, 95% CI: 1.001–1.004, $P = 0.028$), IgM (HR: 1.002, 95% CI: 1.000–1.003, $P = 0.011$), and IgG (HR: 1.001, 95% CI: 1.000–1.001, $P = 0.006$) remained significant in multivariable analysis (Supporting Information S1: Table S4). These findings suggest that immune- and cholestasis-related markers such as ALP, γ -GTP, IgM, and

IgG may help predict the presence of bile duct loss in patients with early-stage PBC.

3.8 | Cumulative Incidences of LRE According to Nakanuma Bile Duct Loss Score in the Subgroup of Patients in ALBI Grade 1 With Nakanuma Stage 1/2

We divided the patients in the subgroup of patients in ALBI grade 1 with Nakanuma stage 1/2 into Nakanuma bile duct loss scores of ≥ 1 and 0. The cumulative incidences of LRE were significantly higher in patients with Nakanuma bile duct loss score of ≥ 1 (log-rank test; $P = 0.012$) (Supporting Information S1: Figure S5).

TABLE 4 | Demographic and clinical characteristics of patients at pathological early-stage (Nakanuma stage 1/2; study 2).

	All patients (<i>n</i> = 230)	Nakanuma bile duct loss score ≥ 1 (<i>n</i> = 86)	Nakanuma bile duct loss score 0 (<i>n</i> = 144)	<i>p</i> -value
	Median (IQR)			
Age, years	57 (51–66)	57 (48–65)	58 (53–66)	0.293
Male, <i>n</i>	35 (14.9%)	10 (11.6%)	25 (17.4%)	0.242
PLT, × 10 ⁴ /μL	22.7 (18.4–26.6)	21.6 (18.2–25.5)	23.2 (18.8–26.9)	0.175
Alb, g/dL	4.2 (4.0–4.4)	4.2 (3.9–4.3)	4.2 (4.0–4.5)	0.081
T-bil, mg/dL	0.74 (0.60–0.97)	0.80 (0.60–1.03)	0.73 (0.57–0.95)	0.228
AST, U/L	37 (27–59)	47 (30–67)	34 (25–51)	0.001
ALT, U/L	38 (25–68)	48 (27–79)	34 (24–56)	0.014
ALP, U/L	400 (305–545)	416 (321–610)	397 (277–524)	0.063
γ-GTP, U/L	122 (72–206)	158 (85–275)	112 (68–178)	0.004
IgG, mg/dL	1525 (1282–1867)	1703 (1366–2063)	1445 (1250–1765)	< 0.001
IgM, mg/dL	238 (133–370)	296 (197–439)	207 (112–293)	< 0.001
ALBI grade 1/2/3, <i>n</i>	180/48/2	60/25/1	120/23/1	0.016
Nakanuma stage 1/2/3/4, <i>n</i>	104/126/0/0	0/86/0/0	97/47/0/0	< 0.001
Fibrosis score 0/1/2/3, <i>n</i>	131/92/7/0	33/51/2/0	98/41/5/0	< 0.001
Bile duct loss score 0/1/2/3, <i>n</i>	144/77/9/0	0/77/9/0	144/0/0/0	< 0.001
Cholangitis activity 0/1/2/3, <i>n</i>	104/41/25/60	23/19/13/31	81/22/12/29	< 0.001
Hepatitis activity 0/1/2/3, <i>n</i>	80/90/47/13	6/41/32/7	74/49/15/6	< 0.001
Second line therapy with bezafibrate	38 (16.5%)	19 (22.1%)	19 (13.2%)	0.079

Note: Bold entries indicate *p* < 0.05.

Abbreviations: γ-GTP, γ-glutamyltranspeptidase; Alb, albumin; ALBI, albumin-bilirubin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IgG, immunoglobulin G; IgM, immunoglobulin M; IQR, interquartile range; PLT, platelets; T-bil, total bilirubin.

TABLE 5 | Univariable and multivariable Cox regression analyses of prognostic factors for observation period before LRE development in patients at pathological early-stage (Nakanuma stage 1/2; study 2).

	Univariable		Multivariable	
	HR (95% CI)	<i>p</i> -value	HR (95% CI)	<i>p</i> -value
Nakanuma bile duct loss score of ≥ 1	6.60 (1.37–31.86)	0.019	6.60 (1.37–31.86)	0.019
Nakanuma hepatitis score of ≥ 1	8.94 (1.04–76.8)	0.046		
IgM, mg/dL	1.002 (1.000–1.004)	0.013		

Note: Bold entries indicate *p* < 0.05.

Abbreviations: 95% CI, 95% confidence interval; HR, hazard ratio; IgM, immunoglobulin M; LRE, liver-related event.

4 | Discussion

4.1 | Main Findings

Multivariable analysis in this study identified Nakanuma bile duct loss score as a significant prognostic factor in both clinical and pathological early-stage PBC patients. Liver fibrosis is generally a poor prognosticator in chronic liver diseases, including PBC. Because the patients included in this study tended to have early-stage PBC, fibrosis understandably did not reach significance as a prognostic factor. Our investigation indicates that evaluating bile duct loss may help the prognosis estimation of early-stage PBC before UDCA

treatment. Pathological bile duct loss could be irreversible even with UDCA treatment unlike pathological cholangitis. This fact may be associated with the mechanism of poor prognosis in PBC patients before UDCA treatment. Additionally, total bilirubin was also a significant independent poor prognostic factor in patients with ALBI grade 1. Serum total bilirubin level has been known as important prognostic factor of PBC. Some prognostic model such as Mayo PBC survival model, MELD (model for endstage liver disease) score, and ALBI score include total bilirubin as one of the variables used in calculations [10, 11]. It is notable that our study showed total bilirubin was also prognostic factor in patients with clinical early-stage.

4.2 | Context With Published Literature

In recent years, PBC is becoming more frequently diagnosed at an early stage [1]. Although no radical PBC treatment exists, the prognosis of UDCA treatment responders is relatively favorable [12–16]. The number of other effective treatment options is increasing, including positive prognosis values for bezafibrate along with improvements in biochemical findings by pemafibrate and obeticholic acid [17–22]. Several highly accurate prognostic models have been developed for PBC based on large patient series that focus on the biochemical response to UDCA therapy [23–28]. However, those models require 6–12 months from the start of UDCA, with some requiring complex calculations. Recent studies reported that ALBI score/grade could be useful in estimating PBC prognosis [29, 30]. ALBI score/grade can be calculated relatively easily from only two items: albumin and bilirubin. Elsewhere, diagnosis at early histological stages was found to carry a favorable prognosis, whereas the cirrhotic phase of PBC was associated with an increased risk of liver decompensation and liver-related death [31, 32]. Liver histology is indeed a strong prognostic factor. However, in current clinical practice, PBC may preferably be diagnosed at an early stage clinically or histopathologically. A simple index such as bile duct loss score to estimate the prognosis of early-stage patients is needed.

Interestingly, multivariable analysis showed that bile duct loss was associated with elevated ALP, γ -GTP, IgM, and IgG (Supporting Information S1: Tables S3 and S4), suggesting a phenotype characterized by more intense or sustained immune-mediated bile duct injury. In this context, bile duct loss may reflect a qualitatively distinct immunopathological state rather than merely disease duration. Nonetheless, lead time bias is also possible: a longer subclinical course (e.g., 20 years) may partially account for poorer outcomes in patients with bile duct loss score of ≥ 1 . These possibilities are not mutually exclusive, and both merit further study to clarify the biological and prognostic meaning of bile duct loss in early-stage PBC.

4.3 | Strengths and Limitations

This study included a relatively large number of PBC patients who were followed over a long observation period with the inclusion of thorough liver pathology analysis. To our knowledge, it is the first to highlight the prognostic significance of bile duct loss in the pathology of early-stage PBC among the scarce studies focusing on patients with this condition.

This study has several limitations. First, we could not fully assess the impact of UDCA responsiveness, and no histological evaluation was performed after UDCA initiation. The retrospective design may also introduce selection and sampling bias. Second, although all histological assessments were performed by an experienced hepatopathologist, the lack of interobserver validation may limit generalizability. Third, orcein staining was not routinely performed and was inconsistently available in archival samples, so it could not be evaluated in this study. Future prospective studies with standardized protocols should address these issues.

4.4 | Future Implications

The system proposed by Nakanuma et al. is useful for separately assessing the degree of liver fibrosis and bile duct loss in histological staging [7–9]. However, the utility of Nakanuma staging for prognosis other than liver fibrosis score has not been reported to date. Our study demonstrated the clinical potential of evaluating bile duct loss score for estimating prognosis in patients with early-stage PBC. Our results suggest that if clinicians perform liver biopsy on PBC patients, especially at the early stage, considering bile duct loss score may help determine prognosis. Further research is needed to explore other prognostic factors, including noninvasive markers, in clinicopathological early-stage PBC patients.

5 | Conclusions

In conclusion, Nakanuma bile duct loss score may be useful to prognosticate outcomes in PBC patients at a clinicopathological early-stage before starting UDCA therapy.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting Information S1: hepr70018-sup-0001-suppl-data.docx.