

# Value of posttransplant protocol biopsies in 2 biliary autoimmune liver diseases

## A step toward personalized immunosuppressive treatment

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### Abstract

The value of protocol liver graft biopsies with good liver function was evaluated in patients with primary sclerosing cholangitis (PSC) or primary biliary cholangitis (PBC).

A total of 250 protocol liver biopsy reports from 182 PSC and PBC patients were compared. Overall histopathological findings and those leading to changes in immunosuppression therapy were retrospectively analyzed.

The mean time to first protocol biopsy after transplantation was 5.5 ( $\pm$ 4.5) years for PSC patients and 9.3 ( $\pm$ 6.6) years for PBC patients. More than 1 abnormal histopathological parameter was found in 43% and 62% of PSC and PBC patients, respectively. However, the histology was interpreted as normal by the pathologist in 78% of PSC and 60% of PBC patients. Immunosuppression therapy was reduced in 10% and increased in 6% patients due to protocol biopsy findings. Biopsies leading to increased immunosuppression therapy had more portal ( $P=.004$ ), endothelial ( $P=.008$ ), interphase ( $P=.021$ ), and lobular ( $P=.000$ ) inflammation.

Mild histopathological findings were frequently found in the protocol biopsies despite the normal biochemistry. PBC patients had more histological abnormalities than those transplanted due to PSC; however, PBC patients had longer follow-up times. Immunosuppression therapy could be safely increased or decreased according to protocol biopsy findings after multidisciplinary meeting discussions.

**Abbreviations:** HCV = hepatitis C-virus, LTx = liver transplantation, MDT = multidisciplinary team, PBC = primary biliary cholangitis, PSC = primary sclerosing cholangitis.

**Keywords:** liver transplantation, primary biliary cholangitis, primary sclerosing cholangitis, protocol biopsy

### 1. Introduction

Percutaneous liver biopsy is an established method for clinical diagnosis (e.g., rejection) after liver transplantation (LTx).

Editor: Maya Saranathan.

Marko Vannas and Helena Isoniemi were partially supported by the Finnish Ministry of Social Affairs and Health through the special funds grant awarded to the Helsinki University Hospital.

The authors have no conflicts of interest to disclose.

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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How to cite this article: Vannas M, Arola J, Nordin A, Isoniemi H. Value of posttransplant protocol biopsies in 2 biliary autoimmune liver diseases: a step toward personalized immunosuppressive treatment. *Medicine* 2022;101:2 (e28509).

Received: 14 April 2021 / Received in final form: 21 November 2021 / Accepted: 17 December 2021

<http://dx.doi.org/10.1097/MD.00000000000028509>

However, its impact as a protocol biopsy taken at predetermined time points is not confirmed.

Many centers have discontinued protocol biopsies, or only use them for hepatitis C virus (HCV) patients after LTx and quite rarely for other diseases.<sup>[1]</sup> In a survey of 35 transplant centers, only 9 reported using protocol biopsies other than for HCV-infected transplanted recipients.<sup>[1]</sup> It is clear that short-term outcomes after LTx have improved over time, but there has not been similar improvement in long-term outcomes.<sup>[2]</sup> It has been suggested that protocol biopsies should be more widely used to improve graft diagnostics in the long-term.<sup>[3,4]</sup> Several studies have shown changes in histology even when liver biochemistry values are normal.<sup>[5]</sup> These findings have led to renewed interest in protocol biopsies, which have shown recurrence of primary liver disease, chronic rejection, chronic graft hepatitis, and other abnormalities.<sup>[6,7]</sup>

Immunosuppression therapy also causes problems in the long-term, but histology can be used to evaluate tolerance to reduce the use of immunosuppressive drugs.<sup>[8]</sup> Changes of liver parenchyma can be used to modify immunosuppression therapy before acute clinical symptoms occur.<sup>[9]</sup> Personalized immunosuppressive therapy is an active research area; thus, it is expected that the “one-size-fits-all” strategy of long-term immunosuppression therapy will be superseded in the future.<sup>[10]</sup> Immunosuppressive medications should be tailored according to various factors, including the inflammatory status of the graft.

Our center, Transplantation and Liver Surgery Clinic, Helsinki University Hospital, has been performing protocol biopsies since 2009.

Primary sclerosing cholangitis (PSC) and primary biliary cholangitis (PBC) are both cholestatic immune-mediated liver diseases that cause liver fibrosis and ultimately lead to cirrhosis.<sup>[11,12]</sup> While they both share similar end-points, it is evident that they are very different diseases as the options for treatment and diagnoses are rather different. Additionally, PSC is frequently found in association with inflammatory bowel diseases and has more serious complications and associated risks, mainly cholangiocarcinoma. Disease recurrence after LTx is more common in PSC than in PBC.

In this mainly cross-sectional retrospective study, we examined the histological findings interpreted at the time of protocol biopsy of these 2 autoimmune diseases, PSC and PBC, mainly affecting the bile ducts. This information was combined with multidisciplinary team (MDT) suggestions for treatment changes. The ultimate goal was to determine the impact and usefulness of the protocol biopsy of well-functioning grafts in the clinical decision.

## 2. Materials and methods

This study includes all PSC and PBC patients transplanted in 1 center and having protocol biopsies. Histopathological findings were compared with other clinical findings and immunosuppression therapy.

### 2.1. Patients

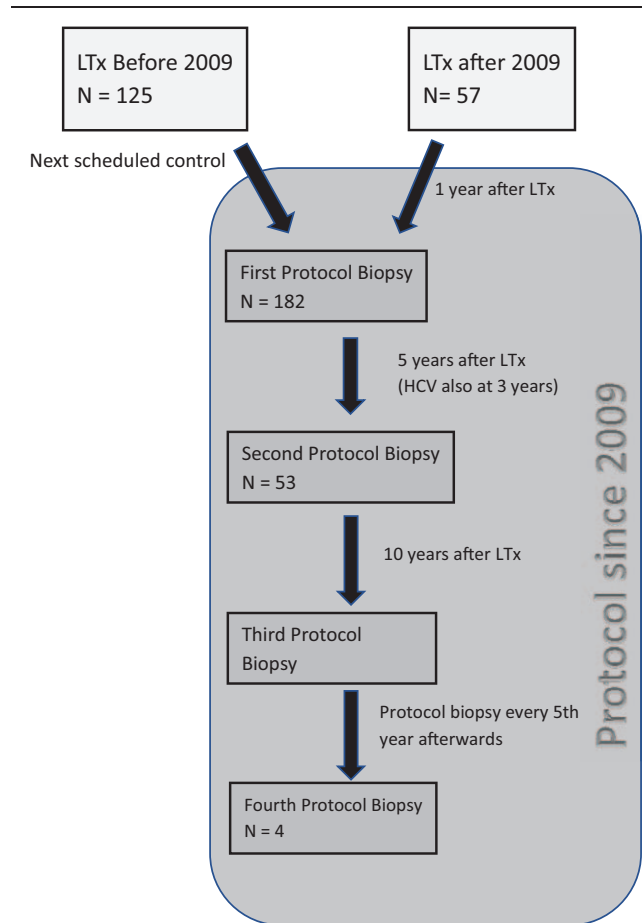
At end of May 2015, there were 153 PSC and 143 PBC patients in the Finnish Liver Transplantation Registry, transplanted since 1982. This registry includes all LTxs performed in the only transplant center in Finland. All patients have had regular follow-up in the transplantation center with 2- to 3-year intervals after the first 2 years. Protocol biopsies were started since 2009.<sup>[13]</sup> Since then, a protocol biopsy was performed at 1 year and every fifth year posttransplant. Hepatitis C virus patients also had biopsy at 3 years. The first protocol biopsy for patients transplanted before 2009 has been performed during the nearest scheduled follow-up visits and then according to the protocol (Fig. 1).

Structured histopathological reports on posttransplantation biopsies interpreted by a liver pathologist have been available in the electronic database of the Department of Pathology since 2004. The present study included 100 PSC and 82 PBC liver transplant patients who had at least 1 protocol biopsy since 2009. Liver transplantation for these 182 patients was performed between 1987 and 2014.

Institutional board of Abdominal Center in Helsinki University Hospital has approved the study.

### 2.2. Immunosuppression

All patients initially received calcineurin inhibitor-based immunosuppression. The majority received and was maintained on cyclosporine in combination with an antimetabolite; azathioprine was the primary drug of choice until 2006 and mycophenolate thereafter. Immunologically unstable patients or those in ongoing trials were treated with tacrolimus. Methylprednisolone was given initially, and when feasible, steroid withdrawal was done within 12 months postoperatively except in patients with underlying autoimmune liver disease. In the long-term, the typical daily dose of methylprednisolone was 2 to 4 mg.



**Figure 1.** Flowchart of the protocol biopsies since 2009. HCV = hepatitis C virus, LTx = liver transplantation.

The modification of patient immunosuppression therapy is discussed in regular multidisciplinary meetings with pathologist, gastroenterologist, radiologist, and transplant surgeon. The histology of the protocol biopsy is discussed with other clinical findings. If histology findings were normal, possible reduction was discussed especially if any side effects of immunosuppression was observed. Immunosuppression therapy was enhanced if signs of chronic graft hepatitis (interface/lobular inflammatory changes) were detected.

**2.2.1. Liver biopsies.** According to our protocol, liver biopsy was performed 1 year and every 5 years after transplantation (also 3 years posttransplantation in HCV patients). Experienced liver radiologists perform all protocol biopsies using the Biopsy device under ultrasound guidance using a 16- or 18-G needle. Biopsies were considered protocol biopsies when they were taken at predetermined times after transplantation and patient biomarkers or clinical signs do not warrant a biopsy. Of all 1096 biopsy reports of PSC and PBC found in the pathology database, we excluded all indication biopsies that were taken due to abnormal liver biochemistry, biopsies at the time of transplantation, and follow-up biopsies, which were scheduled for a later date due to either clinical or histopathological findings, at autopsy or before LTx. The medical records of the remaining 276 reports from 82 PBC and 100 PSC patients marked as

**Table 1**  
**Morphological parameters analyzed in this study.**

Parameter	Grading
Portal inflammation 0–3	Absent (0), mild (1), moderate (2), and Severe (3) (BANFF RAI scoring[1997, Hepatology, 25, 658-63])
Cholangitis 0–3	Absent (0), mild (1), moderate (2), and severe (3) (BANFF RAI scoring[1997, Hepatology, 25, 658-63])
Endothelial Inflammation 0–3	Absent (0), mild (1), moderate (2), and severe (3) (BANFF RAI scoring[1997, Hepatology, 25, 658-63])
Interphase Inflammation 0–3	Absent (0), mild (1), moderate (2), and severe (3) (METAVIR)
Lobular inflammation 0–2	Absent (0), mild (1), severe (2) (METAVIR)
Fibrosis 0–4	METAVIR classification [Bedossa and Poynard, 1996, Hepatology, 24, 289-93]
Steatosis 0–3	0 = 0–4% found in hepatocytes 1 = 5–30% 2 = 31–60% 3 = >60%
Hepatocyte necrosis	Any sign of necrosis
Cholestasis	Any sign of cholestasis
Hemorrhage	Any sign of hemorrhage in the biopsy
Congestion	Any sign of congestion in the biopsy
Arterial obliteration	Any sign of arterial obliteration

RAI = Rejection Activity Index.

protocol biopsies were examined in detail. However, an additional 16 reports were considered to be from follow-up biopsies and 10 were discarded due to inconsistencies. Therefore, the final number of biopsies was 250 from 82 PBC patients (117 biopsies) and 100 PSC patients (133 biopsies). Fifty-three patients had multiple protocol biopsies (Fig. 1). One patient had re-transplantation after the first protocol biopsy and a subsequent protocol biopsy after the re-transplantation. Of the 182 patients included in this study, 81 underwent additional biopsies, either a protocol biopsy (n=40), follow-up biopsy (n=8), or indication biopsy (n=33).

### 2.3. Biochemistry

Hematological laboratory values, hemoglobin, leucocytes and thrombocytes, as well as levels of liver enzymes, bilirubin, electrolytes, and creatinine were obtained from the LTx registry and electronic patient records for relevant biopsy dates.

**2.3.1. Histopathological scoring.** All histological samples were evaluated by an expert liver pathologist at the time of diagnosis. Results were reported in a predefined structured format tailored for LTx biopsies and included 12 histopathological parameters (Table 1). Fibrosis and activity of chronic hepatitis were graded using the METAVIR classification.<sup>[14]</sup> Our reports came involved protocol biopsies, and no transplant rejections were reported.<sup>[15]</sup> Idiopathic posttransplant chronic hepatitis was reported here as chronic graft hepatitis (Fig. 2A and B), as well as the possible recurrence of the original disease (Fig. 2C and D). Protocol biopsies were routinely stained in our laboratory, including hematoxylin and eosin, Herovici, Periodic acid-Schiff-diacetate, and cytokeratin 7. Original reports were used for analysis as the clinical decision was based on their interpretation.

### 2.4. Statistics

Statistics was done using IBM SPSS version 25 (New York, NY), and group comparisons were done using either Fisher exact test or chi-squared test. The *t* test was used to compare means, and the

nonparametric independent-samples median test was used when data were not normally distributed.

## 3. Results

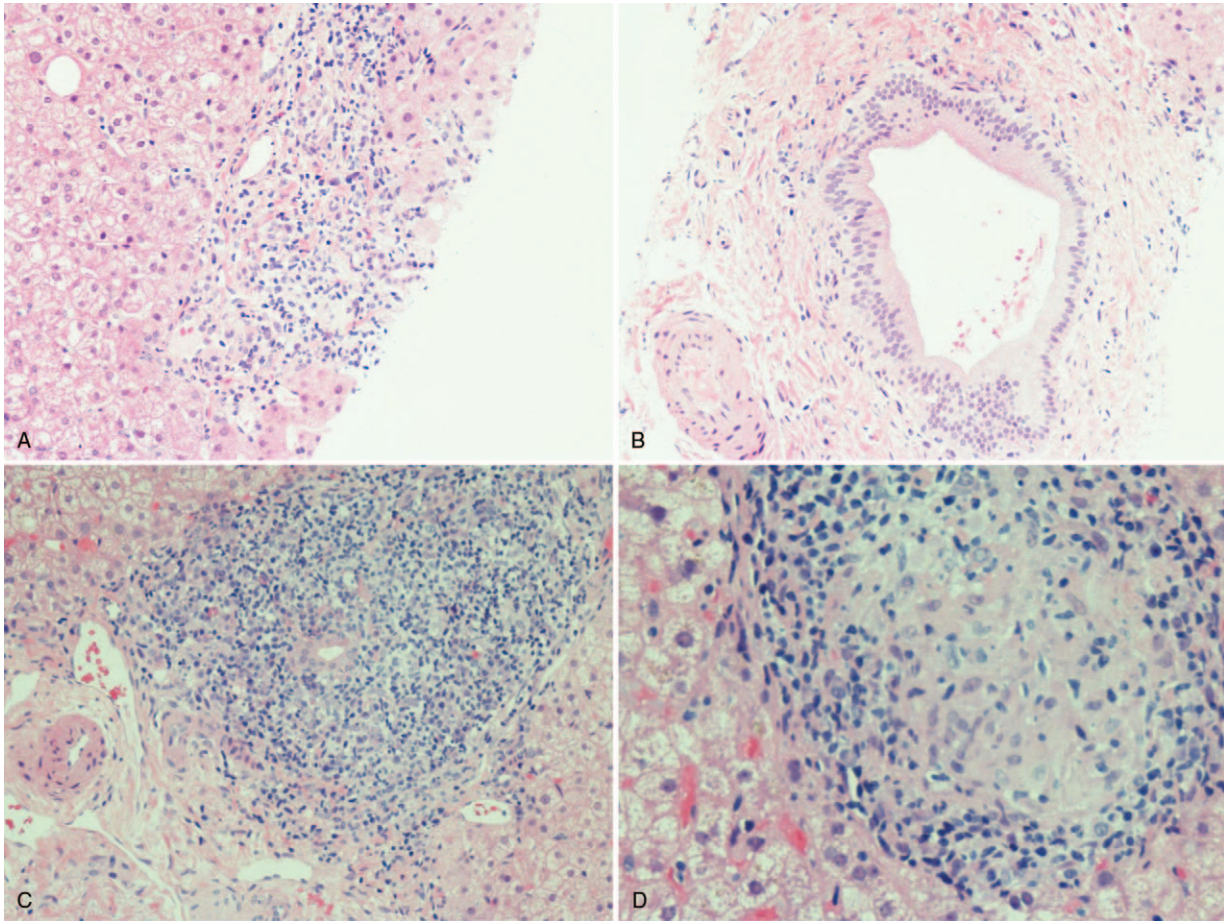
Primary biliary cholangitis patients were older than PSC patients (53.3 years vs 45.1 years,  $P < .001$ ), had longer follow-up times (12.3 years vs 8.3 years,  $P = .000$ ) and longer mean times to first protocol biopsy (9.3 years vs 5.5 years,  $P < .001$ ), and spent a shorter time on the waiting list (42 days vs 81 days,  $P = .001$ ). There were more females ( $P < .001$ ) in the PBC patients and the type anastomoses were mostly different ( $P < .001$ ) (Table 2).

There were more PBC patients who were over 15 years from their LTx (Fig. 3). Laboratory tests at the time of the protocol biopsy were similar in both patient groups (Table 3). Statistically significant differences in the medians of the international normalized ratio and prealbumin and sodium levels were observed, but the 95% confidence intervals were within the normal range in both groups.

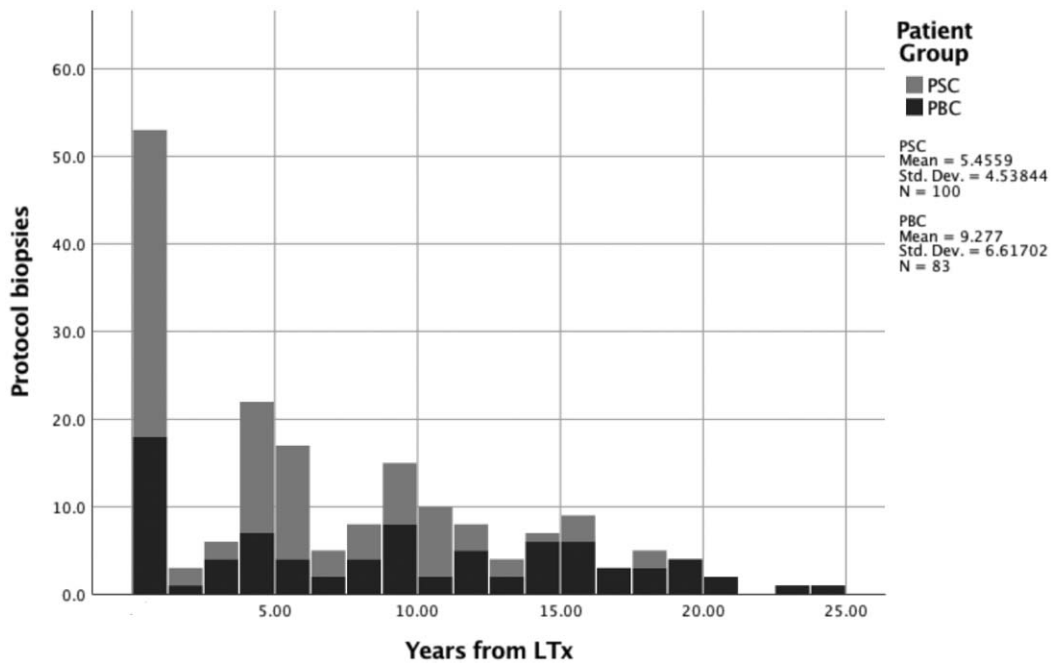
### 3.1. Histopathological parameters

Normal liver histology without any abnormal histopathological parameters was reported in 19% (48/250) of all liver biopsies, 12% of PBC (14/117) patients, and 26% of PSC (34/133) patients. One abnormal histopathological parameter was found in 27% of biopsies in PBC patients (30/117) and 32% of biopsies in PSC (42/133) patients. Multiple abnormal histopathological parameters were observed in 62% of biopsies in PBC (73/117) patients and in 43% of biopsies in PSC (57/133) patients (see Table 1 for parameters and Table 4 for results).

Moderate (20%) and severe (5%) portal inflammation were more frequent in the biopsies of PBC patients compared to those of PSC patients (10% moderate and 1% severe) ( $P = .01$ ). Lymphocytic cholangitis was more frequent in the biopsies of PBC patients than that in PSC patients ( $P = .011$ ; Table 5). Steatosis was more common in PBC patients, although the difference did not reach statistical significance ( $P = .053$ ). Severe steatosis (>60%) was only found in a few patients in both



**Figure 2.** (A and B) Protocol biopsy 3 yrs after liver transplantation due to primary sclerosing cholangitis, mild chronic graft hepatitis (idiopathic posttransplant chronic hepatitis), no sign of recurrence. (C and D) Protocol biopsy 3 yrs after liver transplantation due to primary biliary cholangitis recurrence.



**Figure 3.** Years after liver transplantation to first protocol biopsy. LTx = liver transplantation, PBC = primary biliary cholangitis, PSC = primary sclerosing cholangitis.

**Table 2****Demographics of liver transplantation recipients.**

	PSC LTx (n = 100) Mean (SD)	PBC LTx (n = 83)* Mean (SD)	P
Recipient sex			<.001
Male	62	10	
Female	38	73	
Age at the time of transplantation	45.1(11.8)	53.3 (9.3)	<.001
Recipient BMI	23.9 (4.52)	23.76 (4.46)	.822
Waiting time (d)	81 (90)	42 (51)	.001
Follow-up after LTx (yrs)	8.3 (4.9)	12.3 (7.1)	.000
Protocol biopsy count per patient	1.33	1.41	.421
First protocol biopsy after LTx (yrs)	5.5 (4.5)	9.3 (6.6)	<.001
LTx type			.845
First	92	77	
Re-LTx	8	6	
Donor age	42 (15)	45 (15)	.216
Donor BMI	23.96 (3.2)a	23.39 (3.17)b	.229
Cold ischemia time (h)	5:17.0 (01:55)	5:46 (02:39)	.156
Anhepatic time (min)	55 (13)	54 (11)	.582
Type of anastomosis			<.001
Duct-to-duct	6	80	
Roux-en-Y	94	2	

Mean (SD).

(a) n=98, (b) n=81.

BMI = body mass index, LTx = liver transplantation, PBC primary biliary cholangitis, PSC = primary sclerosing cholangitis.

\* One patient had 2 transplantations with protocol biopsies and is thus included twice.

groups. A total of 7% PBC and 5% PSC patients had more than 30% fat.

**3.1.1. Clinical diagnostics in protocol biopsies.** Histological findings were interpreted as normal or mild and clinically nonspecific in 60% (70/117) of PBC patients and 78% (104/133) of PSC patients. Recurrent disease was diagnosed in 15% (18/117) of PBC patients and 3% (4/133) of PSC patients. Chronic graft hepatitis (including inflammatory activity) was present in 14% (16/117) of PBC patients and 7% (10/133) of PSC patients. Steatohepatitis was only found in 2/133 of PSC patients. Vanishing bile duct syndrome was present in 1% (1/117) of PBC biopsies and 2% (2/133) of PSC biopsies (Table 4).

**3.1.2. Variation based on time from liver transplantation.**

Variation between 1-, 5-, and 10-year protocol biopsies was insignificant, but PSC patients had less abnormal histopathological parameters than that in PBC patients at all times. Biopsy findings were more often interpreted as normal by pathologists in PSC than that in PBC patients at 0 to 3 years ( $P=.008$ ), 3 to 7 years ( $P=.02$ ), and 7 to 12 years ( $P=.019$ ) after LTx. Recurrence based on pathology was more frequent in PBC than that in PSC patient biopsies at 0 to 3 years and 3 to 7 years after LTx ( $P=.043$  and  $P=.015$ ). Chronic graft hepatitis from pathology was more frequently interpreted in PBC patient biopsies at 3 to 7 years ( $P=.008$ ) after LTx but more frequent in PSC patient biopsies taken over 12 years ( $P=.022$ ) after LTx (Table 4).

**Table 3****Biochemistry results at protocol biopsy.**

Laboratory test (normal ranges m/f)	PSC n=133	PBC n=117	P
	Median (95% CI)	Median (95% CI)	
Hemoglobin (mg/L, 134–167/117–155)	126 (124-131)	125 (123-130)	.414
Leukocytes (E9/L, 3.4-8.2)	5.0 (5.0-6.0)	5.0 (5.0-6.0)	.726
Thrombocytes (E9/L, 150-360)	203 (190-221)	183 (175-205)	.096
INR	1.0 (1.0-1.1)	1.0 (1.0-1.1)	.090
Albumin (g/L, 36-48)	36 (36-38)	36 (36-38)	.178
Prealbumin (mg/L, 200-390/170-350)	242 (230-247)	210 (196-237)	.035
ALT (U/L, <50/35)	26 (24-32)	24 (20-28)	.762
AST (U/L, 15-45/15-35)	28 (27-30)	27 (25-29)	.165
ALP (U/L, 35-105)	80 (72-87)	78 (75-89)	.601
GGT (U/L, <60/40)	34 (35-49)	52 (40-57)	.101
Bilirubin (μmol/L, <20)	11 (10-13)	11 (11-13)	.970
Creatinine (μmol/L, 60-100/50-90)	98 (93-104)	96 (89-100)	.940
Urea (mmol/L, 3.5-8.1/3.1-7.9)	7.9 (7.0-8.0)	8.9 (8.0-9.0)	.047
Sodium (mmol/L, 137-145)	140 (140-141)	141 (141-143)	.016

ALT = alanine aminotransferase, AST = aspartate aminotransferase, ALP = alkaline phosphatase, GGT = gamma glutamyl transferase, INR = international normalized ratio.

**Table 4**  
Histopathological findings and pathology interpretations based on patient group and time range.

	0 to 3 yrs from LTx		3 to 7 yrs from LTx		7 to 12 yrs from LTx		Over 12 yrs from LTx		All			
	PSC	PBC	P	PSC	PBC	P	PSC	PBC	P	PSC	PBC	P
	n=43	n=26		n=42	n=25		n=35	n=26		n=133	n=117	
Morphological abnormalities												
None	12 (28%)	4 (15%)		9 (21%)	1 (4%)		9 (26%)	5 (17%)		4 (31%)	4 (11%)	34 (26%) 14 (12%)
One parameter	15 (35%)	4 (15%)		13 (31%)	8 (32%)		10 (29%)	6 (20%)		4 (31%)	12 (33%)	42 (32%) 30 (26%)
Two or more	16 (37%)	18 (70%)		20 (48%)	16 (64%)		16 (46%)	19 (63%)		5 (38%)	20 (56%)	57 (43%) 73 (62%)
PAD interpreted from biopsy												
Normal	37 (86%)	15 (58%)	.008	29 (67%)	10 (36%)	.02	30 (86%)	30 (86%)	.019	8 (62%)	27 (75%)	.357 104 (78%) 70 (57%) .002
Recurrent disease	1 (2%)	4 (15%)	.043	1 (2%)	5 (18%)	.015	2 (6%)	2 (6%)	.156	0 (0%)	4 (11%)	.278 4 (3%) 18 (15%) .001
Chronic graft hepatitis*	3 (7%)	4 (15%)	.262	3 (7%)	8 (29%)	.008	1 (3%)	1 (3%)	.232	3 (23%)	1 (3%)	.022 10 (8%) 16 (13%) .083
Vanishing bile duct synd.	0 (0%)	0 (0%)	-	1 (2%)	0 (0%)	-	1 (3%)	1 (3%)		0 (0%)	1 (3%)	12 (9%) 17 (14%) .235
Steatosis	2 (5%)	3 (12%)	.285	7 (16%)	5 (18%)	.731	1 (3%)	1 (3%)	.026	2 (15%)	3 (8%)	.472 2 (2%) 0 (0%) .5
Steatohepatitis	0 (0%)	0 (0%)	-	2 (5%)	0 (0%)	-	0 (0%)	0 (0%)		0 (0%)	0 (0%)	2 (2%) 1 (1%) 1

LTx = liver transplantation, PBC = primary biliary cholangitis, PSC = primary sclerosing cholangitis.  
\* Idiopathic posttransplant chronic hepatitis.

**3.1.3. Subsequent biopsies.** Follow-up or new protocol biopsies were available from 81 patients. One-third of patients (27/81) had significant/relevant diagnostic findings in the first protocol biopsy; 14 had chronic graft hepatitis, 6 had steatosis, 2

had vanishing bile duct syndromes, 9 had PBC recurrence, and 1 had PSC recurrence. Of the 54 patients who had no abnormalities in the first protocol biopsy, 22% (12/54) developed diagnostic changes in a subsequent protocol biopsy; 7 had chronic graft

**Table 5**  
Comparison of morphological parameters between groups.

Parameter	Grading	PSC (n = 133)		PBC (n = 117)		P
		n	%	n	%	
Portal inflammation 0-3	Absent	62	47%	35	30%	.005
	Mild	56	42%	52	44%	
	Moderate	14	11%	24	21%	
	Severe	1	1%	6	5%	
Cholangitis 0-3	Absent	115	87%	75	64%	.001
	Mild	16	12%	35	30%	
	Moderate	2	2%	6	5%	
	Severe	0	0%	1	1%	
Endothelial inflammation 0-3	Absent	130	98%	113	97%	.578
	Mild	3	2%	4	3%	
	Moderate	0	0%	0	0%	
	Severe	0	0%	0	0%	
Interphase inflammation 0-3	Absent	116	87%	91	78%	.108
	Mild	14	11%	20	17%	
	Moderate	2	2%	6	5%	
	Severe	1	1%	0	0%	
Lobular inflammation 0-2	No	112	84%	99	85%	.266
	Mild	20	15%	14	12%	
	More than mild	1	1%	4	3%	
Fibrosis (METAVIR)	Stage 0	92	69%	71	61%	.395
	Stage 1	28	21%	36	31%	
	Stage 2	10	8%	9	8%	
	Stage 3	2	2%	1	1%	
	Stage 4	1	1%	0	0%	
Vacuolization (fat)	0%-4%	112	84%	83	71%	.053
	5%-30%	15	11%	26	22%	
	31%-60%	2	2%	5	4%	
		4	3%	3	3%	
	>60%					
Hepatocyte necrosis		4	3%	3	3%	.832
Cholestasis		0	0%	2	2%	.130
Hemorrhage		4	3%	5	4%	.592
Congestion		11	8%	10	9%	.937
Arterial obliteration		4	3%	2	2%	.404

PBC = primary biliary cholangitis, PSC = primary sclerosing cholangitis.

**Table 6**  
Treatment changes due to diagnostic interpretations of the protocol biopsy.

Type of immunosuppression/medication change	PSC n=133	PBC n=117
	n (%)	n (%)
Reduced – Group 2 (P=.068)	17 (13%)	7 (6%)
MMF or Aza stopped or reduced	15	7
Steroids stopped	2	0
Triple medication reduced to double	3	2
Double medication reduced to single	3	2
Increased – Group 1 (P=.872)	8 (6%)	7 (6%)
MMF or Aza started, increased or MMF changed to Aza	8	4
Steroid started	1	5
Single medication increased to double/triple	2	3
Double medication increased to triple	5	2
No change – Group 0 (reference group)	108 (81%)	103 (88%)
Calcineurin inhibitors at biopsy (P=.226)	133 (100%)	113 (97%)
Reduced	14 (11%)	8 (7%)
Stopped	0 (0%)	1 (1%)
Increased	0 (0%)	2 (2%)
Added	0 (0%)	0 (0%)

Aza = azathioprine, MMF mycophenolate mofetil, PBC = primary biliary cholangitis, PSC = primary sclerosing cholangitis.

hepatitis, 2 had steatosis, 2 had PBC recurrence, and 1 had PSC recurrence.

**3.1.4. Changes in medication after protocol biopsy.** All included biopsies were divided into 3 groups based on whether or not changes were made to the immunosuppressive regimen: 1 = increase, 2 = decrease, and 3 = no change groups (Table 6). Most patients (211/250) belonged to the no change group, which was used as the reference group. Immunosuppression was decreased in 9.6% (24/250) of cases.

None of the histopathological parameters were statistically different between the decrease and no change groups (Table 7). Eight of the 24 patients with reduction in immunosuppression had follow-up biopsies. An indication biopsy was taken from 1 patient 46 days after the protocol biopsy due to increased biochemical markers. This patient exhibited inflammatory changes in the protocol biopsy, but due to the side effects of the immunosuppressants, immunosuppression therapy was reduced. The remaining biopsies (7 cases) were conducted more than 2 years later. Immunosuppression therapy was increased in 6% (15/250) of cases in which biopsy reports included more portal inflammation (P=.002), interphase (P=.021), and lobular activity (P=.000) compared to the no change group. In 66% (10/15) of cases, additional biopsies were performed, 30% of

**Table 7**  
Comparison of morphological parameters between immunosuppression groups.

Parameter	Grading	Immunosuppression medication						P*	
		No change (n=211)		Increased (n=15)			Reduced (n=24)		
		n	%	n	%	P*	n		%
Portal Inflammation	Absent	81	38%	4	27%	.004	12	50%	.629
	Mild	96	45%	3	20%		9	38%	
	Moderate	28	13%	7	47%		3	13%	
	Severe	6	3%	1	7%		0	0%	
Cholangitis	Absent	163	77%	8	53%	.067	19	79%	.778
	Mild	39	18%	7	47%		5	21%	
	Moderate	8	4%	0	0%		0	0%	
	Sever	1	0%	0	0%		0	0%	
Endothelial Inflammation	Absent	207	98%	13	87%	.008	23	96%	.465
	Mild	4	2%	2	13%		1	4%	
	Moderate	0	0%	0	0%		0	0%	
	Severe	0	0%	0	0%		0	0%	
Interphase Inflammation	Absent	177	84%	8	53%	.021	22	92%	.717
	Mild	26	12%	6	40%		2	8%	
	Moderate	7	3%	1	7%		0	0%	
	Severe	1	0%	0	0%		0	0%	
Lobular inflammation	Absent	182	86%	6	40%	.000	23	96%	.4
	Mild	25	12%	8	53%		1	4%	
	Severe	4	2%	1	7%		0	0%	
	Stage 0	134	64%	9	60%		20	83%	
Stage 1	56	27%	4	27%	4	17%			
Stage 2	17	8%	2	13%	0	0%			
Stage 3	3	1%	0	0%	0	0%			
Fibrosis	Stage 4	1	0%	0	0%	0	0%		
	0%-4%	162	77%	13	87%	.452	20	83%	.68
	5%-30%	37	18%	1	7%		3	13%	
	31%-60%	7	3%	0	0%		0	0%	
>60%	5	2%	1	7%	1		4%		
Hepatocyte Necrosis		7	3%	0	0%	.474	0	0%	.365
Cholestasis		2	1%	0	0%	.705	0	0%	.632
Hemorrhagia		8	4%	1	7%	.582	0	0%	.332
Congestion		19	9%	2	13%	.577	0	0%	.125
Arterial obliteration		5	2%	1	7%	.341	0	0%	.581

\* Compared to the no change group.

which were indication biopsies within 6 months due to clinical symptoms; 70% were other scheduled biopsies ranging from 6 months to 3 years, all of which showed reduced inflammatory changes.

#### 4. Discussion

In the past, liver biopsies were not widely used without clinical symptoms due to the cost and difficulties in histological classification<sup>[1,16]</sup> as well as complications associated with liver biopsy without evidence of usefulness.<sup>[17,18]</sup> Currently, liver biopsies are rather safe and performed using automated biopsy-devices with ultrasound guidance.<sup>[19]</sup> Reported complications are mostly minor, and major complications are rare.<sup>[7,20]</sup> For non-autoimmune diseases, there is a recently introduced guideline by Banff<sup>[8]</sup> for protocol biopsies. The criteria for rejection are the same for protocol biopsies of transplanted PSC and PBC patients. In both diseases, recurrence includes bile duct damage, which is only associated with very unusual chronic rejection so that distinction is not an issue.

One center in Germany reported that 22% of liver biopsies in LTx patients between 2000 and 2013 were protocol biopsies, and 14% of those resulted in adjustment of immunosuppression therapy.<sup>[21]</sup> In our cohort of liver transplanted patients due to 2 different autoimmune diseases, adjustment of immunosuppression therapy was done according to biopsy findings in 16% of patients (Table 6). At follow-up in this German study, it was found that the percentage of patients with elevated alanine aminotransferase levels decreased from 60% to 33% at 12 weeks; however, there were no further biopsies performed. In our study, we performed further control biopsies, which showed that inflammatory changes decreased in 70% of patients who had their immunosuppression therapy increased. Still, clear evidence for protocol biopsies is lacking especially today when more personalized immunosuppression therapies are used.

Comparing PBC and PSC patients in our study, PSC patients had less histopathological findings in well-functioning grafts (Table 4). It has been previously reported<sup>[22]</sup> that abnormalities in histology increase with time after LTx from 65% at the 10-year biopsy to 90% at the 20-year biopsy. However, the authors did not report any differences based on LTx indication. Our findings were similar in PBC patients, of which 62% had 2 or more abnormal histopathological parameters (Table 4). Only 43% of PSC patients had 2 or more abnormal histopathological parameters, but they also had a shorter time on average from LTx to biopsy. However, when comparing histopathological findings at different time points after LTx, the general assumption that PBC patients have more significant findings is true on all time points.

Primary disease recurrence was fairly low in the present study, namely, 15% in PBC patients and only 3% in PSC patients (Table 4). However, our numbers are based on histological findings in protocol biopsies only, and no other considerations for recurrence were taken into account. Since PSC recurrence diagnoses are usually based on radiological findings and not on biopsies as in our study, diagnosis of recurrent PSC is a controversial issue. It has been shown that various factors, such as recurrent bacterial cholangitis or ischemic-type biliary lesions, may cause biliary strictures in transplanted livers and may mimic the recurrence of PSC.<sup>[23]</sup> This also complicates the diagnosis of recurrent PSC from histological findings. In previous studies, magnetic resonance cholangiopancreatography-based PSC re-

currence rates have been higher at 19%.<sup>[24]</sup> Overall recurrence of PSC and PBC after LTx has been reported up to 37% and 43%, respectively.<sup>[25]</sup> PSC is evidently more often clinically symptomatic than PBC when recurrent disease occurs.<sup>[26]</sup> Based on histopathological findings, a previous study reported a significantly higher amount of 29% recurrent disease for PBC.<sup>[4]</sup> In the same study, 38% of PBC patient biopsies had some stage of chronic graft hepatitis, although most (95%) were reported as mild inflammatory activity only. However, the authors also reported that 26% had nearly normal liver histology, whereas in our study, only 12% had normal liver histology, possibly due to our longer follow-up time of 9.3 vs 3.3 years.<sup>[4]</sup> Also, our detailed structural reporting system for every biopsy with 14 different histopathological parameters, of which 12 were used in this study (Table 1), may have also influenced our results, as minor changes were found.

Many studies have reported that recurrent PBC does not have an impact on graft survival<sup>[11]</sup> in the short and medium term. Research on the long-term impact of PBC recurrence on graft survival has not been conducted, but re-transplantation is needed for recurrent disease. When recurrent disease occurs, adding ursodeoxycholic acid and increasing or maintaining adequate immunosuppression therapy for PBC patients may slow the progression or even prevent recurrence<sup>[23,26]</sup>, but long-term evidence for this is lacking.

Steatosis was more common in PBC patients, but none of them were diagnosed with steatohepatitis, possibly due to the fact that PBC patients were generally older and had longer follow-up. Body mass index was similar in both groups. Comorbidities, such as diabetes, were not included in the analysis, which might explain that steatosis was more common in PBC patients. An earlier study<sup>[4]</sup> reported only 3% of fatty liver occurrence in PBC patients, but did not specify the criteria (steatosis percentage) for the diagnosis. Our study population had a similar percentage of over 30% steatosis, 7% in PBC and 5% in PSC patients (Table 5). Indirect signs of chronic rejection (vanishing bile duct syndrome) were equally low in both patient groups.

Immunosuppressive medication is often the reason for patients' discomfort and lower quality of life<sup>[8]</sup> and therefore should be administered in a low dose as possible. In our study, 24 patients had their immunosuppression therapy reduced after protocol biopsy (Table 7), which resulted in transiently increased liver enzymes in 1 patient. This patient already had inflammatory changes in the protocol biopsy; however, due to severe side effects, reduction was required. None of the patients with reduced immunosuppression therapy reported any acute rejection episodes. Other studies<sup>[4]</sup> reported a higher percentage (25%) of reduced immunosuppression therapy based on protocol biopsy compared to our population (9.6%) (Table 6). There are no histological guidelines justifying the reduction of immunosuppression therapy in PBC and PSC patients. Another study suggests regular protocol biopsies for pediatric LTx patients to determine the long-term outcomes and guidance for withdrawal of immunosuppression therapy.<sup>[27]</sup> Our study population only included adult autoimmune liver diseases, and reduction of immunosuppression therapy was carried out with extreme caution as previously suggested by Banff<sup>[8]</sup> for this patient group. All our protocol biopsy results are discussed in MDT meetings, and thus, possible changes in medication were reviewed by a larger audience of experts. No specific cutoff values were identified for protocol biopsies either for reducing or increasing immunosuppression therapy (Table 7).



Personalized immunosuppression therapy has 2 goals, to prevent rejection and thus improve long-term function; and conversely, to individually minimize the adverse effects of drugs in all solid organ transplantations. Nowadays, tacrolimus, a calcineurin inhibitor, is used as an alternative to cyclosporine and mycophenolate mofetil, a purine synthesis inhibitor, as an alternative to azathioprine. Transplantation due to an autoimmune disease may necessitate lifelong steroid administrations after LTx but may carry an increased risk of cardiovascular complications. Selective matching of drugs in a given patient is possible, considering the transplanted organ, immunological status and compatibility, original end-stage organ disease, and adverse effects of a specific drug.

The obvious strength of our study is the structured report done by liver pathologist which enables us to formally assess the findings of the biopsies. All liver transplant patients are followed in the same transplant center which enhances the validity of the findings. Biases in this study include the large time variation from LTx to first biopsy and also a rather small number of patients.

Our data suggest that protocol biopsy may be useful in the decision-making of immunosuppression therapy. In our study, according to the protocol biopsy, immunosuppression therapy was increased in 6% of patients, which is in accordance with another study.<sup>[4]</sup> In 7 of 10 patients, with further follow-up biopsies, findings were diminished, suggesting that an increase in immunosuppression therapy resolved the clinical situation. Previous studies have shown that biopsies are useful in evaluating the effectiveness of immunosuppression therapy 20 years after LTx, resulting in changes of immunosuppressive medication in 35% of biopsy patients.<sup>[22]</sup> This study also found that biopsy seemed to indicate a more frequent increase than a decrease in immunosuppression therapy (Table 4). Increasing immunosuppression therapy based on inflammatory changes in biopsy is easily justified. A normal histology in protocol biopsy will help clinicians reduce immunosuppression therapy safely. Our study found more findings in the protocol biopsies of PBC patients than that of PSC patients. Overall, at least 1 histopathological parameter was abnormal in 2/3 of biopsies; however, biochemistry profiles did not differ in patients with a normal histology and those with pathological findings. Even though histopathological findings were common in the biopsies, they were mostly mild. Findings in protocol biopsies caused medication changes in 16% of patients. Thus, this result justifies the use of protocol biopsies in patients when the primary indications of LTx are PBC or PSC.

Using a predetermined structural report to present histological findings in biopsies is very useful for clinicians, especially in MDT meetings, as it allows other specialists to clearly understand the effect of medication change and the interpretation of the pathologist.

In the future, a separate study of longitudinal protocol biopsies should be carried out. Protocol biopsies can provide important and useful additional information. Mild histopathological findings were frequently found in the protocol biopsies despite the normal biochemistry. Clinical decisions of tailored immunosuppression therapy can be done in the context of protocol biopsies and multidisciplinary meetings.

### Author contributions

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