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2018-01

Hukkinen , M , Kerola , A , Lohi , J , Heikkilä , P , Merras-Salmio , L , Jahnukainen , T , Koivusalo , A , Jalanko , H & Pakarinen , M P 2018 , ' Treatment Policy and Liver Histopathology Predict Biliary Atresia Outcomes : Results after National Centralization and Protocol Biopsies ' , Journal of the American College of Surgeons , vol. 226 , no. 1 , pp. 46-57 . <https://doi.org/10.1016/j.jamcollsurg.2017.09.009>

<http://hdl.handle.net/10138/300142>

<https://doi.org/10.1016/j.jamcollsurg.2017.09.009>

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Treatment Policy and Liver Histopathology Predict Biliary Atresia Outcomes: Results after National Centralization and Protocol Biopsies

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- BACKGROUND:** Different treatment policies can influence biliary atresia outcomes, but the pathophysiology of expanding fibrosis occurring even after successful portoenterostomy remains unclear.
- STUDY DESIGN:** Clearance of jaundice (COJ) (bilirubin <20 $\mu\text{mol/L}$), native liver survival, and overall survival rates of biliary atresia patients were analyzed before and after national centralization of management, as well as in relation to native liver histopathology of protocol biopsies.
- RESULTS:** Of the 59 patients, 35 were managed after centralization and received standardized postoperative adjuvant therapy, including corticosteroids. After centralization, age at portoenterostomy decreased from 73 days to 54 days ($p = 0.014$) and COJ rate increased from 42% to 80% ($p = 0.005$), 5-year native liver survival increased from 38% to 70% ($p = 0.014$), and 5-year overall survival increased from 68% to 94% ($p = 0.007$). High-grade portal inflammation at portoenterostomy predicted COJ (odds ratio 3.66; $p = 0.011$) and slower fibrosis progression ($\beta = -0.74$; $p = 0.005$). Native liver survival was extended in patients with high-grade portal inflammation ($p = 0.002$) and in patients whose bilirubin normalized within 3 months ($p < 0.001$). Portal inflammation and cholestasis reduced only after COJ ($p < 0.001$), and persisting ductal reaction, reflected by cytokeratin 7-positive proliferating bile ductules and periportal hepatocytes, correlated with follow-up fibrosis ($r = 0.454$ to 0.763 ; $p < 0.001$ to 0.003). Cytokeratin 7 immunopositivity of periportal hepatocytes increased after COJ ($p = 0.015$) and was the only predictor of follow-up liver fibrosis ($\beta = 0.36$; $p = 0.002$) in multiple regression.
- CONCLUSIONS:** Biliary atresia outcomes improved significantly after centralization and standardized management. Resolution of cholestasis and reduction of high-grade portal inflammation postoperatively predict slower fibrosis progression and improved native liver survival, and persisting ductal reaction parallels progressive native liver fibrosis despite COJ. (J Am Coll Surg 2018;226:46–57. © 2017 by the American College of Surgeons. Published by Elsevier Inc. All rights reserved.)

Disclosure Information: Nothing to disclose.

Support: This study was supported by the Sigrid Jusélius Foundation, the Finnish Pediatric Research Foundation, and the Helsinki University Central Hospital Grant #TYH2015219.

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Received June 19, 2017; Revised July 28, 2017; Accepted September 7, 2017.

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Biliary atresia (BA) is an idiopathic cholangiopathy of infancy characterized by fibro-inflammatory obstruction of extrahepatic bile ducts, leading to cholestasis and eventually cirrhosis.¹ Adjacent to biliary fibrosis, chronic cholestasis promotes proliferation of bile ductules, called ductal reaction.² The first-line treatment for BA is portoenterostomy (PE), which typically restores adequate bile flow in approximately half of patients.^{3–7} It remains unclear why destruction of intrahepatic bile ducts, which promotes progressive liver damage, continues after resolution of jaundice.⁸ Additional understanding of the pathophysiology driving liver injury is essential for novel approaches designed to extend native liver survival. Biliary atresia is the most common indication for pediatric liver

Abbreviations and Acronyms

BA	= biliary atresia
BASM	= biliary atresia splenic malformation
COJ	= clearance of jaundice
CK7	= cytokeratin 7
HCM	= hepatocyte-cholangiocyte metaplasia
LT	= liver transplantation
OR	= odds ratio
PE	= portoenterostomy

transplantation (LT), which is required in 70% to 80% of patients within 20 years of PE.^{9,10}

After successful PE, plasma bilirubin levels normalize, which postpones the need for LT and improves patient survival.^{11,12} Portoenterostomy success rates are influenced by several patient-dependent factors, such as age at operation,^{3,6,13} anatomy of the biliary tract remnant,^{13,14} coexistent congenital malformations,^{3,6,7} and liver histologic features at PE, including degrees of fibrosis and ductal reaction.¹⁵⁻¹⁸ Postoperative steroids can improve clearance of jaundice (COJ) rates, although their effect on native liver survival remains unestablished.^{19,20} In addition, hospitals with low caseloads can have inferior outcomes compared with more experienced centers,^{4,7,21} which has led to centralization of BA treatment, at least in the UK and Finland.^{4,21}

After national centralization of BA management in Finland to Helsinki University Children's Hospital, results of the initial short-term follow-up demonstrated significantly improved COJ and transplant-free survival rates.⁴ To improve BA management and to better understand the pathophysiology behind progressive liver damage, protocol native liver biopsies at PE and during follow-up were started after centralization.²² Here, we evaluate the effect of centralization on BA outcomes after more than a decade of follow-up and compare the outcomes with those obtained in Helsinki before centralization. In addition, we address predictors of COJ, native liver survival, and overall survival, and analyze how liver histopathology at PE and during follow-up relates to these outcomes.

METHODS

Ethics

This study was approved by the Ethical Committee of Helsinki University Hospital and the Finnish National Authority of Medicolegal Affairs and Health. A consent from participants or their caregivers was obtained before any procedures.

Patients

In 2005, the Ministry of Health centralized BA management in Finland from 5 university hospitals to Helsinki University Children's Hospital, which is a tertiary referral center for pediatric liver diseases with a nationwide LT program since 1987. All BA patients managed in Helsinki during 1987 to 2016 with at least a 4-month follow-up after primary operation were included. Biliary atresia diagnosis was confirmed by operative findings; perioperative cholangiography, when possible; and histopathologic assessment of the biliary remnant. Medical records were reviewed for gestational age and weight, associated congenital malformations, anatomic type of BA,²³ details of operative management, COJ rate, LT listing and performance, and possible death. Biliary atresia splenic malformation syndrome (BASM) was defined as presence of poly- or asplenia.²⁴ Clearance of jaundice was defined as serum total bilirubin <20 µmol/L at any time point postoperatively. Bilirubin levels were recorded at 3 and 6 months after PE, as well as at last follow-up.

Management of biliary atresia

In contrast to the variable follow-up and medical management practices performed before centralization, management and follow-up of BA were standardized in 2005, and a single team led by 1 surgeon operated on patients thereafter. Ursodeoxycholic acid (30 mg/kg/d), thimetoprim-sulfadiazine (4 + 12.5 mg/kg/d), and dexamethasone with decreasing doses (0.6 to 0.2 mg/kg/d, days 5 to 28) were routinely administered postoperatively.²⁵⁻²⁷ Enteral energy provision was aimed at 150 kcal/kg/d with use of carbohydrate and medium chain fatty acid energy supplements.²⁸ Fat-soluble vitamin supplements were continued after discharge together with ursodeoxycholic acid and thimetoprim-sulfadiazine.^{4,28} Follow-ups were arranged at 3-month intervals in local hospitals and at least once a year in Helsinki.

Liver histology

Liver biopsies at PE and at last follow-up were analyzed and the changes occurring in histology over time were evaluated according to COJ status. Routine follow-up biopsies were initiated in 2005 and performed 1, 5, and 10 years after PE or when clinically indicated. Follow-up biopsies were taken percutaneously with ultrasound guidance under general anesthesia for endoscopic variceal surveillance.²⁹ For patients who underwent LT, follow-up liver histology was mainly based on the explant.

In addition to conventional stains, immunostaining for cytokeratin 7 (CK7) was performed using a rabbit anti-human monoclonal antibody and ultra View Universal DAB Detection Kit (Ventana). Two experienced pediatric

pathologists blinded to clinical data re-reviewed the slides until consensus was reached. Specimens were analyzed for portal inflammation (the extent of portal cellular infiltrate, scored 0 to 3), intracellular, intracanalicular, and ductal cholestasis (scored 0 to 3), and fibrosis (Metavir stage 0 to 4).³⁰ Ductal reaction was visualized by CK7 staining in proliferative bile ductules (scored 0 to 2) and adjacent hepatocyte-cholangiocyte metaplasia (HCM) by CK7 staining in periportal hepatocytes (scored 0 to 4).^{15,17,22} The changes of Metavir, CK7 immunostaining, and portal inflammation scores between PE and follow-up were calculated. Fibrosis progression rate (not calculated for patients with Metavir = stage 4 at PE, $n = 7$) was defined as the change of Metavir score divided by the years between PE and follow-up biopsies, and expressed as change in Metavir score/year.

Statistical methods

Continuous data are expressed as median values with interquartile ranges (IQR) and categorical data as frequencies unless otherwise stated. Kruskal-Wallis and Mann-Whitney U tests were used to compare independent continuous variables, Wilcoxon signed-rank test to compare repeated measurements, and Fisher's exact test to compare frequencies between groups. Associations between variables were examined with Spearman rank correlation.

Predictors of COJ, bilirubin levels at 3 months after PE, follow-up Metavir score, and fibrosis progression rate were evaluated with regression analyses. For COJ, odds ratios (OR) with 95% CIs were generated by binary logistic regression, and linear regression was used for continuous variables. Predictors showing significant associations with outcomes variables in simple regression were included in multiple regression analyses.

Cumulative native liver and overall survival rates were analyzed with Kaplan-Meier curves. Univariate predictors of survival were evaluated with the log-rank test. Multivariate survival models were performed by generating hazard ratios with 95% CIs with Cox proportional hazards regression model, adjusted for statistically significant variables from univariate models and for age at PE^{3,6,7} for native liver survival. The level of significance was set at $p < 0.05$ and all analyses were performed with SPSS, version 24 (IBM Corp).

RESULTS

Baseline characteristics

Altogether 63 patients were identified. Two patients were excluded; 1 died of heart defect before operation and the other with multiple severe anomalies died few weeks after

PE; no COJ status for him could be defined. The remaining 61 patients were divided into cohorts A ($n = 25$ patients [41%]) and B ($n = 36$ [59%]), depending on whether they were treated before (cohort A) or after (cohort B) the decree on national centralization came into force in 2005.⁴ Altogether, 59 patients underwent PE and 1 patient from both cohorts underwent primary LT due to delayed diagnosis and liver failure.

Based on annual birth rates in Finland,³¹ the incidence of BA was 1:18,600. Median annual caseload was 1 (IQR 1 to 2) before and 3.5 (IQR 2 to 4.3) after centralization. Cohort B patients underwent PE at younger age, had lower birth weight, and tended to be born earlier and have lower preoperative bilirubin compared with cohort A (Table 1). Median age at PE was 64 (IQR 39 to 85) days.

Liver biopsies at time of PE were available for half (12 of 24) of cohort A and 97% (34 of 35) of cohort B patients. At PE, histologic features consisted of moderate to severe fibrosis, portal inflammation, ductal reaction, and cholestasis (Table 1). Ductal reaction and associated periportal HCM correlated with age at operation ($r = 0.679$; $p < 0.001$ and $r = 0.463$; $p = 0.002$, respectively) and degree of fibrosis ($r = 0.539$; $p < 0.001$ and $r = 0.454$; $p = 0.002$, respectively). Portal inflammation showed no association with baseline characteristics.

Clearance of jaundice

The proportion of patients clearing their jaundice almost doubled after centralization; 28 of 35 (80%) vs 10 of 24 (42%) ($p = 0.005$). In total 26% (10 of 38) achieved COJ later than 3 months after operation, and COJ rate at 3 months was 65% in cohort B vs 32% in cohort A ($p = 0.028$). Median time to COJ showed no difference between cohorts A and B (3.2 [IQR 1.8 to 6.3] months vs 2.3 [IQR 0.84 to 3.6] months; $p = 0.272$). At 3 and 6 months after operation, cohort B patients had lower bilirubin than cohort A patients (11 [IQR 6.0 to 27] $\mu\text{mol/L}$ vs 135 [IQR 12 to 283] $\mu\text{mol/L}$; $p < 0.001$ and 7.5 [IQR 3.5 to 27] $\mu\text{mol/L}$ vs 182 [IQR 8.0 to 239] $\mu\text{mol/L}$; $p = 0.008$).

In logistic regression, operation after centralization (OR 5.60; 95% CI 1.76 to 17.9; $p = 0.004$), high-grade portal inflammation (OR 3.66; 95% CI 1.35 to 9.97; $p = 0.011$), and young age at operation (OR 0.98; 95% CI 0.97 to 1.00; $p = 0.025$) predicted COJ. In multiple regression, only the effect of portal inflammation remained significant (OR 3.82; 95% CI 1.23 to 11.9; $p = 0.020$).

Variables associated with bilirubin levels at 3 months in linear regression were operation before centralization ($\beta = 165$; 95% CI 77.6 to 252; $p < 0.001$), age at PE

Table 1. Baseline Characteristics and Liver Biopsy Findings at Portoenterostomy

Characteristic	Cohort A 1987–2004 (n = 25)	Cohort B 2005–2016 (n = 36)	p Value (cohort A vs B)	Both cohorts (n = 61)
Gestational age, wk, median (IQR)	40 (39–41)	39 (38–40)	0.055	39 (38–40)
Gestational weight, g, median (IQR)	3,260 (3,265–3,865)	3,264 (2,928–3,600)	0.014	3,390 (3,083–3,703)
Female, n (%)	14 (56)	19 (53)	1.000	33 (54)
Type of biliary atresia, n (%)				
1	1 (4.0)	1 (2.8)	1.000	2 (3.3)
2	2 (8.0)	2 (5.6)		4 (6.6)
3	22 (88)	33 (92)		55 (90)
Age at PE, d, median (IQR)	73 (53–101)	54 (28–79)	0.016	64 (40–85)
Associated malformations, n (%)	9 (36)	13 (36)	1.000	22 (36)
Biliary atresia splenic malformation, n (%)	5 (20)	5 (14)	0.727	10 (16)
Preoperative bilirubin, $\mu\text{mol/L}$, median (IQR)	186 (146–214)	145 (111–191)	0.067	169 (132–207)
Liver histology at PE, mean (range)				
Fibrosis, Metavir, 0–4	2.75 (3)	2.43 (3)	0.345	2.51 (3)
Portal inflammation, 0–3	1.58 (3)	2.11 (2)	0.062	1.98 (3)
Cholestasis, mean (range)				
Intracellular, 0–3	1.67 (2)	2.17 (2)	0.078	2.04 (2)
Intracanalicular, 0–3	2.00 (2)	2.40 (2)	0.095	2.30 (2)
Ductal, 0–3	1.67 (3)	1.32 (3)	0.394	1.41 (3)
Cytokeratin 7 immunostaining, mean (range)				
Periportal hepatocytes, 0–4	1.17 (1)	1.27 (3)	0.621	1.24 (3)
Proliferating bile ductules, 0–2	1.50 (2)	1.39 (2)	0.830	1.42 (2)

Results are presented separately for patients treated before (cohort A) and after (cohort B) centralization of biliary atresia management in 2005. Bilirubin and age at operation data given for patients who underwent PE and not primary liver transplantation (n = 59).

CK7, cytokeratin 7; IQR, interquartile range; PE, portoenterostomy.

($\beta = 1.92$; 95% CI 0.61 to 3.23; $p = 0.005$), and portal inflammation grade at PE ($\beta = -53.0$; 95% CI 101 to -5.41 ; $p = 0.030$). Only the effect of centralization remained statistically significant in multiple regression ($\beta = 95.0$; 95% CI 8.91 to 181; $p = 0.031$). Other clinical and histologic findings were unrelated with COJ and bilirubin levels at 3 months (eTable 1).

Native liver survival

Cumulative native liver survival for cohorts A and B were 37.5% ($\pm\text{SE } 9.6\%$) and 77.6% (± 7.5) at 2 years; 37.5% (± 9.9) and 70.2% (± 8.4) at 5 years; and 33.3% (± 9.6) and 65.2% (± 9.2) at 10 years, respectively (Fig. 1). Clearance of jaundice, bilirubin levels at 3 and 6 months post-operatively, portal inflammation grade at PE, and centralization were significant predictors of native liver survival (Fig. 1, Table 2). Among cohort B patients, BASM and higher periportal HCM at PE predicted shorter native liver survival, and higher ductal reaction at PE tended to predict shorter native liver survival (Table 2).

Bilirubin at 3 months after operation was a better predictor compared with bilirubin at 6 months and was therefore chosen for the multivariate analysis. The hazard

of death or transplantation was higher if bilirubin was $\geq 20 \mu\text{mol/L}$ at 3 months after operation (hazard ratio 11.0; 95% CI 2.38 to 50.4; $p = 0.002$), and the effects of centralization, age, or portal inflammation remained insignificant.

Liver transplantation

During follow-up, 22 patients (11 from both cohorts, 44% and 31%, respectively) underwent LT at age 1.15 (IQR 0.77 to 2.46) years. One patient from both cohorts underwent primary LT and 20 patients underwent LT after PE. Of them, the majority (n = 15) received transplants because of liver failure after an unsuccessful PE, and indications for LT after initial COJ were liver failure (n = 1), recurrent variceal bleeding (n = 1), recurrent cholangitis (n = 1), hepatopulmonary syndrome (n = 1), or refractory ascites (n = 1). Two patients with BASM died after LT (2 of 5) compared with 17 transplanted patients without BASM surviving until the end of the follow-up (log-rank test; $p = 0.006$). Centralization, COJ, or baseline characteristics were unrelated with post-LT survival, which was 91% at 2, 5, and 10 years (Fig. 2). One patient required a retransplantation.

Figure 1. Cumulative native liver survival rates. Cumulative native liver survival according to (A) era of treatment, (B) clearance of jaundice (COJ) status, (C) plasma bilirubin levels at 3 months after portoenterostomy (PE), and (D) portal inflammation grade at PE. Subgroups are compared with the log-rank test. Cohort A = 1987 to 2004; cohort B = 2005 to 2016.

Overall survival

During follow-up, 10 (16%) patients died, more frequently in cohort A than B (8 of 25 vs 2 of 36; $p = 0.011$). All deaths occurred at age younger than 2 years, so the cumulative 2-year survival was equal to 5- and 10-year survival rates, that is 68.0% (± 9.3) in cohort A and 93.7% (± 4.3) in cohort B ($p = 0.007$, Table 3). Reasons for death were complications of advanced liver disease ($n = 7$), intestinal perforation ($n = 1$), volvulus ($n = 1$), and heart failure ($n = 1$). Three patients died on LT waiting list and only 1 of the deceased patients had cleared his jaundice after PE.

Univariate analyses suggested survival was better among patients whose bilirubin decreased $<20 \mu\text{mol/L}$ within 3 months of operation ($p = 0.001$) and who were operated

on after centralization ($p = 0.007$) (Table 3). Neither predictor remained significant in multivariate analysis.

Follow-up liver histology

Follow-up biopsies were available for 29% (7 of 24) of cohort A and 86% (30 of 35) of cohort B patients, and were missing for those aged younger than 1.5 years. Patients who cleared their jaundice underwent follow-up biopsies at older median age than others (4.73 [IQR 3.13 to 6.25] years vs 0.91 [IQR 0.70 to 1.51] years; $p < 0.001$).

Fibrosis and periportal HCM increased and ductal reaction persisted in all patients after PE, and cholestasis and portal inflammation decreased only after successful PE and remained unchanged if no COJ was achieved (Fig. 3, Table 4). Follow-up fibrosis scores correlated

Table 2. Univariate Analysis of 5-Year Kaplan-Meier Native Liver Survival with Cumulative 5-Year Survival Estimates

Univariate	Cohort B 2005–2016 (n = 35)			Both cohorts 1987–2016 (n = 59)		
	Patients, n	5-year NL survival, %; mean (SE)	p Value	Patients, n	5-year NL survival, %; mean (SE)	p Value
Age at portoenterostomy			0.674			0.392
64 d or younger	22	72.6 (10.6)		32	62.1 (9.1)	
Older than 64 d	13	64.6 (14.3)		27	44.2 (10.0)	
Biliary atresia splenic malformation			0.023			0.705
No	30	77.0 (8.3)		49	58.0 (7.4)	
Yes	5	40.0 (21.9)		10	50.0 (15.8)	
Associated malformations			0.311			0.995
No	22	74.3 (9.9)		38	54.9 (8.4)	
Yes	13	62.9 (15.0)		21	59.3 (11.3)	
Type of biliary atresia			0.278			0.448
1 or 2	3	100.0		6	83.3 (15.2)	
3	32	66.9 (9.2)		53	52.9 (7.2)	
Bilirubin at 3 mo			0.001			<0.001
<20 µmol/L	22	93.3 (6.4)		29	95.5 (4.4)	
≥20 µmol/L	13	38.5 (13.5)		30	23.3 (7.7)	
Bilirubin at 6 mo*			0.013			<0.001
<20 µmol/L	20	92.3 (7.4)		28	91.2 (6.0)	
≥20 µmol/L	13	46.2 (13.8)		24	33.3 (9.6)	
Fibrosis, Metavir			0.671			0.543
0–2	22	70.7 (10.1)		28	58.6 (9.6)	
3–4	12	68.2 (15.4)		18	73.9 (11.3)	
Portal inflammation			0.056			0.002
0–1	7	44.4 (22.2)		13	35.2 (15.2)	
2–3	27	75.1 (8.9)		33	73.6 (8.0)	
Intracellular cholestasis			0.327			0.887
0–1	10	80.0 (12.6)		16	68.8 (11.6)	
2–3	24	64.2 (11.1)		30	60.8 (9.8)	
CK7 expression in periportal hepatocytes [†]			0.035			0.282
0–1	21	82.0 (9.5)		31	70.9 (8.7)	
2–4	11	48.5 (16.4)		13	49.0 (14.8)	
CK7-positive bile ductulus proliferation [†]			0.070			0.172
0–1	16	84.6 (10.0)		22	74.5 (9.9)	
2	16	56.0 (13.8)		22	54.6 (11.4)	
Centralization			—			0.014
Before	—	—		24	37.5 (9.9)	
After	—	—		35	70.2 (8.4)	

The p values from the log-rank test are reported. Results presented separately for cohort B and for the whole sample.

*Bilirubin data at 6 months missing for patients who received liver transplant (n = 2) or died (n = 4) before 6 months of PE or in whom bilirubin was not measured at 6 months (n = 1).

[†]Two biopsies were not analyzed for CK7 immunostaining scores.

CK7, cytokeratin 7; NL, native liver.

with follow-up ductal reaction ($r = 0.494$; $p = 0.003$) and were closely related with periportal HCM, portal inflammation, and their increase during follow-up (Fig. 4). In simple regression, follow-up fibrosis score was associated with biopsy timing after PE ($\beta = -0.09$; 95% CI -0.18 to -0.003 ; $p = 0.044$), COJ ($\beta = 1.03$;

95% CI 0.16 to 1.91 ; $p = 0.022$), intracellular ($\beta = 0.49$; 95% CI 0.09 to 0.89 ; $p = 0.018$), and intracanalicular ($\beta = 0.41$; 95% CI 0.07 to 0.74 ; $p = 0.018$) cholestasis, ductal reaction ($\beta = 0.86$; 95% CI 0.41 to 1.31 ; $p < 0.001$), as well as follow-up portal inflammation ($\beta = 0.99$; 95% CI 0.62 to 1.36 ; $p < 0.001$) and

Figure 2. Cumulative survival after liver transplantation (LT). Cumulative survival after LT for all transplanted patients from cohorts A and B (n = 22).

HCM scores ($\beta = 0.52$; 95% CI 0.36 to 0.68; $p < 0.001$) and their observed change after PE ($\beta = -0.61$; 95% CI

-0.88 to -0.34 ; $p < 0.001$ and $\beta = 0.41$; 95% CI 0.23 to 0.59; $p < 0.001$). In multiple regression (where absolute scores were used instead of their change, and intra-cellular cholestasis was used to reflect the influence of cholestasis), only the effect of periportal HCM remained significant ($\beta = 0.36$; 95% CI 0.14 to 0.58; $p = 0.002$). Age, histology at PE, time to COJ, or other clinical characteristics were unrelated with follow-up fibrosis.

Progression of histologic fibrosis and its predictors

Compared with patients who did not achieve COJ, those whose bilirubin normalized presented at follow-up with lower degrees of fibrosis ($p = 0.029$, Table 4), tendency toward smaller increase in fibrosis during follow-up (mean 1.83 [range 2.0] vs 0.79 [range 5.0]; $p = 0.057$), and slower fibrosis progression rate ($p < 0.001$, Fig. 5).

In simple regression, variables associated with fibrosis progression rate were biopsy timing after PE ($\beta = -0.12$; 95% CI -0.20 to -0.04 ; $p = 0.006$), COJ ($\beta = 1.81$; 95% CI 1.18 to 2.44; $p < 0.001$), portal inflammation score at PE ($\beta = -0.74$; 95% CI -1.24 to -0.24 ; $p = 0.005$), and change of portal inflammation during follow-up ($\beta = -0.73$; 95% CI -1.24 to -0.23 ; $p = 0.006$). In a multiple regression model, only the effect of COJ remained significant ($\beta = 1.60$; 95% CI 0.84 to 2.35; $p < 0.001$).

Table 3. Univariate Analysis of 5-Year Kaplan-Meier Overall Survival Estimates

Univariate	Cohort B 2005–2016 (n = 36)			Both cohorts 1987–2016 (n = 61)		
	Patients, n	5-y survival, %, mean (SE)	p Value	Patients, n	5-y survival, %, mean (SE)	p Value
Age at portoenterostomy			0.683			0.700
64 d or younger	22	95.2 (4.6)		32	84.7 (7.0)	
Older than 64 d	13	90.9 (8.7)		27	80.5 (7.2)	
Biliary atresia splenic malformation			0.148			0.212
No	31	96.2 (3.8)		51	85.5 (5.1)	
Yes	5	80.0 (17.9)		10	70.0 (14.5)	
Associated malformations			0.653			0.275
No	23	95.0 (4.9)		39	86.4 (5.7)	
Yes	13	91.7 (8.0)		22	77.0 (9.0)	
Bilirubin at 3 mo			0.090			0.001
<20 mmol/L	22	100		29	100	
≥ 20 mmol/L	13	84.6 (10.0)		30	66.7 (8.6)	
Bilirubin at 6 mo*			0.283			0.151
<20 μ mol/L	20	100		28	96.0 (3.9)	
≥ 20 μ mol/L	13	92.3 (7.4)		24	83.3 (7.6)	
Centralization			—			0.007
Before	—	—		25	68.0 (9.3)	
After	—	—		36	93.7 (4.3)	

Results are presented separately for cohort B and for the whole sample. p Values from the log-rank test are reported. Bilirubin levels analyzed only for patients undergoing PE and not primary transplantation (n = 59).

*Bilirubin data at 6 months missing for patients who received liver transplantation (n = 2) or died (n = 4) before 6 months of PE or in whom bilirubin was not measured at 6 months (n = 1).

Figure 3. (A–C) Representative native liver histology from the same patient at portosenterostomy (PE) and (D–F) 2.4 years after successful PE (200× magnification). Portal inflammation (0–3) reduced from grade 3 to 1 (A, D: hematoxylin and eosin). Metavir fibrosis stage (0–4) increased from 2 to 4 (B, E: Herovici stain). Ductal reaction (0–2, dark brown, arrow) increased from grade 1 to 2 and hepatocyte-cholangiocyte metaplasia in periportal hepatocytes (0–4, light brown, asterisk) from 0 to 4 (C, F: cytokeratin-7 staining).

DISCUSSION

This study demonstrates that national centralization of BA management and standardization of care can considerably improve outcomes, even exceeding those reported previously.^{3,7,32,33} Although the incidence of BA in Finland is equivalent to other European countries,^{6,21} the annual caseload inevitably remains lower, as Finland's population approximates 5.5 million. After centralization of management, COJ rate increased from 42% to 80%, showing that excellent PE outcomes can be achieved,

even with relatively low caseloads, if the treatment process is rationally planned. Reflecting COJ rates, both native liver and overall survival estimates improved significantly. In addition, this study provides novel evidence on BA liver histopathology and its relation to the clinical outcomes. High-grade portal inflammation at PE is associated with improved COJ rates and, consequently, with slower progression of fibrosis and longer native liver survival. Periportal HCM was the only predictor of follow-up fibrosis in multiple regression, and the extent of

Table 4. Liver Histologic Features at Time of Portosenterostomy and at Last Follow-Up

Histology	COJ (n = 26)			No COJ (n = 7)			All patients (n = 33)		
	At PE, mean (range)	At follow-up, mean (range)	p Value	At PE, mean (range)	At follow-up, mean (range)	p Value	At PE, mean (range)	At follow-up, mean (range)	p Value
Fibrosis, Metavir, 0–4	2.38 (3)	3.12* (3)	0.009	2.43 (3)	4.00 (0)	0.026	2.39 (3)	3.30 (3)	0.001
Portal inflammation, 0–3	2.27† (2)	1.00 (2)	<0.001	1.71 (2)	1.43 (2)	0.414	2.15 (2)	1.09 (3)	<0.001
Cholestasis									
Intracellular, 0–3	1.96 (2)	0.12‡ (1)	<0.001	2.29 (2)	2.00 (2)	0.414	2.03 (2)	0.52 (3)	<0.001
Intracanalicular, 0–3	2.19 (2)	0.08‡ (2)	<0.001	2.43 (1)	2.43 (2)	1.000	2.24 (2)	0.58 (3)	<0.001
Ductal, 0–3	1.15 (3)	0.00† (0)	<0.001	1.67 (3)	2.00 (3)	0.492	1.25 (3)	0.38 (3)	0.002
Cytokeratin 7 immunostaining									
Periportal hepatocytes, 0–4	1.17 (4)	2.04 (2)	0.015	1.50 (2)	2.67 (2)	0.066	1.23 (2)	2.17 (4)	0.003
Proliferating bile ductules, 0–2	1.13 (2)	1.29 (2)	0.356	1.83 (1)	1.67 (1)	0.564	1.40 (2)	1.23 (2)	0.282

Results are presented separately for patients who cleared or did not clear their jaundice after PE (bilirubin <20 vs ≥20 μmol/L). Data are shown for 33 patients with liver histology available both at baseline and at follow-up. Wilcoxon signed-rank test p values for the difference between histology scores at operation and at follow-up are reported.

*Mann-Whitney U test p value < 0.05 for the differences observed in histologic scores between COJ and no-COJ subgroups.

†Mann-Whitney U test p value < 0.01 for the differences observed in histologic scores between COJ and no-COJ subgroups.

‡Mann-Whitney U test p value < 0.001 for the differences observed in histologic scores between COJ and no-COJ subgroups.

COJ, clearance of jaundice; PE, portosenterostomy.

Figure 4. Cytokeratin (CK7) expression in periportal hepatocytes, portal inflammation score, and their observed change between portoenterostomy (PE) and follow-up biopsies according to follow-up Metavir fibrosis scores. The length of the box represents the interquartile range and the line through the middle of the box the median. Error bars show minimum and maximum values. Kruskal-Wallis test $p < 0.001$ for CK7 expression; $p = 0.003$ for change in CK7 expression; $p < 0.001$ for portal inflammation; and $p = 0.008$ for change in portal inflammation. *Statistically significant difference when compared with Metavir stage = 4 ($p < 0.05$).

both HCM and ductal reaction paralleled closely the degree of fibrosis at PE and its progression during follow-up. These findings suggest progression of liver damage is partly regulated by inflammation and ductal reaction.

After failed PE, BA patients die of liver failure within 2 years unless LT is performed.⁸ The PE success rates typically vary between 40% and 60%,^{3,6,7,32} with best outcomes reported so far from the UK, where BA treatment has been centralized in 3 hospitals,²¹ and from Japan, where BA incidence is remarkably higher compared with Europe.⁵ In this study, COJ and 5-year native liver survival rates almost doubled after centralization. Although age at operation decreased simultaneously, regression models suggested centralization was the most important factor predicting postoperative bilirubin levels. In French and Canadian national cohorts, hospitals with the lowest and highest caseloads achieved similar outcomes.^{6,33,34} Our post-centralization results also suggest that instead of absolute caseload numbers, BA outcomes are influenced by the quality of each treatment aspect, including early diagnosis and appropriate surgical technique, as well as standardized postoperative management and careful follow-up.²⁵⁻²⁷ Postoperative steroids can improve COJ in patients who undergo an early PE,^{19,25} suggesting children managed after centralization at median age of 54 days might have particularly benefited from adjuvant therapy. A combination of steroids, ursodeoxycholic acid,²⁷ and antibiotics^{3,26} has been previously

demonstrated to improve COJ and native liver survival.^{35,36} Unfortunately, because the use and dosage of postoperative medications was variable before centralization, their effect could not be controlled for in statistical analyses.

Bilirubin level at 3 months after operation reflects the success of PE and has outperformed the prognostic value of other factors on native liver survival in several large cohorts.^{14,37-39} Also, in our patients, bilirubin at 3 months was the best predictor of native liver and overall survival, and centralization strongly influenced the postoperative reduction of bilirubin. Patients with BASM had higher post-LT mortality and shorter native liver survival in cohort B than others. In addition to BASM,^{24,32,33} anatomic subtype of BA^{6,13,14} and age at PE^{3,6,7,13} can influence native liver survival. As splenic malformations and BA subtypes 1 to 2 each occur in about 10% of patients,^{24,38} their predictive value is evidently limited. Although increased age at operation relates with shorter transplant-free survival, whether it limits achievement of COJ remains unclear,^{3,7,14,38} and no threshold age beyond which PE would be futile has been set.¹⁰ Although we found bilirubin at 3 months as the best predictor of LT need, as much as 26% cleared their jaundice later. Frequent monitoring of clinical status and laboratory values are needed to distinguish between such patients and those who will require LT during infancy.

Figure 5. Fibrosis progression rate during follow-up in patients who cleared vs did not clear their jaundice after portoenterostomy (PE). Patients with Metavir stage 4 at PE ($n = 7$) are excluded from the analysis. The length of the box represents the interquartile range (IQR) and the line through the middle of each box the median. Error bars show minimum and maximum values. The round dot represents a value outside the upper quartile $+1.5$ times the IQR. Mann-Whitney U test p value is reported. COJ, clearance of jaundice.

Although previous abdominal operation can increase the risk of complications after LT, mortality after primary transplantation in BA is higher compared with LT performed after PE.^{6,12,13} In addition, better LT outcomes are achieved in older children compared with infants,^{11,40} which supports the benefit of primary PE unless patients present with liver failure. As malnutrition and stage of liver disease also influence LT outcomes,^{39,40} management of BA in centers with experienced multidisciplinary teams and a transplantation program likely improves patient survival by ensuring appropriate management, as well as timely listing for LT when needed.¹⁰ Centralization of BA treatment in a hospital with such services might, in part, explain the improved outcomes observed in our series.

Viral, autoimmune, or toxic triggers are thought to give rise to an inflammatory reaction launching destruction of bile ducts in BA.^{1,8,41} Different degrees of portal inflammation were present at diagnosis in virtually all of our patients, supporting a proinflammatory cause. Interestingly, higher inflammation scores strongly predicted COJ, longer native liver survival, and slower fibrosis progression. Portal inflammation reduced only after successful PE, which might have been facilitated by postoperative adjuvant therapy use after centralization.¹⁹ At PE, most BA patients present with some degree of liver inflammation,^{16,41} higher levels of which

have been reported in cytomegalovirus-associated BA.⁴² Besides differential viral cause, a predominantly inflammatory liver histopathology could reflect an earlier and more modifiable stage of BA before irreversible progression to a predominantly fibrotic stage, as suggested by gene expression profiling analyses.⁴¹ We found portal inflammation scores unrelated with age at PE or clinical patient characteristics. Although our sample is limited by its relatively small size and some missing biopsies among cohort A patients, the strong inverse association between inflammation and improved outcomes appears to be a true biologic phenomenon.

The main determinant of the course of BA and need for LT is the progression fibrosis, which pathogenesis is incompletely understood.^{22,43,44} In BA and other cholangiopathies, a characteristic histologic feature evolving adjacent to fibrosis is ductal reaction, which refers to hepatocytes and/or progenitor cells transdifferentiating into CK7-positive cell lineages, toward either intermediate hepatocytes or epithelial cells forming immature biliary ductules.^{17,43,45} Although mechanisms behind ductal reaction-driven fibrogenesis remain unclear, worsening fibrosis correlates closely with increasing ductal reaction in several acquired liver diseases.^{45,46} In BA, degree of biliary proliferation predicts native liver survival and reflects the degree of fibrosis at time of PE.^{15,16} Our results demonstrate that the extent of ductal reaction and associated periportal HCM parallel closely the degree of fibrosis not only at PE but also during the course of BA, suggesting such histopathologic features can promote progressive fibrosis, even when inflammation resolves after successful PE. Notably, in multiple regression, periportal HCM was the only predictor of follow-up fibrosis. In BA animal models, fibrogenic growth factor signaling from ductal reaction seems to activate other cell types to produce extracellular matrix.^{47,48} Further characterization of such interplay^{47,49,50} is crucial to understanding how to prevent progressive liver injury.

CONCLUSIONS

Based on more than a decade of follow-up after national centralization of BA management, outcomes measured as COJ, native liver survival, and overall survival improved significantly. Resolution of cholestasis and reduction of high-grade portal inflammation after PE is associated with slower fibrosis progression and improved native liver survival. Despite COJ, liver fibrosis progressed in parallel with CK7 immunostaining of proliferating bile ductules and periportal hepatocytes during follow-up, suggesting expanding biliary fibrosis in BA is closely coupled with ductal reaction and associated HCM. To what extent

fibrogenesis and ductal reaction are affected by the initial inflammatory reaction remains to be elucidated. While waiting for effective antifibrotic therapies, standardized multidisciplinary treatment protocols, as well as centralization of BA management, can postpone the need for LT and extend patient survival.

Author Contributions

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eTable 1. Baseline Characteristics and Liver Biopsy Findings at Portoenterostomy in Relation to Clearance of Jaundice

Characteristic	No COJ after PE (n = 21)	COJ (n = 38)	p Value
Gestational age, wk, median (IQR)	40 (38–41)	39 (38–40)	0.575
Gestational weight, g, median (IQR)	3,630 (3,315–3,825)	3,250 (3,003–3,640)	0.020
Type of biliary atresia, n (%)			
1	1 (4.7)	1 (2.6)	1.000
2	1 (4.7)	3 (7.9)	
3	19 (90)	34 (89)	
Age at PE, d, median (IQR)	75 (54–100)	56 (29–78)	0.013
Associated malformations, n (%)	6 (29)	15 (39)	0.571
Biliary atresia splenic malformation, n (%)	3 (14)	7 (18)	1.000
Preoperative bilirubin, $\mu\text{mol/L}$, median (IQR)	176 (142–195)	159 (110–213)	0.322
Liver histology at PE, mean (range)			
Fibrosis, Metavir, 0–4	2.54 (3)	2.45 (3)	0.925
Portal inflammation, 0–3	1.46 (3)	2.18 (2)	0.008
Cholestasis, mean (range)			
Intracellular, 0–3	1.92 (2)	2.09 (2)	0.533
Intracanalicular, 0–3	2.15 (2)	2.33 (2)	0.290
Ductal, 0–3	1.67 (3)	1.33 (3)	0.409
Cytokeratin 7 immunostaining, mean (range)			
Periportal hepatocytes, 0–4	1.33 (2)	1.16 (2)	0.427
Proliferating bile ductules, 0–2	1.67 (1)	1.31 (2)	0.177

COJ, clearance of jaundice; IQR, interquartile range; PE, portoenterostomy.