Title: Outcomes of biliary atresia in the Nordic countries - A multicenter study of 158 patients during 2005-2016

Corresponding Author: Dr. Mikko P. Pakarinen, MD PhD

Corresponding Author's Institution: Children's Hospital / University of Helsinki

First Author: Mikko P. Pakarinen, MD PhD

Order of Authors: Mikko P. Pakarinen, MD PhD; Lars Søndergaard Johansen; Jan Svensson; Kristin Bjørnland; Vladimir Gatzinsky; Pernilla Stenström; Antti Koivusalo; Nina Kvist; Markus Almström; Ragnhild Emblem; Sigurdur Björnsson; Torbjörn Backman; Runar Almaas; Hannu Jalanko; Björn Fischler; Jørgen Thorup

Abstract: Background/Purpose: Biliary atresia is the most common reason for newborn cholestasis and pediatric liver transplantation. Even after normalization of serum bilirubin after portoenterostomy, most patients require liver transplantation by adulthood due to expanding fibrosis. We addressed contemporary outcomes of biliary atresia in the Nordic countries.

Methods: Data on center and patients characteristics, diagnostic practices, surgical treatment, adjuvant medical therapy after portoenterostomy, follow-up and outcomes were collected from all the Nordic centers involved with biliary atresia care during 2005-2016.

Results: Of the 154 patients, 148 underwent portoenterostomy mostly by assigned surgical teams at median age of 64 (interquartile range 37-79) days, and 95 patients (64%) normalized their serum bilirubin concentration while living with native liver. Postoperative adjuvant medical therapy, including steroids, ursodeoxycholic acid and antibiotics was given to 137 (93%) patients. Clearance of jaundice associated with young age at surgery and favorable anatomic type of biliary atresia, whereas annual center caseload >3 patients and diagnostic protocol without routine liver biopsy predicted early performance of portoenterostomy. The cumulative 5-year native liver and overall survival estimate was 53% (95%CI 45-62) and 88% (95%CI 83-94), respectively. Portoenterostomy age <65 days and annual center caseload >3 patients were predictive for long-term native liver survival, while normalization of serum bilirubin after portoenterostomy was the major predictor of both native liver and overall 5-year survival.

Conclusions: The outcomes of biliary atresia in the Nordic countries compared well with previous European studies. Further improvement should be pursued by active measures to reduce patient age at portoenterostomy.

Helsinki
24.8.2017

Dear Dr Holcomb,

Thank you very much for the positive response on our revised manuscript entitled
'**Outcomes of biliary atresia in the Nordic countries – A multicenter survey of 158
patients during 2005-2016**'. We have attached detailed answers to the reviewers'
questions and comments. We hope that this re-revised version is acceptable for
publication in the Journal of Pediatric Surgery.

Sincerely,

Mikko Pakarinen, MD, PhD

Section of Pediatric Surgery
Helsinki University Central Hospital
University of Helsinki
Helsinki, Finland

<div align="right">

Helsinki
24.8.2017

</div>

Dear Dr Holcomb,

Thank you very much for the positive response on our revised manuscript entitled '**Outcomes of biliary atresia in the Nordic countries – A multicenter survey of 158 patients during 2005-2016'**. Below you'll find detailed answers to the reviewers' questions and comments.

Reviewer #2: I am content that the revisions required have been done and the questions answered.

Thank you.

Reviewer #3: I would like to thank the authors for their attentive revision to their manuscript entitled "Outcomes of biliary atresia in the Nordic countries -A multicenter study of 158 patients during 2005-2016.". The authors aim was to present the contemporary outcomes following portoenterostomy for biliary atresia over an 11 year period in the Nordic countries in a retrospective, observational multicenter study. The important finding of this paper is that patients who undergo portoenterostomy at a younger age were more likely to clear jaundice and that >3 cases per year predicted younger age at time of surgery. Also, 93% patients received postoperative steroids, ursodeoxycholic acid, and prophylactic antibiotics.
The authors have satisfactorily addressed questions previously raised. I have found a few minor editing points for the authors to consider.

Thank you.

Minor points:
1. Abstract/results. Consider "clearance of jaundice was associated…biliary atresia; whereas…" and "Portoenterostomy at age <65…"

We chose not to change this, because it would alter the meaning of the sentence and would not correctly reflect our findings.

2. Page 2, line 55. preoperative

It was deliberately peroperative, because cholangiography was performed during laparotomy for PE.

3. Page 3, statistical analysis - Please include which statistical program was used.

Statistical programme was added as suggested.

4. Page 4, results - Please change median range from small to large (ie 0.7-3.7)

Changed as suggested.

5.   Page 5, line 46. Consider "biliary tract remnant "was" associated…

Changed as suggested.

Sincerely,

Mikko Pakarinen, MD, PhD

Section of Pediatric Surgery
Helsinki University Central Hospital
University of Helsinki
Helsinki, Finland

TABLE 1. GUIDELINES FOR THE REPORTING OF CLINICAL RESEARCH DATA IN THE *JOURNAL OF PEDIATRIC SURGERY*

Methods:

Reported	Not Applicable	Reporting Detail
x		*The number and practice type of all institutions where cases were performed*
x		*The number of surgeons who actually operated in the study (& relative number of cases for each)*
	x	*The prior experience of participating surgeons in performing the reported intervention*
x		*The precise timeline during which all patients were treated in the study(i.e. Jan 1995 to March 1998)*
x		*A clear description of how patients were selected into the study. This should include relevant inclusion and/or exclusion criteria.*
x		*The number of eligible patients at the study sites excluded during the timeline of the study*
x		*A clear description of the study population from which the patients were selected*
x		*A clear description of the relevant diagnostic criteria used to identify cases*
	x	*A clear description of critical aspects of operative technique and perioperative care*
	x	*Statement as to whether any attempts were made to standardize operative technique or perioperative care (and how this was accomplished)*

Results:

Reported	Not Applicable	Reporting Detail
x		*The range and mean of all relevant demographic and baseline variables*
x		*The range and median (not mean) for length of follow-up reporting*
x		*Relevant outcome variables are presented with appropriate measures of range and variability (i.e. standard deviation)*
x		*Methods for measuring outcomes of interest are clearly described*
x		*Statement regarding whether any data is missing (and how missing data is addressed in the analysis of outcome variables)*
x		*Number and appropriate details regarding all complications*

Additional Details for Studies Reporting More Than One Treatment Group (e.g. Controls):

Reported	Not Applicable	Reporting Detail
x		*Mean and range for all relevant demographic and baseline variables for all treatment groups*
x		*The range and median (not mean) for length of follow-up reporting for each treatment group*
x		*A precise timeline during which all patients were treated for each group*
x		*Outcome variables being compared between groups are presented with appropriate measures of variability (e.g. standard deviation)*
x		*Measures to type II error (P-values) for comparison statistics are presented with actual values if P=.01 or larger (e.g. P=NS and P<.05 are not acceptable)*
x		*A description of how patients were selected into each treatment group*
x		*A statement is made as to whether the same surgeons operated on patients from different treatment groups*

Manuscripts concerning clinical research should follow a uniform set of reporting guidelines. The guidelines, listed above, were developed from sound clinical research principles and are designed to improve the reporting accuracy of clinical data pertaining to surgical conditions. With more accurate and transparent reporting of study methodology and outcomes data, readers of the Journal will be better able to gauge the relevance of reported results to their own clinical practice. Although not all of the recommended reporting guidelines are applicable to every clinical study, it is important that all details relevant to your study are clearly reported in the manuscript. Please check the appropriate boxes to verify compliance with these guidelines and submit with the manuscript. Compliance with these guidelines will be considered by the editor in the final decision regarding publication of your manuscript.

Outcomes of biliary atresia in the Nordic countries – A multicenter study of 158 patients during 2005-2016

Mikko P. Pakarinen[1], Lars Søndergaard Johansen[2], Jan F. Svensson[3], Kristin Bjørnland[4], Vladimir Gatzinsky[5], Pernilla Stenström[6], Antti Koivusalo[1], Nina Kvist[2], Markus Almström[3], Ragnhild Emblem[4], Sigurdur Björnsson[5], Torbjörn Backman[6], Runar Almaas[7], Hannu Jalanko[1], Björn Fischler[3], Jørgen Thorup[2] on behalf of the Nordic Pediatric Surgery Study Consortium

[1]Children's Hospital, University of Helsinki and Helsinki University Hospital, Finland

[2]Department of Pediatric Surgery, Rigshospitalet, Copenhagen, Denmark

[3]Astrid Lindgren Children´s Hospital, Karolinska University Hospital, Sweden

[4]Oslo University Hospital and University of Oslo, Norway

[5]Queen Silvia Children's Hospital, Gothenburg, Sweden

[6]Skane University Hospital in Lund, Lund University, Sweden

[7]Department of Pediatric Research, Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Norway

Corresponding author: Mikko P Pakarinen, P.O Box 281, Stenbäckinkatu 11, 00029 HUS, Helsinki, Finland, mikko.pakarinen@hus.fi, phone: + 358 50 4272981, Fax +385 9 47175737.

Electronic word count: 4890

Number of figures and tables: 3 figures, 6 tables

Abbreviations: BA, biliary atresia; LT, liver transplantation; PE, portoenterostomy; ERC, endoscopic retrograde cholangiography; PTC, percutaneous transhepatic cholangiography; CI, confidence interval; OR, odd ratio; HR, hazard ratio.

Background/purpose: Biliary atresia is the most common reason for newborn cholestasis and pediatric liver transplantation. Even after normalization of serum bilirubin after portoenterostomy, most patients require liver transplantation by adulthood due to expanding fibrosis. We addressed contemporary outcomes of biliary atresia in the Nordic countries. **Methods:** Data on center and patients characteristics, diagnostic practices, surgical treatment, adjuvant medical therapy after portoenterostomy, follow-up and outcomes were collected from all the Nordic centers involved with biliary atresia care during 2005-2016. **Results:** Of the 154 patients, 148 underwent portoenterostomy mostly by assigned surgical teams at median age of 64 (interquartile range 37-79) days, and 95 patients (64%) normalized their serum bilirubin concentration while living with native liver. Postoperative adjuvant medical therapy, including steroids, ursodeoxycholic acid and antibiotics was given to 137 (93%) patients. Clearance of jaundice associated with young age at surgery and favorable anatomic type of biliary atresia, whereas annual center caseload >3 patients and diagnostic protocol without routine liver biopsy predicted early performance of portoenterostomy. The cumulative 5-year native liver and overall survival estimate was 53% (95%CI 45-62) and 88% (95%CI 83-94), respectively. Portoenterostomy age <65 days and annual center caseload >3 patients were predictive for long-term native liver survival, while normalization of serum bilirubin after portoenterostomy was the major predictor of both native liver and overall 5-year survival. **Conclusions:** The outcomes of biliary atresia in the Nordic countries compared well with previous European studies. Further improvement should be pursued by active measures to reduce patient age at portoenterostomy.

Key words: clearance of jaundice, liver transplantation, portoenterostomy, steroids, survival, ursodeoxycholic acid

Retrospective prognosis study: Level II

Introduction

Biliary atresia (BA) is a severe cholangiopathy of infancy and the most common reason for neonatal

cholestasis and pediatric liver transplantation (LT) (1,2). Pathophysiology of BA remains unclear,

but rapidly progressing biliary fibrosis leads to liver failure and death within two years if untreated

(1,2). The first line surgical treatment is portoenterostomy (PE), which typically resolves cholestasis

in around half of the patients (3-6). Even after normalization of serum bilirubin levels,

approximately 70-80% of patients require LT by adulthood due to portally expanding ductal

reaction and fibrosis associated with complications of ensuing portal hypertension (1,2,7,8).

Outcome of PE carries vital prognostic significance in BA, because successful PE circumvents the

high waiting list mortality during infancy and increases survival after LT compared to primary LT

(9-11).

Several patient-dependent factors at the time of PE such as young age, favorable anatomy of the

biliary tract remnant, absence of associated congenital malformations and lower stage of liver

fibrosis associate with increased PE success rates and improved native liver survival (1,3,4,6,12).

Different postoperative adjuvant medications have been applied to improve PE outcomes. Although

randomized trials have failed to show improvement in native liver survival by steroid treatment

after PE (13,14), steroids do seem to increase the proportion of patients who clear their jaundice

(15,16), while both steroids and ursodeoxycholic acid have a beneficial effect on liver biochemistry

(15-17). Antibiotic prophylaxis aims to prevent recurrent cholangitis episodes, which may

jeopardize survival with native liver (3,18). Adjuvant therapy regimens combining all three above

mentioned medications has improved outcomes in small retrospective studies (19,20).

Concentration of clinical experience with BA management has also been shown to improve

1

outcomes, which has led to centralization of BA in several countries such as UK and Finland (21,22).

In recent large European studies the clearance of jaundice rate and the 5-year native liver survival have ranged between 36%-55% and 39%-46%, respectively (3,4,5), whereas no data on collective outcomes in the Nordic countries are available. The Nordic countries has a combined population of 27 million people, share similar healthcare systems, socioeconomic status and educational level, and perform LT within a joint organ exchange organization (11). The principal aims of this study were to characterize current management practices, analyze recent outcomes of BA and assess factors affecting timing and success rate of PE and native liver survival in the Nordic countries.

Patients and methods

Study design and data collection

This was an international multicenter retrospective observational survey. All children with confirmed BA born in the Nordic countries during 1.1.2005-30.6.2016 and followed-up at least for four months were included. Data were collected locally by pediatric surgeons, pediatric hepatologists and pediatricians in all Nordic centers involved with the treatment of BA according to a collectively approved questionnaire and data extraction sheet. The participating centers included one from Denmark (Copenhagen), Finland (Helsinki) and Norway (Oslo), and three from Sweden (Stockholm, Gothenburg, Lund), while no BA patients were reported from Iceland during this time period. All participating centers, excluding Lund, also perform pediatric LT within the Scandiatransplant organ exchange organization (11). The diagnosis of BA was confirmed based on clinical history, laboratory results, surgical findings, imaging such as peroperative cholangiography, endoscopic retrograde cholangiography (ERC), percutaneous transhepatic cholangiography (PTC),

2

liver biopsy and histopathological assessment of the biliary remnant according to local preferences. Patients, who had undergone PE but proved to have Alagille syndrome (n=3) were excluded.

Clinical practices concerning diagnostic modalities, surgical approach to PE and medical therapy and esophageal variceal surveillance after PE were enquired from each center. Data registered for each patient included gender, ethnicity, timing and type of initial operative management (PE, LT or no operation), anatomic type of BA: extrahepatic bile duct obstruction at the level of the common bile duct (type 1), the hepatic duct (type 2) or the porta hepatis (type 3) according to the Japanese Society of Pediatric Surgeons classification (23), observed associated congenital anomalies, presence of asplenia or polysplenia, use of adjuvant medical therapy after PE including steroids, ursodeoxycholic acid or antibiotic cholangitis prophylaxis, clearance of jaundice, endoscopically detected esophageal varices and listing, performance and timing of LT, and data on death. Withdrawal of active treatment and reason for that was also registered. Latest available serum bilirubin concentrations and blood platelet levels while living with native liver were recorded as surrogates of bile flow and portal hypertension among native liver survivors (24). Clearance of jaundice was defined as decrease in total serum bilirubin below 20 µmol/l at any time point after PE. Physiological foramen ovale, common anatomic variations of cardiac valves or vesicoureteral reflux were not considered congenital malformations.

Ethics

The ethical review boards at participating hospitals approved the study.

Statistical analysis

Continuous data are expressed as medians with interquartile ranges (IQR) and categorical data as frequencies unless otherwise stated. Mann-Whitney U test was used to compare continuous

3

variables, and χ^2 test to compare frequencies between groups. Cumulative native liver survival

(from birth to end of follow-up, LT or death), survival after LT (from LT to end of follow-up or

death) and overall survival (from birth to end of follow-up or death) rates were analyzed using

Kaplan Meier curves with 95% confidence intervals (CI) and log-rank test. In multivariate analyses,

odd ratios (OR) with their 95% CIs were generated with logistic regression models for predictors of

age at PE and clearance of jaundice, and hazard ratios (HR) with their 95% CIs with Cox

proportional hazards regression models for predictors of 5-year native liver survival. The

proportional hazard hypotheses were confirmed graphically and all variables in univariate analyses

were entered to multivariate analyses. The variables for multivariate analyses were chosen based on

earlier literature (3-6). The level of significance was set at p<0.05. Statistics were calculated with

StatView® 512 software (Brain Power, Calabasas CA, USA).

Results

Center characteristics

The median number of patients treated in the participating six centers annually ranged from 0.7 to

3.7, while only two centers managed over three patients annually (Supplementary Table 1).

Cholescintigraphy, operative cholangiography and ultrasound were uniformly used diagnostic

investigations, whereas preoperative diagnostic liver biopsy, MRI, PTC and ERC were less

commonly employed. In five centers one surgeon or surgical team operated all BA patients and

none used laparoscopic surgical approach for PE. Five centers reported routine steroid use after PE,

while ursodeoxycholic acid, antibiotic prophylaxis and supplemental fat-soluble vitamins were

regularly prescribed after the surgery in all centers. Two centers followed an endoscopic

surveillance protocol for esophageal varices.

Patient characteristics

4

In total, 158 patients were identified (Fig. 1). Treatment was withdrawn in four cases (2.5%) due to untreatable severe associated malformations affecting the heart or the central nervous system, and they were excluded from further analyses. Of the remaining 154 patients, 148 underwent PE and six (3.9%) had a primary LT. During the study period, 58 patients had undergone a secondary LT after PE, and 137 (89%) of the actively treated patients were alive. Median follow-up time among all patients was 4.9 (1.8-7.9) years.

The majority of patients were of European origin with a slight female predominance (Table 1). The most unfavorable anatomic type of BA (type 3) was observed in 82% of patients and 27% of patients had any associated congenital malformations, which affected the spleen in 12% and the heart in 6.5%. The vast majority of patients had received steroids, ursodeoxycholic acid and antibiotics following PE in order to improve bile flow and prevent cholangitis (Table 1). Of the 148 patients, who underwent PE, 137 (93%) had been treated with all these three adjuvant medications postoperatively.

Clearance of jaundice

In total, 95 of the 148 patients (64%) normalized their serum bilirubin concentration after PE, while living with their native livers. The patients who cleared their jaundice were median 10 days younger at PE [58 (31-74) days] than those who did not [68 (45-84) days, p<0.037]. In addition to younger age at surgery, favorable anatomy of the biliary tract remnant (BA types 1 and 2) was associated with increased clearance of jaundice rate after PE, and favorable anatomic type of BA remained as the only significant prognosticator in multivariate analysis (Table 2). Gender, ethnicity, associated congenital malformations, splenic malformations or annual center caseload were not significantly associated with clearance of jaundice.

Native liver survival and function

5

Including the six patients who underwent primary LT, the 5- and 10-year native liver survival

estimates were 53% (95%CI 45-62) and 45% (95%CI 35-55), respectively (Fig. 2). The respective

native liver survival estimates were 55% (95%CI 47-64) and 47% (95%CI 37-57) following PE. In

univariate analysis PE age <65 days, annual center caseload >3 and normalization of serum

bilirubin after PE were predictive for long-term native liver survival (Table 3 and Fig. 3). In

multivariate analysis, clearance of jaundice and annual center caseload >3 remained highly

predictive for the 5-year native liver survival, while the male gender emerged as an additional

statistically significant protective factor. Ethnicities, anatomic types of BA or associated congenital

malformations, including the splenic ones, were not associated with native liver survival.

Among the 82 patients, who were currently alive with their native livers, median serum bilirubin

concentration and blood platelet level was in the normal range (Table 1). Of them, 16% had

endoscopically verified esophageal varices, and only two were listed for LT.

Age at PE

Among the 148 patients, who underwent PE, the surgery was performed at mean age of 60 (range 4-

165) days (Table 1). Considering the prognostic importance of the timing of PE for clearance of

jaundice and native liver survival, factors associated with the PE age were addressed further. As

shown in Table 4, associated congenital malformations, especially the splenic ones, and annual

center caseload >3 patients were associated with a greater chance to undergo PE before the age of

65 days, whereas routine use of diagnostic preoperative liver biopsy markedly increased the risk of

delayed surgery. The absolute age at PE was median 48 (30-69) days in patients with associated

congenital anomalies versus 67 (39-83) days (*p*=0.019) in those without; 44 (27-58) days in patients

with splenic malformations versus 67 (39-80) days (*p*=0.018) in those without; 52 (29-80) days in

centers with >3 annual patients versus 69 (45-79) days (*p*=0.092) in centers with <3 patients; and 72

6

(67-82) days in centers routinely using diagnostic preoperative liver biopsy and 52 (28-78) days

(*p*=0.0002) in centers not routinely using preoperative liver biopsy. In multivariate analysis, routine

use of preoperative diagnostic liver biopsy was the most significant predictor of the PE age,

increasing the risk of delayed surgery by 4.3 fold (Table 4).

Liver transplantation

Six patients with a delayed diagnosis were transplanted primarily without a prior PE at median age

of 193 (154-281) days. In total, eight patients (5.1%) died without receiving a liver graft and nine

patients died after LT (Fig. 1). Overall median age at LT was 0.82 (IQR 0.58-1.76, range 0.40-10.1)

years. Including all 64 transplanted patients, both the 5- and 10-year survival estimates after LT

were 84% (95%CI 74-94) (Fig. 2). The respective figures were 85% (95%CI 75-95) among the

patients, who had undergone a prior PE before transplanted. Ethnicities, gender, anatomic types of

BA, clearance of jaundice, associated congenital anomalies or splenic malformations were not

associated with the 5-year survival after LT (data not shown).

Overall survival

The 5- and 10-year overall survival estimates were 88% (95%CI 83-94) and 87% (95%CI 81-93),

respectively (Fig. 1). The 5-year overall survival was higher among patients who cleared their

jaundice after PE [98% (95%CI 95-100)] than among those who did not [73% (95%CI 61-86)],

p<0.0001, and tended to be lower among the patients with splenic malformations [77% (95%CI 57-

97)] than in patients without [90% (95%CI 84-95)], *p*=0.064. In univariate analysis ethnicities,

gender, anatomic types of BA, annual center caseload or associated congenital anomalies were not

associated with the 5-year overall survival (data not shown).

Discussion

The results of this study show that the clearance of jaundice rate, the 5-year native liver survival after PE and overall survival in the Nordic countries compared well with previous European studies (Table 5). Following PE, serum bilirubin concentration normalized in 64%, and 55% of the Nordic patients were alive with their native livers at 5 years. Their median serum bilirubin and blood platelet levels were in the normal range, reflecting well-preserved native liver function. The diagnostic and management practices of BA were relatively uniform in the Nordic countries, and the great majority of the patients received a combination adjuvant therapy after PE, including steroids, ursodeoxycholic acid and antibiotics.

Although only around 20-30% of the BA patients reach adulthood without undergoing LT (7,25), extended native liver survival following successful PE has the potential to improve overall prognosis of BA in several ways. Firstly, it eliminates the increased waiting list mortality during infancy after failed PE (9,11), which was reflected in the present study by the 98% 5-year overall survival among the patients who cleared their jaundice. Secondly, successful PE and subsequently prolonged survival with the native liver seem to improve both the graft and patient survival rates after LT performed later in life (10). Thirdly, extended survival with native liver shortens the cumulative life-time exposure to toxic side-effects of immunosuppressive medication by postponing the need for LT.

Similarly to previous studies (3,4,26), our findings suggest that patient age at surgery is the major prognostic factor for the success of PE. In the present study, younger age at PE associated with both more frequent clearance of jaundice and better long-term native liver survival. As expected, clearance of jaundice, in turn, was clearly the most important predictor of long-term native liver survival. In the present study, the median patient age at PE (64 days) was 5 to 10 days higher than

8

in other recent European reports from France, the Netherlands and England and Wales (Table 5), offering a logical and realistic target for further improvement of the outcomes. While no screening programs for BA have been yet established in the Nordic countries, all newborns visit a primary care physician at the age of 4-6 weeks. Identification of cholestatic infants at this point at the latest should leave enough time for completion of a well-organized diagnostic protocol and timely performance of PE before the age of two months. General awareness of newborn cholestasis and the practice of routine measurement of serum conjugated bilirubin in all jaundiced newborns older than two weeks could be endorsed by active education (27-29). Stool color card screening has improved timing of PE, clearance of jaundice and native liver survival in Taiwan and Japan, where BA incidence is considerably higher than in Europe (30,31).

Despite early recognition of cholestatic newborns, the in-hospital delay related to diagnostic protocols or logistic problems may also unnecessarily delay the primary surgical care. We found that routine use of preoperative diagnostic liver biopsy associated with delayed performance of PE, whereas annual caseload >3 patients associated with timely surgery. However, our survey did not look for differences in the age at first referral to tertiary centers. Theoretically, the two centers practicing routine liver biopsies may have received their referrals at a later age than the others. Furthermore, the recently published joint European and North American guidelines do in fact recommend the use of liver biopsy for evaluation, in particular because of its high specificity for BA (12,29). Taken together, our findings imply, that any chosen diagnostic pathway should be critically evaluated and optimized to circumvent all avoidable delays, while increasing number of patients may help to put final touches in these processes.

Besides age at PE, annual center caseload >3 patients associated with improved native liver survival in the present survey. Centralization of BA treatment and increasing caseloads has been shown to improve native liver and overall survival in England and Wales as well as in Finland and Denmark

9

(21,22,32). In contrast, no differences in outcomes were observed between hospitals with the lowest and highest caseloads in France and Canada ranging from below two to over 20 patients per year (33,34). BA treatment results are collectively influenced by the quality of each step of the care, including early diagnosis, appropriate surgery, postoperative management and follow-up. Because caseload was not associated with normalization of serum bilirubin after PE, other steps of the care pathway than surgical management are possibly more sensitively affected by the caseload. In order to maximize surgical experience, the relatively low caseload in each country was compensated by concentrating PE operations to one surgeon in most Nordic centers.

In this study the presence of associated congenital anomalies, including the splenic malformations, was not associated with decreased clearance of jaundice or native liver survival rates in contrast to some previous studies (4). This could be explained by the observation that these patients underwent PE at younger age than others possibly following an earlier BA diagnosis inflicted by discovery and investigation of their associated congenital anomalies. The prevalence of splenic malformations and congenital cardiac defects was comparable to previously reported figures (35,36), while occurrence of any associated malformation was somewhat higher than in previous studies (35-37).

There is evidence on postoperative steroids improving clearance of jaundice rate and liver biochemistry following PE (15,16), although randomized trials have failed to demonstrate benefit of postoperative steroids on native liver survival (13,14). Ursodeoxycholic acid has a favorable effect on serum liver biochemistry (17), while antibiotic prophylaxis may reduce recurrent cholangitis episodes and improve native liver survival after successful PE (3,18). Interestingly, two retrospective studies have reported improvements in clearance of jaundice and native liver survival rates after PE with postoperative adjuvant therapy consisting of steroids, ursodeoxycholic acid and antibiotics (19,20). Although our study was not design to address the effectiveness this triple

adjuvant therapy, it could be one factor contributing to the achieved outcomes. Obviously, a randomized controlled trial would be the optimal way to address this question further.

This study had several limitations. Because only two centers followed an endoscopic surveillance protocol, the incidence of varices is most likely markedly underestimated. We did not collect data on cholangitis episodes, serum bilirubin concentrations at set time points or the time taken to achieve clearance of jaundice and thus were not able address their effects on survival outcomes. Nor did we collect details or duration of postoperative medical therapy following PE. However, availability and accuracy of such data always remains imperfect in a retrospective study design and our major aim was to assess management characteristics and outcomes of BA in the Nordic countries.

In conclusion, these findings demonstrate that current outcomes of BA in the Nordic countries are well comparable to previous European studies and annual caseload is highly predictive for the 5-year native liver survival. Further improvement should be pursued by multimodal active measures to reduce patient age at PE. The high rates of clearance of jaundice and native liver survival after PE were achieved with the use of steroids, ursodeoxycholic acid and antibiotics as a postoperative adjuvant medical therapy in over 93% of patients

Figure legends

Fig. 1. Biliary atresia patients treated in the Nordic countries.

Fig. 2. Survival outcomes of 154 actively treated biliary atresia patients in the Nordic countries. Kaplan Meier survival curves are shown for (A) native liver survival (B) post liver transplantation survival and (C) overall survival.

11

Fig. 3. Native liver survival after portoenterostomy. Kaplan Meier survival curves and log-rank p-values are shown according to (A) age at portoenterostomy (B) annual center caseload and (C) clearance of jaundice.

References

1. Hartley JL, Davenport M, Kelly DA. Biliary atresia. Lancet 2009;374:1704-1713.

2. Asai A, Miethke A, Bezerra JA. Pathogenesis of biliary atresia: defining biology to understand clinical phenotypes. Nat Rev Gastroenterol Hepatol 2015;12:342-352.

3. de Vries W, de Langen ZJ, Groen H, Scheenstra R, Peeters PM, Hulscher JB, Verkade HJ. Biliary atresia in the Netherlands: outcome of patients diagnosed between 1987 and 2008. J Pediatr 2012;160:638-644.

4. Chardot C, Buet C, Serinet MO, Golmard JL, Lachaux A, Roquelaure B, et al. Improving outcomes of biliary atresia: French national series 1986-2009. J Hepatol 2013;58:1209-1217.

5. Davenport M, Ong E, Sharif K, Alizai N, McClean P, Hadzic N, et al. Biliary atresia in England and Wales: results of centralization and new benchmark. J Pediatr Surg 2011;46:1689-1694.

6. Superina R, Magee JC, Brandt ML, Healey PJ, Tiao G, Ryckman F, et al. The anatomic pattern of biliary atresia identified at time of Kasai hepatoportoenterostomy and early postoperative clearance of jaundice are significant predictors of transplant-free survival. Ann Surg 2011;254:577-585.

7. de Vries W, Homan-Van der Veen J, Hulscher JB, Hoekstra-Weebers JE, Houwen RH, Verkade HJ, et al. Twenty-year transplant-free survival rate among patients with biliary atresia. Clin Gastroenterol Hepatol 2011;9:1086-1091.

8. Sundaram SS, Mack CL, Feldman AG, Sokol RJ. Biliary atresia: Indications and timing of liver transplantation and optimization of pretransplant care. Liver Transpl 2017;23:96-109.

9. Arnon R, Annunziato RA, D'Amelio G, Chu J, Shneider BL. Liver Transplantation for Biliary Atresia: Is There a Difference in Outcome for Infants? J Pediatr Gastroenterol Nutr 2016;62:220-225.

10. Neto JS, Feier FH, Bierrenbach AL, Toscano CM, Fonseca EA, Pugliese R, et al. Impact of Kasai portoenterostomy on liver transplantation outcomes: A retrospective cohort study of 347 children with biliary atresia. Liver Transpl 2015;21:922-927.

11. Malenicka S, Ericzon BG, Jørgensen MH, Isoniemi H, Karlsen TH, Krantz M, et al. Impaired intention-to-treat survival after listing for liver transplantation in children with biliary atresia compared to other chronic liver diseases: 20 years' experience from the Nordic countries. Pediatr Transplant 2017 Mar;21(2). doi: 10.1111/petr.12851. Epub 2016 Dec 13.

12. Russo P, Magee JC, Anders RA, Bove KE, Chung C, Cummings OW, et al. Key Histopathologic Features of Liver Biopsies That Distinguish Biliary Atresia From Other Causes of Infantile Cholestasis and Their Correlation With Outcome: A Multicenter Study. Am J Surg Pathol 2016;40:1601-1615.

13. Davenport M, Stringer MD, Tizzard SA, McClean P, Mieli-Vergani G, Hadzic N. Randomized, double-blind, placebo-controlled trial of corticosteroids after Kasai portoenterostomy for biliary atresia. Hepatology 2007;46:1821-1827.

14. Bezerra JA, Spino C, Magee JC, Shneider BL, Rosenthal P, Wang KS, et al. Use of corticosteroids after hepatoportoenterostomy for bile drainage in infants with biliary atresia: the START randomized clinical trial. JAMA 2014;311:1750-1759.

15. Davenport M, Parsons C, Tizzard S, Hadzic N. Steroids in biliary atresia: single surgeon, single centre, prospective study. J Hepatol 2013;59:1054-1058.

16. Chen Y, Nah SA, Chiang L, Krishnaswamy G, Low Y. Postoperative steroid therapy for biliary atresia: Systematic review and meta-analysis. J Pediatr Surg 2015;50:1590-1594.

17. Willot S, Uhlen S, Michaud L, Briand G, Bonnevalle M, Sfeir R, et al. Effect of ursodeoxycholic acid on liver function in children after successful surgery for biliary atresia. Pediatrics 2008;122:e1236-41.

18. Decharun K, Leys CM, West KW, Finnell SM. Prophylactic Antibiotics for Prevention of Cholangitis in Patients With Biliary Atresia Status Post-Kasai Portoenterostomy: A Systematic Review. Clin Pediatr (Phila) 2016;55:66-72.

19. Stringer MD, Davison SM, Rajwal SR, McClean P. Kasai portoenterostomy: 12-year experience with a novel adjuvant therapy regimen. J Pediatr Surg 2007;42:1324-1328.

20. Meyers RL, Book LS, O'Gorman MA, Jackson WD, Black RE, Johnson DG, Matlak ME. High-dose steroids, ursodeoxycholic acid, and chronic intravenous antibiotics improve bile flow after Kasai procedure in infants with biliary atresia. J Pediatr Surg 2003;38:406-11.

21. Davenport M, De Ville de Goyet J, Stringer MD, Mieli-Vergani G, Kelly DA, McClean P, Spitz L. Seamless management of biliary atresia in England and Wales (1999-2002). Lancet 2004;363:1354-1357.

22. Lampela H, Ritvanen A, Kosola S, Koivusalo A, Rintala R, Jalanko H, et al. National centralization of biliary atresia care to an assigned multidisciplinary team provides high-quality outcomes. Scand J Gastroenterol 2012;47:99-107.

23. Ohi R, Ibrahim M Biliary atresia. Semin Pediatr Surg 1992;1:115-124.

24. Ng VL, Haber BH, Magee JC, Miethke A, Murray KF, Michail S, et al. Medical status of 219 children with biliary atresia surviving long-term with their native livers: results from a North American multicenter consortium. J Pediatr 2014;165:539-546.

25. Lykavieris P, Chardot C, Sokhn M, Gauthier F, Valayer J, Bernard O. Outcome in adulthood of biliary atresia: a study of 63 patients who survived for over 20 years with their native liver. Hepatology 2005;41:366-371.

26. Serinet MO, Wildhaber BE, Broué P, Lachaux A, Sarles J, Jacquemin E, et al. Impact of age at Kasai operation on its results in late childhood and adolescence: a rational basis for biliary atresia screening. Pediatrics 2009;123:1280-1286.

27. Wang KS. Newborn Screening for Biliary Atresia. Pediatrics 2015;136:e1663-1669.

28. Harpavat S, Ramraj R, Finegold MJ, Brandt ML, Hertel PM, Fallon SC, et al. Newborn Direct or Conjugated Bilirubin Measurements As a Potential Screen for Biliary Atresia. J Pediatr Gastroenterol Nutr 2016;62:799-803.

29. Fawaz R, Baumann U, Ekong U, Fischler B, Hadzic N, Mack CL, et al. Guideline for the Evaluation of Cholestatic Jaundice in Infants: Joint Recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. J Pediatr Gastroenterol Nutr 2017;64:154-168.

30. Lien TH, Chang MH, Wu JF, Chen HL, Lee HC, Chen AC, et al. Effects of the infant stool color card screening program on 5-year outcome of biliary atresia in Taiwan. Hepatology 2011;53:202-208.

31. Gu YH, Yokoyama K, Mizuta K, Tsuchioka T, Kudo T, Sasaki H, et al. Stool color card screening for early detection of biliary atresia and long-term native liver survival: a 19-year cohort study in Japan. J Pediatr 2015;166:897-902.

32. Kvist N, Davenport M. Thirty-four years' experience with biliary atresia in Denmark: a single center study. Eur J Pediatr Surg 2011;21:224-228.

33. Serinet MO, Broue P, Jacquemin E, Lachaux A, Sarles J, Gottrand F, et al. Management of patients with biliary atresia in France: results of a decentralized policy 1986-2002. Hepatology 2006;44:75-84.

34. Schreiber RA, Barker CC, Roberts EA, Martin SR, Canadian Pediatric Hepatology Research Group. Biliary atresia in Canada: the effect of centre caseload experience on outcome. J Pediatr Gastroenterol Nutr 2010;51:61-65.

35. Davenport M, Tizzard SA, Underhill J, Mieli-Vergani G, Portmann B, Hadzic N. The biliary atresia splenic malformation syndrome: a 28-year single-center retrospective study. J Pediatr 2006;149:393-400.

36. Guttman OR, Roberts EA, Schreiber RA, Barker CC, Ng VL. Biliary atresia with associated structural malformations in Canadian infants. Liver Int 2011;31:1485-1493.

37. Schwarz KB, Haber BH, Rosenthal P, Mack CL, Moore J, Bove K, et al. Extrahepatic anomalies in infants with biliary atresia: results of a large prospective North American multicenter study. Hepatology 2013;58:1724-1731.
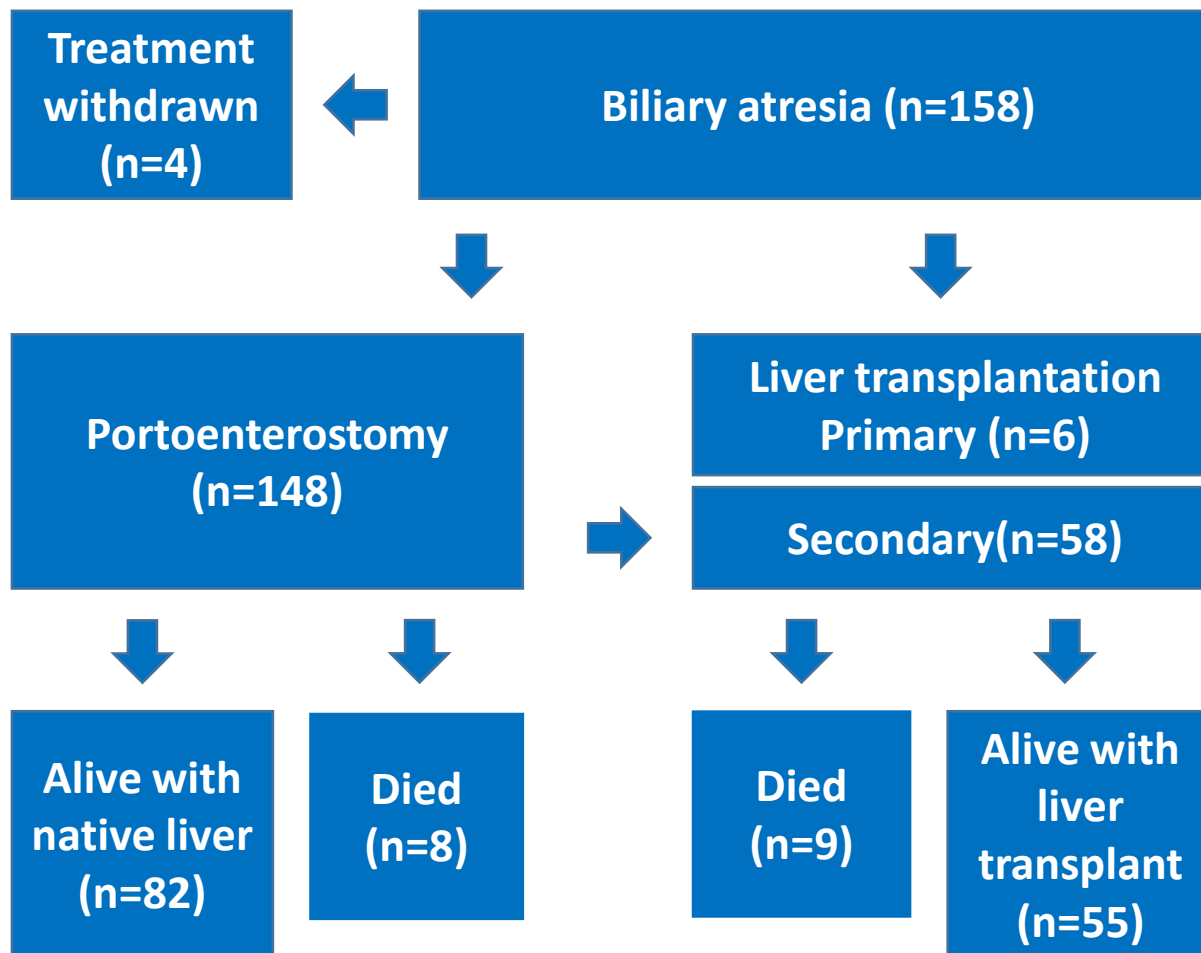
Figure 1

Figure 1

Figure 2
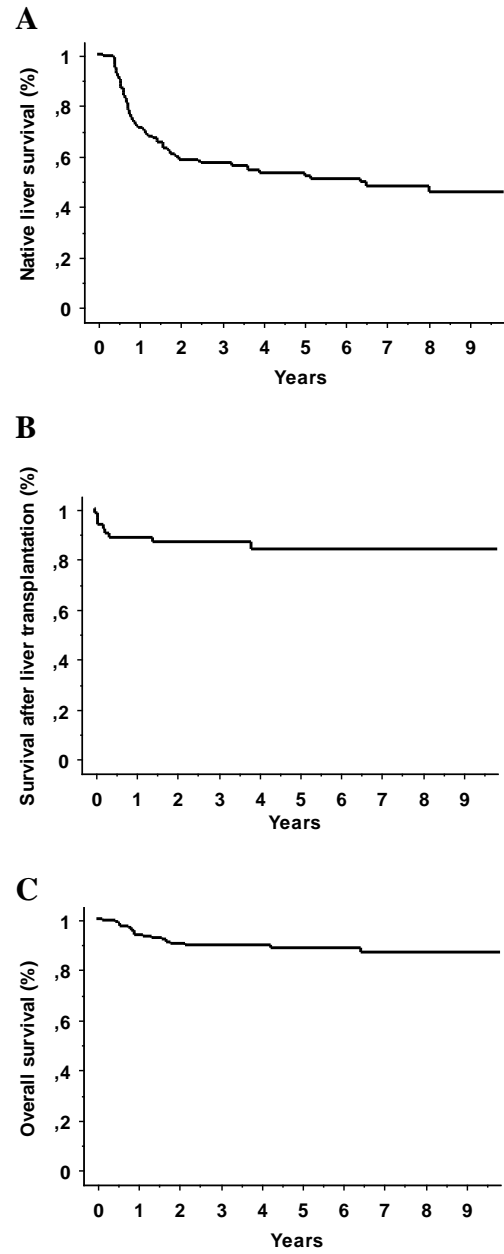
Figure 2.

Figure 3
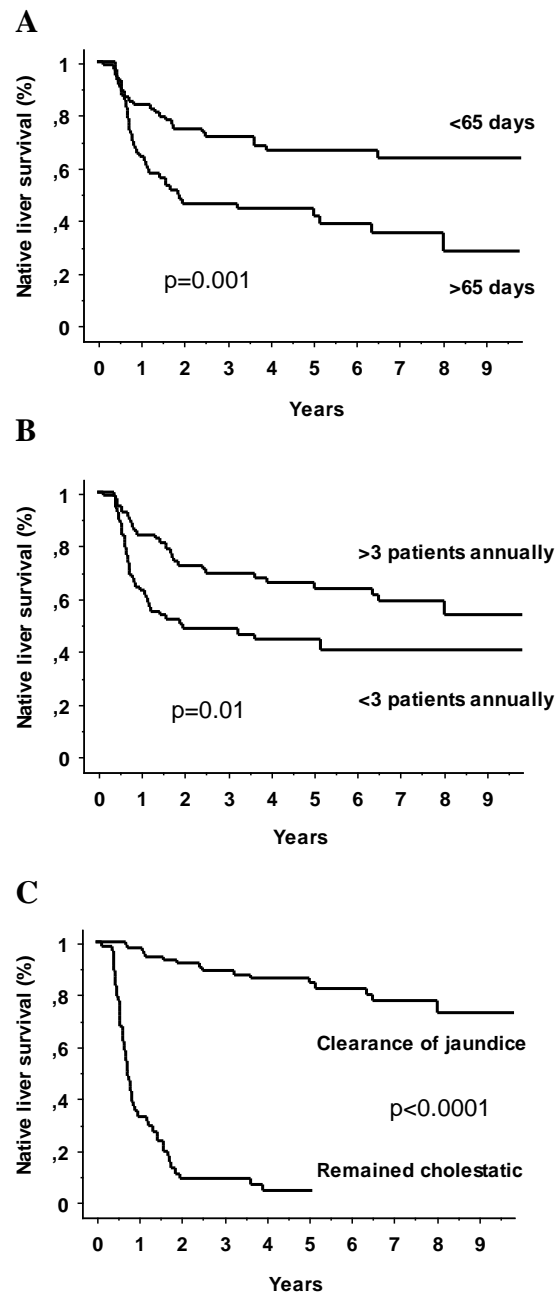
Figure 3.

Table 1

Table 1. Characteristics of actively treated biliary atresia patients during 2005-2016 in the Nordic countries.

Number	154
Females, n (%)	83 (54)
European ethnicity, n (%)	124 (81)
Anatomic type of BA, n (%)	
Type 1	10 (6.4)
Type 2	15 (9.7)
Type 3	126 (82)
Unknown	3 (1.9)
Associated congenital malformations, n (%)	42 (27)
Asplenia or polysplenia	19 (12)
Cardiac defect	10 (6.5)
Underwent portoenterostomy, n (%)	148 (96)
Age at portoenterostomy, days, median (interquartile range)	64 (37-79)
Medical therapy after portoenterostomy, n (%)	
Corticosteroids	137 (93)
Ursodeoxycholic acid	144 (97)
Antibiotics	145 (98)
Normalized serum bilirubin <20 μmol/l, n (%)	95 (64)
Native liver survivors, n (%)	82 (53)
Follow-up age, years, median (interquartile range)	4.9 (2.4-7.3)
Serum bilirubin, μmol/l, median (interquartile range)	6 (4-15)
Blood platelets, 10^9/l, median (interquartile range)	230 (128-303)
Esophageal varices, n (%)	13 (16)

Table 2

Table 2. Predictors for normalization of serum bilirubin concentration (<20 µmol/l) after portoenterostomy during 2006-2016 in the Nordic countries. Clearance of jaundice rates were compared with χ^2 test and odd ratios for multivariate analysis were generated with logistic regression model.

Predictor	Clearance of jaundice, n (%)	p-value	Odd ratio (95% CI)	p-value
Portoenterostomy age				
<65 days	55/77 (71)	0.056	1.98 (0.92-4.24)	0.079
>65 days	40/71 (56)			
Gender				
Female	50/79 (63)	0.807	0.95 (0.46-1.98)	0.900
Male	45/69 (65)			
European ethnicity				
Yes	76/119 (64)	0.868	0.92 (0.36-2.34)	0.863
No	19/29 (66)			
Anatomic type of BA				
type 1 or 2	20/24 (83)	0.031	3.70 (1.14-12.0)	0.030
type 3	74/123 (60)			
Associated malformations				
Yes	27/41 (66)	0.794	1.09 (0.39-3.03)	0.868
No	68/107 (63)			
Splenic malformations				
Yes	11/18 (61)	0.771	0.70 (0.18-2.81)	0.619
No	84/130 (65)			
Center annual caseload				
>3	54/79 (68)	0.258	1.53 (0.73-3.20)	0.262
<3	41/69 (59)			

Table 3

Table 3. Predictors for native liver survival after portoenterostomy during 2006-2016 in the Nordic countries. Five-year native liver Kaplan Meier survival rates were compared with log-rank test and hazard ratios for multivariate analysis were generated with Cox regression model.

Predictor	n	5-year survival (%) (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Portoenterostomy age					
<65 days	77	66 (55-78)	0.005	1.51 (0.80-2.87)	0.207
>65 days	71	44 (32-56)			
Gender					
Female	79	51 (39-63)	0.301	0.47 (0.26-0.85)	0.012
Male	69	60 (48-72)			
European ethnicity					
Yes	119	54 (45-64)	0.692	0.70 (0.35-1.41)	0.318
No	29	60 (42-79)			
Anatomic type of BA					
type 1 or 2	24	66 (44-87)	0.187	1.36 (0.58-3.18)	0.485
type 3	123	53 843-62)			
Associated malformations					
Yes	41	62 (46-78)	0.612	1.02 (0.43-2.45)	0.966
No	107	53 (43-63)			
Splenic malformations					
Yes	18	49 (24-74)	0.546	0.75 (0.25-2.24)	0.601
No	130	56 (47-65)			
Center annual caseload					
>3	79	66 (54-77)	0.004	3.46 (1.76-6.81)	0.0003
<3	69	44 (32-56)			
Clearance of jaundice					
<20 µmol/l	95	86 (78-94)	<0.0001	32.0 (14.8-69.2)	<0.0001
≥20 µmol/l	53	4.3 (0-10)			

Table 4

Table 4. Predictors of portoenterostomy age <65 days in patients with biliary atresia during 2006-2016 in the Nordic countries. Clearance of jaundice rates were compared with χ^2 test and odd ratios for multivariate analysis were generated with logistic regression model.

Predictor	Portoenterostomy age < 65 days, n (%)	p-value	Odd ratio (95% CI)	p-value
European ethnicity				
Yes	65/119 (55)	0.210	1.17 (0.48-2.87)	0.730
No	12/29 (41)			
Anatomic type of BA				
type 1 or 2	12/24 (50)	0.799	0.64 (90.24-1.73)	0.376
type 3	65/123 (53)			
Associated malformations				
Yes	29/41 (71)	0.005	1.46 (0.53-3.74)	0.495
No	48/107 (45)			
Splenic malformations				
Yes	16/18 (89)	0.0008	5.82 (0.99-34.2)	0.051
No	61/130 (47)			
Center annual caseload				
>3	51/79 (65)	0.0011	1.51 (0.64-3.59)	0.350
<3	26/69 (38)			
Liver biopsy based diagnosis				
Yes	7/34 (21)	<0.0001	0.23 (0.08-0.70)	0.010
No	70/114 (61)			

Table 5

Table 5. Recently reported outcomes of biliary atresia in Europe.

Origin (reference)	Period	Cohort size	Median age at PE (days)	Clearance of jaundice (%)	5-year native liver survival (%)	5-year overall survival (%)
England and Wales (5)	1999-2009	439	54	55	46 (95%CI 41-51)*	90 (95%CI 88-93)
France (4)	2003-2009	339	59	36	39 (SE 3.3%)†	89 (SE 1.9%)
Netherlands (3)	1998-2008	104	59	38	42 (95%CI 32-52)†‡	76 (95%CI 68-85)‡
Nordic countries (present)	2005-2016	154	64	64	53 (95%CI 45-62)*	88 (83-94)

PE, portoenterostomy

LT, liver transplantation

*Includes all patients

†Includes patients who underwent PE

‡4-year survival

Supplementary Table 1. Characteristics of centers treating biliary atresia during 2005-2016 in the Nordic countries.

Number	6
Annual caseload, median (range)	2.6 (0.7-3.7)
Number of centers with annual caseload >3, n	2
Diagnostic modalities routinely used to diagnose/rule out biliary atresia, n	
Isotope excretion cholescintigraphy	6
Operative cholangiography	6
Ultrasound	6
Liver biopsy	2
Magnetic resonance imaging	2
Endoscopic retrograde cholangiography	1
Number of operating surgeons, median (range)	1 (1-4)
Laparoscopic portoenterostomy, n	0
Medical therapy routinely prescribed after portoenterostomy, n	
Steroids	5
Ursodeoxycholic acid	6
Antibiotics	6
Fat-soluble vitamin supplementation	6
Endoscopic surveillance protocol for esophageal varices, n	2

JOURNAL OF PEDIATRIC SURGERY (JPS)
Author Disclosure of Relevant Financial Relationships

NAME: Mikko Pakarinen

TITLE OF ARTICLE: Outcomes of biliary atresia in the Nordic countries – A multicenter study of 158 patients during 2005-2016

Per ACCME regulations, in order to provide Continuous Medical Educational credit, the Journal of Pediatric Surgery must ensure that anyone who is in a position to control the content of the education activity has disclosed to us all relevant financial relationships with any commercial interest (see below for definitions).
Please indicate below any commercial interests that would pertain to the content of the article being submitted to JPS.

[X] **None of the authors have anything to disclose (if you are the corresponding author).**

[ ] **I do not have anything to disclose (if you are an individual author).**

[ ] **I am disclosing all relevant financial relationships with any commercial interests that pertain to this article (see below).**

- List the names of any proprietary entities producing marketing, re-selling, or distributing health care goods or services consumed by, or used on patients with which you or your spouse/partner have, or have had, a **relevant** financial relationship within the past 12 months.
- Explain what you or your spouse/partner received (ex: salary, honorarium etc).
- Specify your role.

Commercial Interest	Nature of Relevant Financial Relationship (Include all those that apply)	
	What I or spouse/partner received	**My role**
Example: Company 'X'	*Honorarium*	*Speaker*

What was received: Salary, royalty, intellectual property rights, consulting fee, honoraria, ownership interest, stocks, stock options or other ownership interest, excluding diversified mutual funds), or other financial benefit.

My Role(s): Employment, management position, (e.g., independent contractor (including contracted research), consulting, speaking and teaching, membership on advisory committees or review panels, board membership, and other activities.

Through our review process, should it be determined that a bias is apparent within the content of the article, you will be requested to revise your manuscript prior to publication.

If your article describes the use of a device, product, or drug that is not FDA approved or the off-label use of an approved device, product, or drug or unapproved usage, it is your responsibility to disclose this information within the manuscript.

Glossary of Terms

Commercial Interest
A commercial interest is any entity producing, marketing, re-selling, or distributing health care goods or services consumed by, or used on, patients. The ACCME does not consider providers of clinical service directly to patients to be commercial interests.

Financial relationships
Financial relationships are those relationships in which the individual benefits by receiving a salary, royalty, intellectual property rights, consulting fee, honoraria, ownership interest (e.g., stocks, stock options or other ownership interest, excluding diversified mutual funds), or other financial benefit. Financial benefits are usually associated with roles such as employment, management position, independent contractor (including contracted research), consulting, speaking and teaching, membership on advisory committees or review panels, board membership, and other activities from which remuneration is received, or expected. ACCME considers relationships of the person involved in the CME activity to include financial relationships of a spouse or partner.

Relevant financial relationships
ACCME focuses on financial relationships with commercial interests in the 12-month period preceeding the time that the individual is being asked to assume a role controlling content of the CME activity. ACCME has not set a minimal dollar amount for relationships to be significant. Inherent in any amount is the incentive to maintain or increase the value of the relationship. The ACCME defines "'relevant' financial relationships" as financial relationships in any amount occurring within the past 12 months that create a conflict of interest.

Conflict of Interest
Circumstances create a conflict of interest when an individual has an opportunity to affect CME content about products or services of a commercial interest with which he/she has a financial relationship.

Journal of Pediatric Surgery

Author Contribution Form (*)

Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the manuscript contents. Allowing one's name to appear as an author without contributing significantly to the study or adding the name of an individual that has not contributed or has not agreed to the study in its current form is considered a breach of appropriate authorship and publication ethics. No individual other than the authors listed below should have contributed substantially to the preparation and revision of the manuscript. Ghost-writing is not acceptable. Individuals who do not meet the criteria for authorship can be recognized and listed in the acknowledgments. This includes individuals that allow their clinical experience to be included in the study, those that provide only technical assistance, copyediting, proofreading or translation assistance, or a departmental leader that provided only general support including a department Chair.

For multi-institutional studies, groups of individuals and institutions that have materially contributed to the study but, whose contributions do not justify authorship may be listed in an appendix under a heading such as participating or clinical investigators. Their function should be described for example as a scientific advisor, critically reviewed the study proposal or participating institution.

Type the name of each author next to the appropriate following categories:

Study conception and design: *All authors*

Data acquisition: *All authors*

Analysis and data interpretation: *MP*

Drafting of the manuscript: *MP*

Critical revision: *All authors*

Submit this form with the manuscript.

(*): Modified from the authorship requirements of the J Am Coll Surg