BILIARY ATRESIA

Treatment Results and Native Liver Function

HANNA LAMPELA

ACADEMIC DISSERTATION

To be publicly discussed, with the permission of the Faculty of Medicine, University of Helsinki, in the Niilo Hallman Auditorium, Children’s Hospital, on 1st of March 2013, at 12 noon.

Helsinki 2013
“And if I have the gift of prophecy,

and know all mysteries and all knowledge;

and if I have all faith, so as to remove mountains,

but have not love, I am nothing.”

1. Corinthians 13:2
CONTENTS

ABSTRACT 6
LIST OF ORIGINAL PUBLICATIONS 8
ABBREVIATIONS 9
INTRODUCTION 10

REVIEW OF THE LITERATURE 11
History 11
   Early descriptions 11
   Development of operative techniques 11
Classifications 13
Epidemiology 14
   Incidence 14
   Seasonality 14
Etiology 14
   Isolated BA 14
   Congenital BA 15
Diagnosis 16
   Symptoms and screening 16
   Diagnostic tools and differential diagnostics 17
Treatment 20
   Kasai portoenterostomy 20
Adjuvant medical therapy 23
   Corticosteroids 23
   Choleretics 23
Nutritional therapy 24
Complications 24
   Portal hypertension and esophageal varices 24
   Cholangitis 26
   Other complications 26
   Carcinoma 27
Liver transplantation 28
Outcomes 29
  Factors associated with portoenterostomy success 29
  Native liver and overall survival 29
  Health and quality of life among adult native liver survivors 30

AIMS OF THE STUDY 31

PATIENTS AND METHODS 32
  Patients and study design 32
  Follow-up data collection 33
  Histology and immunohistology 33
  Liver function test and scores 34
  Ethics 35
  Statistics 35
  BA treatment protocol 36

RESULTS 37
  Epidemiology 37
    Seasonality and geographic distribution 38
  Outcomes 39
    Portoenterostomy success and native liver survival 40
    Liver transplantations for BA 42
    Deaths and overall survival 43
    Liver function and general health among native liver survivors 44
    Esophageal varices 44
    Liver histology 46
    Follow-up tools 48

DISCUSSION 50

CONCLUSIONS 55

ACKNOWLEDGEMENTS 56

REFERENCES 59
**ABSTRACT**

**Background.** Biliary atresia (BA) is a devastating disease of infancy where the bile ducts are occluded and destroyed by a fibroinflammatory process. BA is rare but still the most common indication for childhood liver transplantation (LT). BA treatment is started with a portoenterostomy (PE) operation, adjuvant medical therapy, and nutritional treatment and continued with LT if the PE fails.

**Aims.** The aim of this study was to investigate the incidence of BA in Finland and to evaluate the outcomes of Finnish BA patients in the era of liver transplantation, with special emphasis on the effects of treatment centralization in 2005. Furthermore, the occurrence and predictors for esophageal varices and associated gastrointestinal bleeding were assessed, the relations between liver histology and clinical outcome variables evaluated, and noninvasive follow-up tools identified.

**Patients and methods.** BA patients born in Finland between 1987 and 2010 were identified from the national Register of Congenital Malformations and Children’s Hospital BA database. All hospital records were reviewed for diagnosis confirmation associated structural abnormalities, treatment, follow-up data, upper gastrointestinal endoscopies, gastrointestinal bleeding episodes, LT, and outcome. Liver biopsies taken at PE and LT were reviewed together with follow-up biopsies taken at a median 4.2 years after successful PE.

**Results.** BA was diagnosed in 74 children. The incidence of BA was 1:19 900 live births. Anomalies associated with laterality disorders (heart defects, intestinal rotation disorders, poly- or asplenia, pancreatic anomalies, vascular anomalies, situs inversus) were observed in 17 (23%) patients and ten of these (14% of the cohort) met the criteria for BA splenic malformation. Births with BA were more common in autumn-winter than in spring-summer (p=0.013).

Before centralization, 52 BA patients were treated in five centres with a median of zero (0-3) patients/year and after 2005, 22 patients were treated in one centre with a median of four (2-5) patients per year, p<0.001. After centralization, the clearance of jaundice rate improved significantly from 29% to 73%, p=0.001. This improvement translated into increased native liver survival: before centralization the native liver survival at four years was 21% (actual) and after centralization 73% (95% confidence interval, CI 54-91%, actuarial), p<0.001. Importantly, overall survival improved significantly [50% vs. 86% (95% CI 74-98%), p=0.006].

Esophageal varices were equally common after failed (64%) and successful PE (53%). After failed PE varices bled more often (16/28 vs. 0/19 patients, p<0.001) and appeared significantly earlier, at eight months (4-23) vs. 19 months (4-165), p=0.004. Eradication of varices by sclerotherapy succeeded in no failed PE patient while eradication was achieved in six of ten successful PE patients, p=0.001.

A native liver biopsy taken from 23 patients with normal, or near normal, bilirubin levels (≤35 μmol/L) at a median of 4.2 years after successful PE revealed cirrhosis in 12 (52%), cholestasis in 4 (17%), and perportal cytokeratin 7 immunopositivity indicating cholangiocytic transformation of the hepatocytes in 14 (61%). In 19 patients with a liver biopsy taken both at PE and follow-up, liver fibrosis progressed or remained cirrhotic in
12 (63%) and diminished or remained mild in 7 (37%). The patients whose liver fibrosis progressed had higher serum bilirubin levels at follow-up [median 15 μmol/L (3-35) vs. 5 μmol/L (2-11), p=0.006].

For the risk of being listed for LT or dying within one year, pediatric/model for end-stage liver disease score over 3 gave 83% sensitivity and 84% specificity, and galactose half-life over 12.0 minutes gave 83% sensitivity and 64% specificity. The combination of normal serum bilirubin (<20 μmol/L) and galactose half-life under 12.0 minutes was present in none of the patients who were listed for LT or died within one year of measurements. Of the non-invasive follow-up tools studied, aspartate transferase to platelet ratio index APRI over 1.0 gave 91% sensitivity and 83% specificity for esophageal varices among 23 native liver survivors of whom 11 (48%) had varices. APRI also correlated with histological liver fibrosis score (r=0.634, p=0.001) and a value of over 0.60 gave 93% sensitivity and 67% specificity for advanced liver fibrosis (Metavir fibrosis score ≥3).

Conclusions. BA incidence and the associated anomaly pattern were similar to other European and North American countries. The clear improvement in results observed after treatment centralization in 2005 supports centralizing BA treatment to designated multidisciplinary teams, despite the fact that annual caseload remained internationally low. Esophageal varices are common in BA. Endoscopic surveillance could be allocated to patients with elevated serum bilirubin levels or clinical signs of portal hypertension like splenomegaly or thrombocytopenia. The non-jaundiced BA native liver survivors suffer from a concealed cholestasis, possibly contributing to the progression of liver fibrosis. The pediatric/model for end-stage liver disease score and galactose half-life, alone or in combination with serum bilirubin level, appear useful in assessing the one-year prognosis of BA native liver survivors. APRI seems a useful non-invasive tool for predicting liver fibrosis and varices. Multicenter collaboration would be desirable in order to further improve treatment results and to obtain adequate patient numbers in future studies.
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by their Roman numerals I-IV:


These articles are reproduced with the kind permission of their copyright holders. Some previously unpublished data are also presented.
ABBREVIATIONS

APRI  aspartate transferase to platelet ratio index
AST  aspartate transferase
AUROC  area under receiver operating curve
BA  biliary atresia
BASM  biliary atresia splenic malformation
CK7  cytokeratin 7
CI  confidence interval
ERCP  endoscopic retrograde cholangiopancreatography
LT  liver transplantation
MELD  model for end-stage liver disease
MRCP  magnetic resonance cholangiopancreatography
NA`K`ATPase  sodium potassium adenosine triphosphatase
OR  odds ratio
PE  portoenterostomy
PELD  pediatric end-stage liver disease
SD  standard deviation
UDCA  ursodeoxycholic acid
UK  United Kingdom
US  United States
INTRODUCTION

Biliary atresia is a neonatal inflammatory cholangiopathy, which results in total obstruction and destruction of the extrahepatic biliary tree with variable extension to intrahepatic bile ducts (1). Without treatment, BA leads to liver failure and death within the first two years of life. The only effective treatments for BA are surgical drainage of the bile and liver transplantation (2). BA is the most frequent chronic cholestatic disorder in children, leading to need for LT in most affected individuals, and thus BA is the most common indication for childhood liver transplantation (3).

A disease is considered rare when it affects less than 1:2000 individuals (4). According to the World Health Organization, over 5000 rare disorders exist and in Europe, over 30 million patients have a rare disease. Problems that affect patients with rare disorders, and medical professionals treating them, include limited information on rare diseases, limited availability of adequate treatments, and difficulties in obtaining research financing. Despite the fact that most European health care systems cover treatment costs for rare disorders, affected families report considerable burden of lost social and economical opportunities as well as delays in diagnostics and bad experiences of medical care (4). BA, with an incidence of 1:17000 - 19000 live births in Europe, is a rare disease (1). As 5.4 million children are born annually in the European Union area (5), BA is diagnosed in approximately 300 babies each year.

In BA, early diagnosis and access to surgery in an experienced centre are key events that define survival with native liver (1). As results of BA surgery improve, an increasing proportion of BA patients survive with their own liver into adolescence or even adulthood (6). Most native liver survivors have a slowly progressive liver disease with a risk of portal hypertension, liver failure, and even malignancy (7). Besides lifelong dependency on immunosuppressive drugs, pediatric liver transplant recipients face the risks of allograft ageing and the adverse effects associated with long term immunosuppression (8).

The aim of this study was to define the incidence of BA in Finland and outcomes of BA patients born between 1987 and 2010 with special emphasis on the effects of treatment centralization from five centres to one in 2005. In addition, the prevalence of esophageal varices among BA patients and the effects of the current endoscopic screening and treatment protocol are investigated here. Liver function and histology among the native liver survivors is also reported. Furthermore, noninvasive follow-up tools are sought.
REVIEW OF THE LITERATURE

History

Early descriptions

In 350 BCE, Aristotle described agenesis of the gallbladder. The first English language description of a probable BA is found in a textbook “Principles of Midwifery, Including the Diseases of Women and Children”, written by Professor John Burns from the Department of Surgery, University of Glasgow in 1817 (9). He wrote: “The jaundice of infants is a disease attendant with great danger, especially if it appears very soon after birth, and the stools evince a deficiency of bile; for we have then reason to apprehend some incurable state of the biliary apparatus.” Dr. Charles West (1816-1892), the founder of the first children’s hospital in England (Hospital for Sick Children at Great Ormond Street), described the typical BA symptoms: “On November 8, 1855, I saw a female child aged 13 weeks; the only child of healthy parents. It was born at full term, though small, apparently healthy. At 3 days however, it began to get yellow and at the end of three weeks was very yellow. Her motions at no time after the second day appeared natural on examination, but were white, like cream, and her urine was very high coloured. (10)” In 1892 John Thomson from Scotland described 50 BA patients and concluded that the bile ducts were narrow due to defective development, and the obliteration and the eventual disappearance of the ducts resulted from inflammation spreading to the walls of the ducts (11).

Development of operative techniques

In 1940 William Ladd from Harvard Medical School in Boston published a series of 45 BA infants of whom in 9 he had found a “correctable” type of BA and performed a choledochoduodenostomy (12). Five patients survived 5 to 13 years postoperatively. Patients were divided into an “uncorrectable” type with no extrahepatic ductular remnants of the biliary tract and a “correctable” type with a preserved, open part of the common hepatic duct where to suture the intestine. In Ladd’s later series, however, only 12 of 146 (8%) patients became jaundice-free whereas all the rest died (13). These experiences led Willis J. Potts, chief surgeon of Children's Memorial Hospital in Chicago, to declare, in his book The Surgeon and the Child, in 1959: “Congenital atresia of the bile ducts is the darkest chapter in pediatric surgery. The etiology is unknown... In the light of our present knowledge, unless bile can be shunted to the gastrointestinal tract, early death is inevitable.(14)”

Meanwhile in Sendai, Japan, surgeons Shigetsugu Katsura and Morio Kasai (Figure 1), desperate over an “uncorrectable” biliary atresia case, incised the porta hepatis and placed an unopened duodenum over it (15, 16). Postoperatively bile appeared in the stools and jaundice cleared. Their second similarly-treated patient also excreted some bile into stools, but eventually died. In the autopsy Kasai found a biliary fistula formed between the
intrahepatic bile ducts and the duodenum. Impressed of the force that the bile had found its way out of the liver, he performed a hepatic portoenterostomy to a third patient with an “uncorrectable” BA – the operation succeeded. Kasai first published the novel technique of excising the portal plate and suturing a jejunal loop to the liver hilum in Japanese in 1959, then in German in 1963, and in English in 1968 (16). In Kasai’s hands 70% of the patients cleared their jaundice but less than 20% survived jaundice-free more than two years (17). In other centres the figures were even lower, and despite a successful PE the liver cirrhosis progressed slowly to great disappointment in most patients (17-19). It took several years for the method to spread around the world and gain acceptance (20). We must keep in mind that in the 1970s, liver transplantation (LT) as a cure for terminal liver disease was way out of sight. Some surgeons thought that performing a PE, even if it was successful, meant just prolonging a BA child’s suffering (19, 20). The great majority of BA patients, with or without undergoing a PE operation, were to die.

It was not until the mid 1980s that LT would bring a remedy for BA patients. In 1963 Thomas Starzl performed the first human LT in Denver on a three year old boy with BA – the patient died on the operating table. Starzl simultaneously reported two adult patients in whom LT technically succeeded but who died of pulmonary emboli at postoperative days seven and 22. (21) The first LT recipient with a prolonged survival of 13 months was reported in 1967 (22). In early 1980s the invent of immunosuppressant cyclosporine made long term survival after LT possible (23) and in 1983 the US National Institutes of Health confirmed LT as appropriate therapy for selected patients with end-stage liver disease, including BA (24). The first Nordic LT was performed in Helsinki in 1982, and a nationwide Finnish pediatric LT program started in Helsinki in 1987 (25).

BA has onset either during foetal development or postnatally. The exact cause of BA still remains unknown. To date, BA treatment is sequential with PE as the first line treatment, followed by LT if PE fails or cirrhosis develops after successful PE.

Classifications

BA may be isolated or associated with other structural anomalies. Isolated BA can be anatomically classified into subtypes according to the most proximal site of bile duct disruption: in type 1 (approximately 5% of BA cases) a biliary lumen exists from the liver to the common bile duct, in type 2 the biliary lumen extends to the common hepatic duct (rare, about 2%), and in the most common type 3 (>90%) no luminal biliary tract outside the liver can be observed and the portal plate in the porta hepatis is a solid, fibrous mass (1). In some publications, type 2 is called BA with patent distal extrahepatic bile ducts (26).

BA with other structural anomalies is called either developmental or congenital BA or BA splenic malformation (BASM). The definition of BASM has varied but mostly BASM includes biliary atresia with a splenic malformation (poly- or asplenia) with or without situs inversus (estimated frequency within the BASM syndrome 37%), intestinal malrotation (60%), absent inferior vena cava (70%), preduodenal portal vein (40%), cardiac anomalies (45%), or pancreatic anomalies (11%), Figure 2. Since the spleen anomaly is a defining criterion for BASM, patients with biliary atresia who present no spleen anomaly, but have one or more anomalies, are often referred as BA with other anomalies. (1) In the cystic variant of BA, a cystic dilatation is observed somewhere along the remnants of the extrahepatic biliary tract (27).

![Figure 2. Anomalies observed in congenital biliary atresia. A. polysplenia; B. absent inferior vena cava; C. aberrant arterial supply with left hepatic artery from either left gastric artery or from superior mesenteric artery; D. preduodenal portal vein; E. intestinal malrotation. Reproduced with kind permission from Pediatrics in Review, Vol 20, Page 366, Copyright 1999 by the AAP.](image-url)
Epidemiology

Incidence

BA is a rare disorder. European and North American countries report a BA incidence of between 1:14000 and 1:20000 live births (28-34). In Japan the reported incidence is approximately 1:9000 live births (35-37). The highest incidence, approximately 1:3000 live births, is in French Polynesia (38, 39) and Taiwan (40). Reasons for high BA incidence observed on the west side of the Pacific Ocean remain unknown. Reports of gender distribution are conflicting: some centres report a slight majority of girls (54-69%) (30, 31, 33, 38), whereas a register study from Sweden showed female minority (40%) (29). In all series, isolated BA has a clear majority; the proportion of patients with BASM varies from 5% to 14% (30-33) and BA with other anomalies from 13% to 20% (30, 31, 35).

Seasonality

The possibility of a viral infection playing a role in BA pathogenesis raises a question of seasonal variation in BA incidence. European series report no significant monthly or seasonal variation in the incidence of live births with BA compared to general live birth rate (31, 38) – a small, non-significant accumulation of births with BA was observed in November and March in Sweden (29) and in September in England and Wales (30). A registry report from eight states in the US identified infants conceived in the spring season to have a higher risk for BA (OR 2.33, 95% CI 1.05-5.16) than infants conceived in the winter (41). One of these registry report studies from New York state showed significant variation in monthly incidence, but conflict was evident when two different statistical methods used in the study showed two completely separate peak seasons for BA conception (either from March to August or in December) (42). A similar discrepancy is also present in reports from Japan showing either no seasonality (36) or a BA birth peak in the cold season December-March (37). In French Polynesia, a significant clustering of births with BA was observed in the dry season from July to November (39).

Etiology

Isolated BA

The exact events in the development of BA are largely unclear. An increasing amount of data exists, however, on a combined genetic susceptibility and an infectious, most likely viral, trigger followed by an autoimmune-type of inflammation in the biliary tree (1, 43, 44). The data on the potential genetic susceptibility factors of BA are scarce due to the
rarity of the disease and thus small patient populations. Single nucleotide polymorphisms potentially associated with BA have been observed in genes CD14 and macrophage migration inhibitory factor (44). Similarly, a genome-wide association study of 200 Chinese BA children reported high prevalence of single nucleotide polymorphism rs17095355 on 10q24 (45) but the relevance of this finding is unclear. Several animal studies have explored reo-, rota-, and cytomegaloviruses causing conditions mimicking human BA; in the recent years probably the most common model has been the rotavirus-induced murine BA (43, 46). In human studies, in contrast, no viral particles have been found in liver or biliary tract tissue samples from BA patients (1). At BA manifestation, the immune system may have already cleared the initial viral trigger (47). After the virus has been cleared, autoreactive CD4+ and CD8+ lymphocytes that are found in the inflammatory portal area, infiltrate and possibly continue to damage and destroy the biliary epithelium (43, 44, 48). Since June 2009, the World Health Organization has recommended rotavirus vaccination against diarrhoeal disease to be included in all national immunization programmes (49). Whether the use of rotavirus vaccination will decrease BA incidence, remains to be seen. One study from Taiwan reported a significantly decreased incidence of BA since 2007; the incidence showed slight negative correlation with the rotavirus vaccine coverage rate (50).

**Congenital BA**

BASM, a syndromic form of BA, already starts to develop in the first trimester of pregnancy and has been associated with a higher incidence of maternal diabetes (51). Bile duct obstruction and abnormalities in organ symmetry were observed in mice with a mutation in the Inversin gene, which regulates laterality. However, no mutations in the Inversin gene were observed in children with BA and laterality disorders (44). A Canadian study with 328 BA patients of whom 44 (13%) had associated anomalies included a cluster hierarchical analysis revealing two anomaly subgroups of: BA with only heart defects and BA with abdominal vascular anomalies and disordered laterality (52). Since spleen abnormality did not present a definitional criterion, the Canadian group suggested that the BASM acronym be changed to indicate BA structural malformation. A large series of 270 BA patients from England investigating the BA cystic variant found that 11% had the cystic variant; 66% of the cysts were walled with fibroinflammatory material, and 25% contained bile (27).
Diagnosis

Symptoms and screening

The cardinal signs of BA in an infant are persistent jaundice, caused by bilirubin accumulated in blood and tissues, pale stools, and dark urine. In BA bile is accumulated in blood and tissues causing jaundice as bile cannot enter the intestine and thus the stools of a milk-fed infant lack the typical yellow-greenish color and remain near white or beige and are called acholic (Figure 3). The excess amount of water-soluble conjugated bilirubin in the blood is partly excreted by the kidneys making urine dark. Except for jaundice, BA infants usually appear misleadingly healthy during the first months of life (53). If the early signs go unnoticed, liver failure develops presenting as failure to thrive due to catabolic metabolism, deficient absorption of lipids, liver and spleen enlargement, and ascites (1). Very rarely, the first alarm is a hemorrhage, either subcutaneous or intracranial, due to fat soluble vitamin K deficiency associated coagulopathy. In the Netherlands, 8% of BA patients presented with an intracranial hemorrhage – all had received oral prophylactic vitamin K as neonates (54). Oral or absent neonatal vitamin K prophylaxis is a risk factor for hemorrhage, especially in infants with unrecognized cholestasis due to poor intestinal absorption of fat-soluble vitamins (1). In Finland, neonates have received intramuscular vitamin K prophylaxis since the 1950’s (55).

The Taiwanese use stool color cards for screening for BA with favorable results (40, 56). At newborn discharge, the parents are given a stool color card (Figure 3) and advised to observe their baby’s stool color. The parents are asked to take the card to the baby’s one-month health check or mail it to the stool color registry centre. If the parents choose an abnormal stool color, the baby is referred to a pediatric unit and followed by the registry centre until a definite diagnosis is reached or BA ruled out. Abnormal stool color showed 90% sensitivity and 100% specificity for BA, and 90% of the BA cases were screened out before 60 days of age. A stool color card pilot program has also been initiated in Switzerland (57). In the 1990s, a study from England measured bile acid concentrations from dried blood spots obtained from newborns at seven to ten days of age. Patients with either BA or other cholestatic liver disease had significantly higher conjugated bile acid concentrations than their healthy peers but unfortunately the concentration difference was not enough for a mass screening (58).
Diagnostic tools and differential diagnostics

In most BA patients, nothing in particular is observed during pregnancy. A non-specific cyst may be observed in the upper right quadrant of the abdomen in the cystic variant in a second trimester ultrasound examination. In a study from England, a cyst was identified on antenatal sonography in 41% of cystic BA patients (27). In congenital BA, the associated anomalies like heart defects may be observed during pregnancy.

A large proportion of newborns develop physiological jaundice and at two weeks up to 15% will still be jaundiced (59). If the jaundice of a term infant persists after two weeks, it is prolonged. In benign physiologic jaundice, the hyperbilirubinemia consists of unconjugated bilirubin (60). To reveal cholestatic jaundice, the serum conjugated bilirubin should also be measured. If the serum concentration of conjugated bilirubin exceeds 20 μmol/L, or the proportion of conjugated bilirubin exceeds 20%, the possibility of a severe cholestatic condition should be suspected and the patient timely evaluated in a pediatric unit (53, 61, 62).

In infancy, the range of diagnoses underlying cholestasis is wider than ever later in life. The challenge in diagnosing BA is that, besides operative cholangiography, no specific diagnostic test exists. Yet, the process of ruling out or identifying BA should be prompt and cause minimal delay to operative treatment (1). Table 1 summarizes the main causes of neonatal cholestasis.

Besides conjugated bilirubin, other liver biochemical variables are also elevated in BA (1), but only γ-glutamyl transferase levels have been significantly higher when compared to other cholestatic diseases (63). The liver’s synthetic function is usually well preserved. Liver biochemistry observed at first admission among Children’s Hospital BA patients after 2005 is presented in Table 2.

Figure 3. The abnormal, acholic stool colors in three upper pictures and the normal infant stool colors in lowest four. Reproduced with kind permission from Pediatrics, Vol 117(4), Page 1148, Copyright 2006 by the AAP.
Table 1. Causes of neonatal cholestasis (59, 71).

<table>
<thead>
<tr>
<th>Causes of Neonatal Cholestasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile duct obstruction</td>
</tr>
<tr>
<td>Biliary atresia</td>
</tr>
<tr>
<td>Choledochal cysts</td>
</tr>
<tr>
<td>Alagille syndrome</td>
</tr>
<tr>
<td>Caroli’s disease</td>
</tr>
<tr>
<td>Inspissated bile</td>
</tr>
<tr>
<td>Cholelithiasis</td>
</tr>
<tr>
<td>Neonatal sclerosing cholangitis</td>
</tr>
<tr>
<td>Spontaneous perforation of bile ducts</td>
</tr>
<tr>
<td>Tumor/mass</td>
</tr>
<tr>
<td>Congenital hepatic fibrosis</td>
</tr>
<tr>
<td>Systemic infections</td>
</tr>
<tr>
<td>Bacterial sepsis</td>
</tr>
<tr>
<td>Urinary tract infection</td>
</tr>
<tr>
<td>Hepatitic infection</td>
</tr>
<tr>
<td>Echo-, Adeno-, Coxsackie-virus</td>
</tr>
<tr>
<td>TORCH syndrome^1</td>
</tr>
<tr>
<td>Human herpesvirus 6, Varicella-zoster, HIV, Hepatitis B</td>
</tr>
<tr>
<td>Cholestatic syndromes</td>
</tr>
<tr>
<td>Progressive familial intrahepatic cholestasis</td>
</tr>
<tr>
<td>Dubin-Johnson syndrome</td>
</tr>
<tr>
<td>Arthrogryposis, renal dysfunction, and cholestasis syndrome</td>
</tr>
<tr>
<td>Metabolic disorders</td>
</tr>
<tr>
<td>a1-antitrypsin deficiency</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>Tyrosinemia</td>
</tr>
<tr>
<td>Galactosemia</td>
</tr>
<tr>
<td>Niemann-Pick disease</td>
</tr>
<tr>
<td>Farber’s disease</td>
</tr>
<tr>
<td>Bile acid synthetic disorders</td>
</tr>
<tr>
<td>Glycogen storage diseases</td>
</tr>
<tr>
<td>Hypopituitarism</td>
</tr>
<tr>
<td>Hypothyroidism</td>
</tr>
<tr>
<td>Urea cycle disorders</td>
</tr>
<tr>
<td>Mitochondrial disorders</td>
</tr>
<tr>
<td>Chromosomal disorders</td>
</tr>
<tr>
<td>Trisomy 21, 13, 18</td>
</tr>
<tr>
<td>Turner syndrome</td>
</tr>
<tr>
<td>Toxic</td>
</tr>
<tr>
<td>Parenteral nutrition</td>
</tr>
<tr>
<td>Foetal alcohol syndrome</td>
</tr>
<tr>
<td>Vascular disorders</td>
</tr>
<tr>
<td>Budd-chiari syndrome</td>
</tr>
<tr>
<td>Extrahepatic portal vein occlusion</td>
</tr>
<tr>
<td>Congenital portocaval shunt</td>
</tr>
<tr>
<td>Haemangiomas</td>
</tr>
<tr>
<td>Congestive heart failure</td>
</tr>
<tr>
<td>Perinatal asphyxia</td>
</tr>
</tbody>
</table>

^1TORCH acronym for Toxoplasmosis, Other agents, Rubella, Cytomegalovirus, Herpes simplex.
Table 2. Liver biochemical variables among 21 biliary atresia patients at first admission in Children’s Hospital after 2005.

<table>
<thead>
<tr>
<th>Item</th>
<th>Median</th>
<th>Range</th>
<th>Interquartile range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin, μmol/L</td>
<td>168</td>
<td>51-337</td>
<td>141-208</td>
<td>4-20</td>
</tr>
<tr>
<td>Conjugated bilirubin, μmol/L</td>
<td>116</td>
<td>35-224</td>
<td>91-147</td>
<td>0-5</td>
</tr>
<tr>
<td>Aspartate transferase, U/L</td>
<td>152</td>
<td>28-370</td>
<td>83-271</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Alanine transferase, U/L</td>
<td>86</td>
<td>9-276</td>
<td>38-175</td>
<td>&lt;40</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/L</td>
<td>626</td>
<td>170-2008</td>
<td>403-927</td>
<td>115-460</td>
</tr>
<tr>
<td>γ-glutamyl tranferase, U/L</td>
<td>496</td>
<td>175-1346</td>
<td>281-831</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Prothrombin ratio, %</td>
<td>82</td>
<td>6-136</td>
<td>60-93</td>
<td>70-130</td>
</tr>
<tr>
<td>Factor V, %</td>
<td>124</td>
<td>88-196</td>
<td>111-139</td>
<td>79-128</td>
</tr>
<tr>
<td>Prealbumin, mg/L</td>
<td>98</td>
<td>57-120</td>
<td>84-110</td>
<td>95-280</td>
</tr>
</tbody>
</table>

The evaluation of an infant with conjugated hyperbilirubinemia and pale stools usually proceeds with an imaging study in order to verify the patency of the bile ducts. The problem with all the imaging methods is the lack of complete specificity (63). In BA, abdominal ultrasound typically shows enlarged liver, no biliary tree dilatation, and a small or absent gallbladder in 80% of cases. In 20%, gallbladder and common bile duct are patent and visible by ultrasound despite obstruction in the common hepatic duct, looking misleadingly normal (1, 63). A triangular cord sign (reported sensitivity around 80%) may be observed by ultrasound: the sign represents the fibrous mass at the porta hepatis at the site of the former bile duct (59, 64). In cystic BA the cyst may be visible by ultrasound but indistinguishable from a choledochal cyst (59). In cholescintigraphy, the liver uptakes the injected radioactive tracer (often technetium 99m) and normally excretes the tracer into the intestine. Cholescintigraphy is highly sensitive for BA but the specificity is low (65). Data regarding the usefulness of magnetic resonance cholangiopancreatography (MRCP) is limited (65, 66).

In a large German series of 140 cholestatic infants in whom BA was suspected, endoscopic retrograde cholangiopancreatography (ERCP) excluded BA in 34 (25%), who thus avoided an unnecessary laparotomy. ERCP failed technically in 18 (13%) and in 88 (63%) no common bile duct could be visualized; all these patients underwent laparotomy and BA was confirmed in 80 (75%) (67). In a Czech series of 104 cholestatic infants ERCP failed in 9 (9%), excluded BA in 24 (23%), and showed 86% sensitivity and 94% specificity for BA (51 cases) (68). Neither of these series reported serious post-ERCP complications.

Percutaneous liver biopsy is used by most centres in the differential diagnostic path of a cholestatic infant. The liver histological assessment has approximately 90-100% sensitivity and 80-98% specificity for biliary obstruction (65). In a study presenting 891 interpretations of 97 liver biopsies of cholestatic infants, the features indicating BA were bile plugs in bile ducts and canaliculi, portal tract edema, severe portal fibrosis, and bile duct proliferation, whereas sinusoidal fibrosis ruled against BA. Considerable interobserver variability was observed: the percentage of agreement in the different features assessed varied from 43% to 93% (69). In cases of extrahepatic cholestasis,
Alagille syndrome should be excluded with spine x-rays to rule out butterfly vertebrae, ophthalmologist consultation for posterior embryotoxon, and heart ultrasound for cardiac anomalies (70). Cardiac evaluation also reveals heart defects associated with congenital BA (52).

A diagnostic strategy should be chosen to cause minimal delay of possible operative treatment. If BA cannot be excluded after the imaging studies, operative cholangiography is performed before proceeding to portoenterostomy. Operative cholangiography, either during laparoscopy or laparotomy, is considered the gold standard for definitive BA diagnosis (1). If BA is confirmed, the abdominal cavity is carefully explored for associated structural abnormalities.

## Treatment

### Kasai portoenterostomy

The portoenterostomy (PE) operation, introduced by Morio Kasai (16), is the gold standard of BA operative treatment. Conventionally, PE is performed through laparotomy. During the recent years, reports of laparoscopically performed PE have emerged (72-74). In a prospective trial comparing conventional and laparoscopic PE, the native liver survival after laparoscopic operation was significantly reduced, and the trial was stopped (75); similar results were observed in a retrospective series (76). No difference in LT duration between patients with previous laparoscopic or open PE was observed (77).

A wide transverse upper abdominal incision is used for PE. The liver may be completely mobilized for adequate exposure. A fibrotic remnant – representing what is left of the extrahepatic bile ducts - usually connects the site of the gallbladder to the liver hilum (Figure 4A). This fibrous tissue is carefully dissected free from the underlying vessels and followed to the porta hepatis. If present, the liver bridge between segments three and five is divided for full exposure. The portal vein bifurcation is mobilized and branches to the caudate lobe divided to allow excision of the entire portal plate from behind the portal bifurcation, extending to the arterial branches (Figure 5). At the level of the liver capsule, the fibrous tissue representing the portal plate is cut parallel to the liver capsule (Figure 4B). An exit for the bile is created by anastomosing a retrocolic 40cm Roux loop of the jejunum to the edges of the porta hepatis (Figure 4C and D) (78, 79).

Possible short-term postoperative complications include temporary increase in ascites formation, cholangitis, and bile leaks (80). Intra-abdominal adhesions and incisional hernias may also develop (81). The reports on postoperative problems after PE focus on liver function deterioration and data on surgical complications is scarce.
Occasional reports describe hepatic portocholecystostomy for the rare type 2 BA (approximately 2%) with patent extrahepatic bile ducts (gallbladder, cystic duct, and common bile duct) (82). Despite reports of a reduced rate of cholangitides when compared to PE, prolonged anastomotic leaks and most importantly inferior rate of jaundice clearance have kept the popularity low. In histological observations, the seemingly patent common bile ducts showed inflammation and fibrosis, ultimately leading to narrowing and inadequate bile flow. (26, 78)

Surgical revision of the PE has been uncommon because of poor results and the potential of repeated abdominal surgery to cause technical difficulties in the subsequent LT (78). However, a recent study from Cincinnati reported 75% rate of jaundice clearance among patients who underwent PE revision. Half of the revised patients required no further surgery during the average follow-up of seven years. Among those who underwent LT after revision, the transplantation time was longer but the outcomes were similar compared with non-revised patients. The Cincinnati group concluded that PE revision may be a good option for selected patients with jaundice recurrence after an initially successful PE. (83)
Adjuvant medical therapy

Corticosteroids

Corticosteroids have been widely used after the PE operation for several years (84). Steroids were originally thought to benefit BA patients by enhancing bile flow independent of bile salts by both inducing \( \text{Na}^+\text{K}^+\text{ATPase} \) enzyme to increase transport of electrolytes in the canaliculi and by suppressing the inflammatory response (85, 86). BA patients’ intrahepatic biliary epithelium is shown to express \( \alpha \)-type glucocorticoid receptors in correlation with the severity of the liver injury but the clinical significance of the finding remains obscure (87). The evidence supporting steroid use in BA is still rather weak (88). In the only currently available randomized controlled trial the first treatment arm with 34 BA patients received a relatively low steroid dose of two and later one mg/kg/day of oral prednisolone for weeks two to four after PE and the second treatment arm with 37 BA patients received placebo. The primary outcomes were clearance of jaundice (defined as plasma total bilirubin \( <20 \mu\text{mol/L} \)) rate and native liver survival at 6 and 12 months after PE. No significant difference between the groups was observed. However, the steroid group showed a trend towards lower plasma bilirubin levels at one month after PE: 66 \( \mu\text{mol/L} \) (50-108) vs. 92 \( \mu\text{mol/L} \) (51-160), \( p=0.06 \). (89) In a prospective open-label pilot study of 49 BA patients, 20 patients received a high dose (10 mg/kg intravenously) of methylprednisolone on days 1 to 5 after PE and 1 mg/kg orally on days 6 to 28. When compared with 29 patients with no steroid treatment, no difference in serum bilirubin levels or native liver survival was evident at two years (90). In North America, a multicenter, randomized, double-blinded, placebo-controlled trial for steroid therapy after PE is currently ongoing (http://clinicaltrials.gov, NCT00294684).

Choleretics

Ursodeoxycholic acid (UDCA) is a hydrophilic bile acid that normally comprises around 3% of the human bile-acid pool. UDCA is often used following PE (80), but clinical trials are lacking and the possible benefit remains unclear (3). UDCA stimulates bile secretion, protects cholangiocytes from the cytotoxic effects of the hydrophobic bile acids, and protects hepatocytes from undergoing bile acid induced apoptosis. In other cholestatic liver diseases, especially primary biliary cirrhosis, UDCA improves liver biochemistries and may slow down disease progression (91). In one prospective study of 16 BA children, the patients first took UDCA for 18 months, and then the UDCA treatment was discontinued leading to worsening of liver function in 13 patients. After resuming UDCA treatment, liver function improved again and UDCA treatment was evaluated to be beneficial for BA patients. (92)

Phenobarbitone induces bile acid-independent bile secretion and induces hepatic \( \text{Na}^+\text{K}^+\text{ATPase} \) activity and has been used as a choleretic (93). However, evidence of its benefits is lacking. In a study where 27 BA patients were treated with phenobarbitone for
three months after PE and compared with 26 untreated patients, phenobarbitone treatment had no effect on jaundice clearance (94). In antiepileptic use, phenobarbitone treatment has associated with decreased bone mineral density: increased bone turnover and secondary hypoparathyroidism are caused by cytochrome P450 enzyme induction and enhanced vitamin D clearance (95). Because of reduced bile flow and impaired micelle formation, BA patients are especially prone to vitamin D deficiency.

**Nutritional therapy**

BA patients are at risk of nutritional deficiencies. Children with BA have higher resting energy expenditure than healthy peers (96). Even after successful bile drainage the bile acid concentration in the bowel contents remains below normal leading to fat malabsorption and decreased energy intake. The low bile acid content in the bowel also impairs the micelle formation necessary for absorption of fat-soluble vitamins A, D, E, and K, leading to low serum vitamin concentrations. (96, 97) The evidence for nutritional therapy recommendations is relatively weak. A nutritionist should counsel all parents of a BA infant. The nutritional therapy of a BA infant should include up to 50% higher energy intake than age-recommended and a supplementary carbohydrate product of glucose polymers may be used. Supplementation of fat-soluble vitamins is necessary and the lipid content of the diet may be increased by using a medium and short chain triglyceride formula. (96, 98) Lipids are needed during growth as important constituents of cell membranes. Total fat intake is favorably increased with medium chain triglycerides, which are transported in the portal venous flow with no need to form micelles in the intestine (99). Especially when PE fails or cirrhosis develops despite successful PE, these suppletations should be continued and growth and diet assessed regularly. For the patients with only mild liver fibrosis after a successful PE, serum levels of fat-soluble vitamins should be measured regularly and suppletations used if needed, combined with a wholesome diet (97).

**Complications**

**Portal hypertension and esophageal varices**

Increasing liver fibrosis raises the portal venous pressure and may lead to formation of esophageal varices. Most complications of cirrhosis, like variceal hemorrhage and ascites, are associated with portal hypertension (100). In a randomized controlled trial of 213 cirrhotic adults, patients with hepatic venous pressure gradient exceeding 10 mmHg had a significantly higher incidence of varices than patients with a gradient below 10 mmHg (101). In children, measurement of the hepatic venous pressure gradient is highly arduous. Two studies of BA patients report conflicting results on the predictive value of actual
portal venous pressure measured by a catheter placed in the recanalized umbilical vein at the PE operation: Shalaby and co-workers observed no association between portal venous pressure and clearance of jaundice (102) whereas Duché and co-workers report that patients with higher portal venous pressure have a lower chance of native liver survival and a higher risk for varices and variceal bleeding (103).

In upper gastrointestinal endoscopy, esophageal varices may be graded according to the Baveno consensus statement as small (grade 1), medium (grade 2), or large (grade 3) (104). In a prospective study of 139 BA children, 28 (20%) presented with gastrointestinal bleeding at the median age of 17 months (range from 4 months to 5 years and 10 months). The presence of ascites, serum bilirubin concentration >20 μmol/L, prothrombin ratio <80%, and portal vein diameter >5 mm were significant risk factors for bleeding. In total, 125 patients presented with gastrointestinal bleeding, splenomegaly, or thickening of the lesser omentum in abdominal ultrasound and underwent upper gastrointestinal endoscopy. Of the 88 (70%) whom suffered esophageal varices; 74 were under two-years of age. In 59 of the 88 (67%), the varices were small. Esophageal varices showed no association with the serum bilirubin concentration but esophageal red markings and portal hypertensive gastropathy were more common among patients with a serum bilirubin concentration exceeding 100 μmol/L. (105) Among adult native liver survivors, the reported incidence of portal hypertension varies from 50% to 96% (106-108).

Adult cirrhotic patients with medium or large varices are recommended primary prophylaxis of variceal bleeding using either non-selective β-blockers or endoscopic band ligation (100). In children, evidence-based recommendations for screening and treatment of esophageal varices are scarce (100, 109). Adult patients with large varices, red markings on the varices, or poor liver function are at the highest risk of bleeding (100) Duché and co-workers observed similar findings among BA patients (105). Both endoscopic injection sclerotherapy and variceal ligation (for children ≥3 years) have been used successfully among BA patients as primary prophylaxis for preventing variceal bleeding (105, 110-114). Sclerotherapy is also reported as favorable secondary prophylactic treatment (111, 115, 116); the reported complications of sclerotherapy include esophageal ulceration and strictures (110, 111).

Non-invasive methods for predicting the presence of varices are under research. In two studies with 49 and 31 BA patients aged from five months to 25 years, liver stiffness measured by transient elastography before upper gastrointestinal endoscopy predicted esophageal varices with 87-97% sensitivity and 80-88% specificity (117, 118). Gana and coworkers presented 108 children with either chronic liver disease or portal vein thrombosis (30 BA patients) who underwent an upper gastrointestinal endoscopy, coupled with blood tests within three weeks and an abdominal ultrasound within four months of the endoscopy. Platelet count to age-adjusted spleen length ratio was the best predictor of esophageal varices with an AUROC of 0.84 (95% CI 0.75-0.93), followed by a clinical prediction rule consisting of platelet count, age-adjusted spleen length, and albumin (AUROC 0.80; 95% CI 0.70-0.91), and platelet count alone (AUROC 0.79; 95% CI 0.69-0.90). (119)
Cholangitis

Cholangitis is a frequent event after a successful PE for BA and it is most frequent in the first postoperative year (1). Cholangitis is treated with intravenous antibiotics, however there is no specific diagnostic test for cholangitis nor is the mechanism of development completely clear. In addition to the classical triad of fever, jaundice, and abdominal pain (120), cholangitis symptoms may include acholic stools and deterioration in liver function tests. The reported incidence of cholangitis among BA children varies from 40% to 59% (35, 121, 122) and among adult native liver survivors from 30% to 50% (106-108). In some studies, cholangitis was more common among patients with poor bile flow (121, 122). Some centres prescribe prophylactic antibiotics to BA patients (1) although the evidence base is weak: in two studies with 37 patients each, the patients with no prophylactic antibiotics suffered more cholangitides (123, 124). To prevent cholangitis episodes, surgeons have also tried two modifications of the PE operation: an intestinal valve added to the Roux limb of the jejunum, or a temporary external biliary stoma. In a large study with 735 patients from the Japanese BA registry, patients with these modifications or the original PE operation all had similar cholangitis incidence, approximately 40% (35). BA patients may develop bile lakes (intrahepatic collections of stagnant bile), which may be emptied either by percutaneous transhepatic drainage or, if located close to the PE site, by operative drainage to the jejunal limb attached to the liver hilum (125). Two studies of bile lakes or intrahepatic biliary cysts (incidence 16-22%) report that a single bile lake is an insignificant prognostic factor but multiple complicated lakes are associated with more cholangitis episodes and more importantly, a poorer long term prognosis (126, 127). Houben and co-workers described three BA patients, age eight to 17 years, with an onset of cholangitis episodes after several trouble-free years (128). In these three patients, bile had accumulated into the Roux limb of the jejunum due to obstruction of the limb in the mesocolic window and treated by a reoperation.

Other complications

Itch (pruritus) is a frequent and disabling complication of cholestatic liver diseases (129). In a multicenter study with 22 adult BA native liver survivors, 8 patients (40%) reported frequent itching and 5 (23%) itched during cholangitis episodes (107). The mediators of itch are not completely understood but it is hypothesized that increased central opioidergic tone plays a key role. Cholestasis causes pruritogens to accumulate in blood, enter the brain, and change neurotransmission, leading to scratching behavior; pruritogens also stimulate itch receptors in the skin’s neural fibers (129). The severity of itch does not, however, correlate with serum bile acid levels (130, 131). In a study of 13 itching BA children, 12 had a complete or partial response to rifampin therapy (132). Naltrexone, which restrains the overactive endogenous opioid system, seems useful for resistant cholestatic itch but has been mainly studied in adults (129) whereas experience among BA children is restricted to case reports (133).
Hyperbilirubinemia during infancy may cause green staining of the permanent teeth (134). In a study of 31 BA patients undergoing LT at age 0-6 years, 19 (61%) had varying degrees of green staining of the teeth and gingivae (135).

Chronic liver failure is associated with a metabolic bone disease named hepatic osteodystrophy, which in children includes decreased bone mass, fractures, rickets, spine deformities, and impaired growth (136). Data on the frequency and severity of these complications among BA patients is very limited. In one study, 16 non-jaundiced BA patients of a mean age of 7.3 years had significantly lower bone mineral content when compared to controls (137).

In general, literature on skills development and health-related quality of life among BA patients is scant. BA infants with failed PE awaiting LT have decreased scores for mental and motor development in comparison to healthy peers, and the scores seem to drop even further at transplantation. The delay in development correlates with the severity of the liver disease. The developmental scores return to pre-LT levels in approximately one year after LT and after this, developmental catch-up takes place (98, 138-140). In severely liver diseased children in general, developmental delays are worse if the disease starts in the first year of life like BA (98), and global health-related quality of life improves after LT (141). Kosola and coworkers studied 57 Finnish pediatric LT recipients at 10.7 ± 6.6 years posttransplant (32% had BA). One third of the school-aged children was one grade behind (controls: 5%), and a half of these children scored low in at least one domain of health-related quality of life, whereas the other half had all domains in the normal range of controls. Adults scored lower than controls only in physical functioning and general health: LT recipients believed their health would get worse. Still three quarters of the LT recipients thought they were as, or almost as, healthy as other people and two thirds thought their health was excellent. (142)

Carcinoma

Hepatocellular carcinoma is a well-known complication of chronic liver disease and liver cirrhosis (143). Five hepatocellular carcinomas occurred among 387 BA patients from England; two were observed during follow-up and treated with LT, and three were observed in explanted livers after LT (144). A case report describes very early occurrence of carcinoma in a BA patient at ten months of age (145). BA associated hepatocellular carcinoma is rare, but will probably become a more important problem in the near future now that a larger proportion of BA patients survive longer with their native liver. Early detection of BA associated hepatocellular carcinoma is difficult: only some produce α-fetoprotein (two out of three in an Italian report and two out of five in the British report) and small carcinomas are non-detectable by abdominal ultrasound (144, 146). The writers of the UK report recommend that all BA patients have a serum α-fetoprotein measurement and liver ultrasound every six months (144). BA patients often develop benign macroregenerative nodules in their livers: in a Chinese study of 144 BA patients undergoing LT, the incidence of these nodules was 5% (147). Attempts to distinguish these nodules from
carcinomas may be made by contrast-enhanced computed tomography or magnetic resonance imaging and tumor biopsy (143).

Liver transplantation

LT as a treatment option has completely changed the life prospects of BA patients. Approximately 80% of BA patients require a liver transplant by adulthood and infants whose PE operation fails usually before two years of age (1, 148). If the PE is successful, LT timing is more difficult: the risk of death with no LT should exceed the risks of transplantation, but if the patient is too sick when listed for LT, the risk of waiting list mortality grows. Indications for LT include recurrent jaundice, synthetic liver failure (low cholesterol, serum albumin, clotting factors), repeated cholangitis or variceal bleeding episodes, intractable ascites, intolerable itch, and failure to thrive (148, 149). In the US, since 2002 donor livers are allocated based on the model for end-stage liver disease (MELD) score (150) for >12-year olds and pediatric end-stage liver disease (PELD) score (149) for <12-year olds. High MELD and PELD scores at listing are associated with increased waiting list mortality (151, 152). In a study of 1247 pediatric LT recipients, only those with high PELD scores (>17) demonstrated survival benefit within the first post-LT year (153).

In a United Network for Organ Sharing, a US series of 1976 patients who received LT for BA during the years 1988-2003, 73% were under two-year-olds (154). The small size of most recipients leads to the problem of the deceased adult donor liver graft being too large (>4% of body weight) and thus 25-60% received either a split liver or a reduced size graft (8, 154, 155). In BASM patients, the LT operation may be technically demanding due to the associated anomalies (Figure 2), especially regarding absent inferior vena cava and aberrant hepatic artery (156, 157). Infants and patients with a severe growth failure are at a greater risk of dying both while waiting for the transplant and also after LT (8, 154, 155). After LT for BA, the reported actuarial one, five, and ten year patient survival rates are 85-95%, 82-90%, and 81-88% (8, 154, 155, 158).

The shortage of deceased donor organs has driven the development of the living-donor liver transplantation concept especially in the United States: in the years 1988-2003, 17% of BA LT recipients received a live donor partial liver transplant (154). However, in the US, a decrease is seen from 120 live donor pediatric LTs in the peak year 2000 to 51 in 2009, possibly reflecting the risks of donor morbidity and mortality (159). In 2010, a living donor was used in 4% of all LTs in the US (159), 7% in the Eurotransplant area (160), and 3% (8 LTs in Sweden) in Scandinavia (161). In Finland, all liver grafts have thus far been from deceased donors.

Recently, a study with 611 recipients of living related parental liver grafts reported significantly decreased risk of graft failure among BA patients if the graft was from the mother (162). Increased amounts of maternal cells (maternal microchimerism) have been found in the livers of BA children (163). The maternal cells are hypothesized to be tolerogenic, thus leading to better outcomes when receiving a maternal liver graft (162).
Outcomes

The treatment results can be measured from different viewpoints. Age at PE operation represents the health care system’s efficiency in diagnosing BA patients from the large pool of jaundiced infants. Clearance of jaundice rate is mostly related to the quality of the surgical treatment. The postoperative care, follow-up, and treatment of disease complications are all reflected in the native liver survival rate. In addition to these factors, overall survival is affected by mortality related to associated anomalies, LT program accessibility, and LT related factors including waiting list and post-LT mortality.

Factors associated with portoenterostomy success

Young age at PE (<50-70 days) has been associated with longer native liver survival (2, 31-34, 56, 164-167) but age has had no effect in some studies (168, 169). In one study, only BASM patients had better native liver survival if younger at PE (170). In many centres, patients presenting with delayed diagnosis (>100-120 days) are transplanted with no PE attempt as the PE is considered hopeless (32-34). Patients operated on in England at ≥100 days, however, had five and ten year native liver survival rates of 45% and 40% (171). It seems reasonable to also attempt PE among infants with a diagnostic delay, especially since previous PE operation has no reported effect on the post-transplant survival (155, 158). Age at PE has remained unchanged (median at 50-60 days) over the past decades in many centres (34, 164, 172), but Taiwan has reported younger median age at PE after the initiation of a stool color card screening program (56, 173).

Reports of the effect of centre caseload are conflicting: in England and Wales the results of BA treatment improved significantly after treatment centralization into three high volume centres (168, 169) but in reports from France, Canada, and Switzerland, centre caseload had no effect (31, 34, 167). In some studies, advanced liver fibrosis at PE has been associated with inferior native liver survival (174-178) but this finding was not confirmed in a recent study with 244 BA patients from the US (2). Extensive bile ductular proliferation in the liver biopsy taken at PE has also been associated with poor prognosis (179, 180). The prognosis of BASM patients is also conflicting: in some reports BASM patients have inferior native liver survival compared with isolated BA patients (2, 34, 51, 181, 182), but in other reports BASM and isolated BA patients’ prognosis is similar (31, 32, 52, 168, 169). BASM patients’ native liver survival may be reduced by morbidity and mortality associated with other structural abnormalities, for example severe cardiac anomalies.

Native liver and overall survival

The reported jaundice clearance rates (36-57%), four year native liver survival rates (37-60%), and overall survival rates (73-92%) from the largest available series are summarized in Table 3. In most reports, a plateau of native liver and overall survival can
be seen after two to three years of age. Approximately 70-80% of the patients alive with a native liver at two years of age are still alive with the native liver at twenty years (183). The reported proportion of patients surviving until adulthood with their native liver is approximately 25% with the largest series consisting of 63 patients (106, 108, 184).

Table 3. BA outcome measures in reports from Europe, North America, and Japan.

<table>
<thead>
<tr>
<th>Era</th>
<th>Area</th>
<th>Number of patients</th>
<th>Clearance of jaundice, %</th>
<th>4-year native liver survival, %</th>
<th>4-year overall survival, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989-1999</td>
<td>Japan (35)</td>
<td>1381</td>
<td>57</td>
<td>60¹</td>
<td>75¹</td>
</tr>
<tr>
<td>1999-2002</td>
<td>Canada (167)</td>
<td>230</td>
<td>nr</td>
<td>~40</td>
<td>83</td>
</tr>
<tr>
<td>1999-2009</td>
<td>England and Wales (6)</td>
<td>443</td>
<td>55</td>
<td>46¹</td>
<td>90¹</td>
</tr>
<tr>
<td>1986-2002</td>
<td>France (34)</td>
<td>743</td>
<td>36</td>
<td>41</td>
<td>78</td>
</tr>
<tr>
<td>1987-2008</td>
<td>The Netherlands (32)</td>
<td>214</td>
<td>36</td>
<td>46</td>
<td>73</td>
</tr>
<tr>
<td>2004-2010</td>
<td>US (2)</td>
<td>136</td>
<td>46</td>
<td>47²</td>
<td>nr</td>
</tr>
<tr>
<td>1994-2004</td>
<td>Switzerland (31)</td>
<td>48</td>
<td>40</td>
<td>37</td>
<td>92</td>
</tr>
</tbody>
</table>

¹ Five-year; ² Two-year; nr = not reported

Health and quality of life among adult native liver survivors

The largest series of adult native liver survivors comprises 63 patients (106-108, 183-186). Most (64-97%) adult BA native liver survivors have cirrhosis. Sixty to 90% either have a job or are students. In most series, individual patients have severe developmental delays due to an intracranial hemorrhage in infancy. All series include a few women who have successfully delivered healthy babies, who are mostly somewhat small for their gestational age; temporary worsening of portal hypertension during pregnancy is often described. Quality of life has been studied in two reports. In the Netherlands, female BA adults had lower general health perception compared to peers but males consider their health equal to peers (106). BA adults in Japan and England report slightly lowered quality of life compared to the normal population (183). Only one study has compared the course of life between 25 adult BA native liver survivors and 15 LT recipients. The psychosexual development was delayed in the LT recipient group whereas the native liver survivors reported more gambling and substance abuse. (187)
AIMS OF THE STUDY

An infant struck by BA faces considerable morbidity and a risk of dying. Despite the relief PE offers, most need LT before adulthood. Although a rare disease, BA remains the most common indication for LT in childhood. The incidence of BA in Finland was previously unknown, as were the outcomes of BA patients. BA treatment was centralized to Helsinki University in 2005 with a decree from the Ministry of Social Affairs and Health. The general aim underlying this study is a will to improve the outlook for infants suffering from BA.

The specific aims were:

1. To study the epidemiology of BA in Finland

2. To investigate the effects of the 2005 centralization of BA treatment outcomes

3. To study the natural history of esophageal varices in BA and effects of the current screening and prophylaxis protocol on associated bleeding

4. To describe longitudinal changes in liver histology after successful PE and study the associations of liver histology with clinical outcome variables, such as portal hypertension, cholangitis, and liver function

5. To evaluate non-invasive markers of disease progression for the follow-up of BA patients and timing of LT
PATIENTS AND METHODS

Patients and study design

The study cohort comprises all Finnish BA patients born between 1987 and 2010. To identify all BA patients, the Children’s Hospital database was searched for all patients with any liver or biliary tract disorder during those years. In addition to this data search the National Institute for Health and Welfare’s Register of Congenital Malformations was similarly searched for cases born in Finland between 1987 and 2008. After treatment centralization in 2005, all BA patients have been prospectively entered into the hospital BA database. Diagnosis of BA was based on operative cholangiography, operative findings, and liver histology, and in few cases, autopsy reports. The design of studies I-IV is outlined in Table 4.

Table 4. Study design outline. LT = liver transplantation, PE = portoenterostomy.

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main issue</td>
<td>Incidence, survival, effects of centralization</td>
<td>Esophageal varices and associated bleeding</td>
<td>Liver histology after successful PE</td>
<td>Gal½ measurement as a follow-up tool</td>
</tr>
<tr>
<td>Study design</td>
<td>Prospective / retrospective, observational</td>
<td>Retrospective, observational</td>
<td>Cross-sectional</td>
<td>Retrospective, observational</td>
</tr>
<tr>
<td>Number of BA patients</td>
<td>72</td>
<td>47</td>
<td>44</td>
<td>36</td>
</tr>
<tr>
<td>Inclusion criteria</td>
<td>Born in Finland</td>
<td>Born 1987-2008; underwent upper gastrointestinal endoscopy in Children’s Hospital</td>
<td>Native liver survival of minimum 1.5 years or LT</td>
<td>Gal½ measurement; minimum 1 year of follow-up after Gal½ if no LT or death</td>
</tr>
<tr>
<td>Controls</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10 pediatric donor livers</td>
</tr>
<tr>
<td>Other patients</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>56 with other chronic liver disease and Gal½ measurement</td>
</tr>
<tr>
<td>Follow-up endpoints</td>
<td>Death or December 2010</td>
<td>LT, death, or December 2009</td>
<td>LT, death, or April 2012</td>
<td>Last follow-up visit, LT, death, or December 2011</td>
</tr>
</tbody>
</table>
Follow-up data collection

Hospital records were reviewed for complete follow-up data, no patient was lost to follow-up. The recorded demographic data included antenatally observed abnormalities, gestational age, birth weight, birth place, date of birth, number of preceding siblings, and maternal age. Preoperative (blood count and liver biochemistry, imaging studies for diagnosis and associated anomalies, clinical observations) and operative findings (cholangiography, BA type, operation type, associated anomalies) as well as histology reports were recorded. BASM was defined as BA associated with poly- or asplenia, or intestinal malrotation, or both (51). Postoperative clearance of jaundice was defined as serum bilirubin concentration less than 20 μmol/L. Serum total and conjugated bilirubin concentrations were recorded at 3, 6, 24, and 60 months as well as the time to the first recorded normal (<20 μmol/L) serum bilirubin concentration. Cholangitis episodes, reoperations, ultrasonic signs of portal hypertension (repeated abdominal ultrasounds for spleen size and ascites), upper gastrointestinal endoscopy findings and sclerotherapy details, blood count, liver biochemistry, and growth were recorded. Postoperative use of corticosteroids as well as other medications used during follow-up was recorded. Data regarding LT included date and reason for listing, and date of LT. For deceased patients, date and reason of death, and autopsy findings were recorded. The follow-up ended at death or the end of the whole study on 31st May 2012.

Histology and immunohistology

Pathology reports of liver biopsies and biliary tract remnants obtained at PE were reviewed for diagnosis confirmation in study I. In study III, the native liver biopsies obtained at PE, LT, or at follow-up were prospectively assessed and scored according to a recently validated scoring system for BA differential diagnostics, including several features of cholestasis, fibrosis, inflammation, and reactive changes (69). In addition, fibrosis was assessed according to Metavir (188) (Table 5) and Ishak (189) scoring systems. The distribution of portal area inflammatory cells was quantified as percentages of lymphocytes, neutrophils, macrophages, eosinophils, and plasma cells. Slides stained with cytokeratin 7 (CK7) were scored for immunopositivity of periportal hepatocytes and for proliferating bile ducts. The histological features assessed are described in more detail in study III. For CK7 immunostaining, SP52 monoclonal antibody and ultraView Universal DAB Detection Kit (Ventana, Tucson, Arizona, USA) were used.

<table>
<thead>
<tr>
<th>Table 5. The Metavir liver fibrosis scoring system</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>
Liver function test and scores

Blood count, plasma concentrations of total and conjugated bilirubin, alanine transferase, aspartate transferase (AST), alkaline phosphatase, \( \gamma \)-glutamyl transferase, prealbumin, albumin, factor V, and bile acids, and prothrombin ratio were measured using standard hospital laboratory methods and age-appropriate reference ranges were used. Galactose half-life was measured by Tengström’s method (190), described in more detail in study IV. For studies III and IV, AST to platelet ratio index (APRI) was calculated according to Wai and coworkers (191):

\[
APRI = \frac{AST \text{ level} + 50}{\text{Platelet count}} \times 100.
\]

For study IV, the pediatric end-stage liver disease (PELD) scores were calculated for children under twelve years and model for end-stage liver disease (MELD) scores for ages twelve and older using previously described formulae (150, 152).

\[
\begin{align*}
\text{PELD score} &= 0.480 \times Ln(\text{bilirubin mg/dL}) \\
&\quad + 1.857 \times Ln(\text{INR}) \\
&\quad - 0.687 \times Ln(\text{albumin g/dL}) \\
&\quad + 0.436 \ (\text{if age} < 1 \text{ year}) \\
&\quad + 0.667 \ (\text{if growth failure} < -2 \text{ standard deviations}) \\
&\quad \times 10
\end{align*}
\]

\[
\begin{align*}
\text{MELD score} &= 0.957 \times Ln(\text{creatinine mg/dL}) \\
&\quad + 0.378 \times Ln(\text{bilirubin mg/dL}) \\
&\quad + 1.120 \times Ln(\text{INR}) \\
&\quad + 0.643 \\
&\quad \times 10
\end{align*}
\]
**Ethics**

The Ethics Committee for Pediatrics, Adolescent Medicine and Psychiatry in the Hospital District of Helsinki and Uusimaa approved the study (protocol number 345/13/03/03/03/2008). The epidemiological study obtained a research permit from the Ministry of Social Affairs and Health (STM/3685/2009). All live patients, controls, and parents of minors signed an informed consent form after reading and thoroughly discussing the purpose and methods of the study.

**Statistics**

Summary statistics are reported as median and range (or interquartile range in study IV). Statistical tests with nonparametric assumptions were used because most of the data showed non-normal distribution when tested by Shapiro-Wilkins W-test. Categorical variables were compared with Chi square or Fisher’s exact test, unpaired samples with Mann-Whitney U-test, and paired samples with Wilcoxon signed ranks test. Actuarial survival figures with 95% confidence intervals were calculated by the Kaplan-Meier method. Risk factors in studies I and II were assessed by binary logistic regression analyses. The discriminatory potentials of the follow-up tools in studies III and IV were analyzed with area under the receiver operating characteristic (AUROC) curves and sensitivities, specificities, and positive and negative predictive values were calculated for optimal cut-offs. In studies III and IV with multiple comparisons, the Bonferroni corrected level of statistical significance was used in order to avoid the effects of mass significance. SPSS Statistics (IBM, Somers, N.Y., USA) was used for analyses.
BA treatment protocol

Since treatment centralization in 2005, one multidisciplinary team has treated all BA patients to promote care uniformity. The team consists of three pediatric surgeons, of whom two are present in each PE operation, transplant pediatricians, pediatric gastroenterologists, pathologists, and nutritionists. The suspected BA patients are prioritized to all necessary imaging studies and operation in order to minimize in-hospital delay to PE. Main diagnostic modalities included scintigraphy, liver biopsy, and operative cholangiography, and more recently a selective use of ERCP. PE was performed through laparotomy as described previously (79). The adjuvant medical therapy regime (192) is presented in Table 6. A high-energy diet (minimum 150 kcal/kg/day) was recommended, including supplementations with medium chain fatty acids and a glucose polymer carbohydrate product. The BA patients are followed every three months. The follow-up visit includes clinical examination, laboratory tests (total and conjugated bilirubin, liver biochemistries, clotting factors, vitamin A, D, E, and K concentrations, blood count, α-fetoprotein), abdominal ultrasound, and magnetic resonance imaging of the liver if bile lakes or nodular lesions were suspected in the ultrasound examination. Yearly galactose challenge and upper gastrointestinal endoscopy surveillance for varices were performed, more recently accompanied with elastography.

Table 6. Children’s Hospital adjuvant medical treatment protocol for biliary atresia patients after portoenterostomy (August 2012).

<table>
<thead>
<tr>
<th>Medication</th>
<th>Duration</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone</td>
<td>Postoperative days 5-20</td>
<td>0.6 mg/kg/day days 5-10, 0.4 mg/kg/day days 11-15, 0.2 mg/kg/day days 16-20</td>
</tr>
<tr>
<td>Phenobarbitone</td>
<td>Start with oral feedings, stop at discharge</td>
<td>5 mg/kg/day</td>
</tr>
<tr>
<td>Ursodeoxycholic acid</td>
<td>Start with oral feedings</td>
<td>30 mg/kg/day</td>
</tr>
<tr>
<td>Thrimetoprim-sulfadiazine</td>
<td>Start with oral feedings</td>
<td>4 + 12.5 mg/kg/day</td>
</tr>
<tr>
<td>Vitamins A, D, E, and K</td>
<td>Start with oral feedings, adjust dosage according to serum concentrations</td>
<td></td>
</tr>
</tbody>
</table>
RESULTS

Epidemiology

BA was diagnosed in 73 children (43 females, 59%) born in Finland between 1987 and 2010. After the closure of the data collection for study I, which comprises 72 patients, 1 additional patient was diagnosed with BA. BA incidence was 1:19900 live births and a median of 3 (0-6) BA patients were born annually. The incidence remained unchanged during the study period. The hospital BA database was cross-checked with the Register of Congenital Malformations data for the years 1987-2008 to validate the comprehensiveness of national centralization. The Register of Congenital Malformations data revealed two cases of trisomy 18 associated absence of bile ducts (excluded) but no BA cases were observed among stillbirths or fetuses from selective terminations of pregnancy. Fifty-two (71%) patients had isolated BA, ten (14%) were classified as BASM, and eleven (15%) had other structural abnormalities. The anomaly combination of each patient is depicted in Table 7. Altogether 17 patients (23%) had structural abnormalities associated with disordered laterality (cardiac malformations, intestinal malrotation, missing inferior vena cava, polysplenia, asplenia, pancreatic anomalies, abnormal situs).

Table 7. Associated anomalies observed among 21 biliary atresia patients. Each row represents the findings of one patient. Shading in the first column indicates patient classified as having biliary atresia splenic malformation (BASM).

<table>
<thead>
<tr>
<th>BASM</th>
<th>Heart defect</th>
<th>Mal- or non-rotation</th>
<th>Missing inferior vena cava</th>
<th>Poly- or asplenia</th>
<th>Annular pancreas</th>
<th>Situs inversus</th>
<th>Other structural abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Duodenal atresia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Jejunal atresia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sacral hypoplasia, patent urachus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Butterfly vertebra, imperforate anus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Aplasia of the thymus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vestibular anus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cleft palate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Megaureter</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Eventration of the diaphragm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Developmental hip dysplasia</td>
</tr>
</tbody>
</table>
Seasonality and geographic distribution

More BA patients were born during autumn and winter (September-February) than in spring and summer (March-August) when compared to overall distribution of live births, p=0.013, Figure 6. To assess geographic distribution of BA patients in Finland, the BA patients’ birth places were marked on a population density map. No visual clustering of BA cases could be observed.

**Figure 6.** All live births per month compared to live births with biliary atresia per month between 1987 and 2010 in Finland.
Outcomes

The outcome of the entire cohort of 73 BA patients (51 born before treatment centralization in 2005 and 22 after) is outlined in Figure 7. Of BA patients born before 1987 in Finland, none is known to be alive with native liver, six received LT at median age of 10 (range from 5 to 15) years, and four are alive at the median age of 29 years (range 26-33 years).

Figure 7. Outcome of 73 biliary atresia patients born in Finland before and after treatment centralization in 2005. Of 31 liver transplantations (LT) 26 were after a failed portoenterostomy and five after a successful portoenterostomy.
Portoenterostomy success and native liver survival

Sixty-five out of 73 patients underwent an operative attempt to relieve their jaundice at the median age of 64 days (range 7-159 days). Of eight patients who did not undergo operative treatment, six had congenital BA, \( p=0.006 \). A detailed record of BA type was available for 53 patients: 1 (2%) type 1, 7 (13%) type 2, and 45 (85%) type 3. Of 65 operations, 59 were portoenterostomies and 6 variable type operations performed before 2005 (2 colecystoportostomies, 1 colecystoenterostomy, 1 omento-porto-duodenopexy, and 2 explorations). Treatment centralization had no effect on age at PE; the median age before the year 2005 was 65 days (range 15-159 days) vs. 59 days (range 7-140 days) after centralization, \( p=0.316 \). Respectively, the proportion of late PEs (≥100 days) was 20% vs. 10%, \( p=0.480 \). After centralization, the median time from the first admission to the Children’s Hospital to PE was 5 days (range from 1 to 43 days, interquartile range 1 to 8 days).

In total, 27 (42%) patients cleared their jaundice (serum bilirubin below 20 μmol/L) by a median of 3 (range 1 to 33) months after surgery. The clearance of jaundice rate significantly improved after treatment centralization from 27% before the year 2005 to 71% after centralization, \( p=0.001 \).

At four years, 22% of BA patients treated before 2005 were living with their native liver. Among those treated after centralization, the actuarial four-year native liver survival of 73% (95% CI 54-91%) was significantly (\( p<0.001 \)) higher, presented in Figure 8. Among the patients undergoing a drainage operation, native liver survival was similar in congenital and isolated BA, presented in Figure 9.

Before treatment centralization, 25 out of 44 patients were operated on in the Children’s Hospital in Helsinki. After centralization, the treatment results also improved significantly in the Children’s Hospital: clearance-of-jaundice rate rose from 36% to 71% (\( p=0.021 \)) and the actuarial four-year native liver survival from 36% to 73% (\( p=0.021 \)). The age at PE, however, showed no significant change among patients treated in the Children’s Hospital: before centralization the median age was 72 (29-159) days and after 59 (7-140), \( p=0.094 \).
Figure 8. Actuarial native liver survival of 51 biliary atresia patients born 1987-2005 and 22 born 2005-2010.

Figure 9. Actuarial native liver survival of 50 isolated and 15 congenital biliary atresia patients who underwent a surgical bile drainage operation.
Liver transplantations for BA

Deceased donor LT was received by 31 (41%) patients at the median age of 1.1 years (range 0.4-14.0 years) and at the end of the study 21 patients (68%) were alive. The median age of LT survivors was 12.3 years (range 1.6-24.7 years) old. The BA patients’ post-LT survival (68%) was similar to that of other pediatric LT recipients: of 78 pediatric patients receiving LT during 1987-2010 for reasons other than BA, 56 (72%) were alive at the end of the study (p=0.816). Between 1987 and 2010 32% of pediatric LTs in Finland were performed on BA patients.

Between 1987 and 2004, 18 BA patients received LT and the mortality was 44% (n=8). All deaths were LT related and all but one of the deaths occurred within seven months of the first LT. One patient suffered from chronic rejection due to non-adherence at puberty and died at retransplantation of liver and a kidney. At the end of the study, ten (56%) patients who received LT between 1987 and 2004 were alive at a median of 17 years (range 10.5-23.1 years) after LT.

In 2005 or after, 13 BA patients received LT of whom 2 died (mortality 15%). One patient died of graft dysfunction and viral infection at five months after LT and one patient died of a sudden midgut volvulus at three months after LT. At the end of the study, 11 (85%) were alive at a median of 5.3 years (range 0.2-7.1 years) after LT. Post-LT survival in the eras before and after 2005 is presented in Figure 10.

Figure 10. Survival after liver transplantation among 18 biliary atresia patients transplanted between 1987 and 2004 and 13 transplanted in 2005 or after.
Deaths and overall survival

After treatment centralization, overall survival improved significantly (p=0.007). At four years, 50% of patients born before 2005 were alive. For those born after treatment centralization in 2005, the actuarial four-year survival was 86% (95% CI 74-98%) (Figure 11).

Before centralization, seven (14%) patients died at the median age of 3 months (range 0-10 months) without undergoing any operative treatment for BA: three of a complex cardiac anomaly and four of liver failure. Ten (20%) patients died at the median age of 8 months (range 5-13 months) after undergoing a failed PE but no LT. Seven out of ten patients died of liver failure without being listed for LT based on being considered inoperable in the first years of the pediatric LT program and one was not listed because of multiplicity of other anomalies and health problems. The last two were listed for LT but died without receiving a transplant at two and four months after being listed. Before 2005, 26 patients received LT and 9 (35%) died. At the end of the study 25 (49%) of the patients born before treatment centralization are alive, 8 with native liver and 17 with LT (Figure 7).

After treatment centralization 3 (14%) out of 22 patients had died: 1 of a severe cardiac anomaly during a PE attempt, 1 of liver failure after being evaluated for LT but declined due to severe deficits in social network support, and 1 of a midgut volvulus three months after LT. At the end of the study 19 (86%) patients born 2005-2010 were alive, 15 with native liver and 4 after LT.

![Figure 11. Overall survival of 73 biliary atresia patients born 1987-2005 and 2005-2010.](image)
Liver function and general health among native liver survivors

At the end of the study, 23 BA patients were living with their native liver at the median age of 5 years (range 1.9-21.3 years). At the last follow-up visit, all patients had stable liver function and 18 (78%) had serum bilirubin below 20 μmol/L, Table 8. Growth was within normal limits in all. Two had recently suffered recurrent cholangitides and four used antipruritic agents (4 hydroxizine, 3 rifampin, 1 phenobarbitone). Two had difficulties eating and two had behavioral problems. Despite the use of vitamin D supplementation in all but two patients, serum 25-OH vitamin D level was below the target of 80 nmol/L in 12 (52%) patients.

Table 8. Liver biochemistry, signs of portal hypertension, and medications among 23 native liver survivors.

<table>
<thead>
<tr>
<th>Item</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin, μmol/L</td>
<td>12 (3-76)</td>
<td>4-20</td>
</tr>
<tr>
<td>Conjugated bilirubin, μmol/L</td>
<td>5 (1-50)</td>
<td>0-5</td>
</tr>
<tr>
<td>Aspartate transferase, U/L</td>
<td>59 (25-160)</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Platelet count, E9/L</td>
<td>172 (33-360)</td>
<td>200-450</td>
</tr>
<tr>
<td>25-OH vitamin D, nmol/L</td>
<td>79 (40-121)</td>
<td>target ≥80</td>
</tr>
<tr>
<td>Galactose half-life, minutes</td>
<td>11.0 (7.5-22.0)</td>
<td></td>
</tr>
<tr>
<td>APRI</td>
<td>1.1 (0.2-4.6)</td>
<td></td>
</tr>
<tr>
<td>Enlarged spleen</td>
<td>13 (57%)</td>
<td></td>
</tr>
<tr>
<td>Esophageal varices</td>
<td>11 (48%)</td>
<td></td>
</tr>
<tr>
<td>Height, SD</td>
<td>-0.2 (-1.7-1.8)</td>
<td>-2 – 2</td>
</tr>
<tr>
<td>Relative weight, %</td>
<td>5 (-8-25)</td>
<td>-20-20</td>
</tr>
<tr>
<td>UDCA, mg/kg/day</td>
<td>21 (0-36)</td>
<td></td>
</tr>
<tr>
<td>Vitamin D substitution</td>
<td>21 (91%)</td>
<td></td>
</tr>
<tr>
<td>Vitamins A, E, K substitution</td>
<td>18 (78%)</td>
<td></td>
</tr>
<tr>
<td>Prophylactic antibiotics</td>
<td>21 (91%)</td>
<td></td>
</tr>
</tbody>
</table>

Data as median (range) or frequency (percentage). APRI = aspartate transferase to platelet ratio index, UDCA = ursodeoxycholic acid.

Esophageal varices

Among 47 consecutive BA patients in study II, 28 (60%) had esophageal varices: classified as grade 2 in 10 and grade 3 in 18 at first observation at the median age of 11 months (range 4-165 months). Varices occurred at a similar frequency after both successful (10 of 19, 53%) and failed (18 of 28, 64%) PE, p=0.547. Despite identical surveillance protocol, the varices were observed significantly later after successful PE, compared to failed: 19 months (range 4-165 months) vs. 8 months (range 4-23 months), p=0.004.

Bleeding from varices occurred in 13 (28%) patients who all had a failed PE. The median age at the first bleeding episode was 10 months (range 5-20 months); four patients bled before the first surveillance endoscopy at 7 months (range 7-10 months). All bleeders received sclerotherapy with four week intervals, but in 12 of 13 cases, bleeding recurred after initiating the sclerotherapy treatment. In a logistic regression analysis, serum
bilirubin concentration >40 μmol/L at three months after PE was a risk factor for upper gastrointestinal bleeding with an odds ratio of 17 (95% CI 1.7-17.5), p=0.007.

After successful PE, ten patients developed varices: in two, jaundice had recurred before the varices were encountered, and eight developed varices while their liver function was stable. These eight had significantly lower platelets, higher serum bilirubin, and higher conjugated bilirubin than the patients with a successful PE and no varices at last endoscopy [127 E9/L (27-187) vs. 224 (44-405) E9/L, p=0.036, 17 μmol/L (5-19) vs. 8 (2-16) μmol/L, p= 0.045, and 7 μmol/L (2-10) vs. 2 (1-8) μmol/L, p=0.050, respectively]. After successful PE, the patients with varices showed a tendency to decreased native liver survival when compared to the patients without varices (Figure 12).

The varices of 27 patients were treated with sclerotherapy in 115 sessions (median per patient 2, range 1 to 19). Sodium tetradecyl sulphate was injected into a median of two (range one to seven) sites per session. Sclerotherapy was repeated every two to four weeks until the varices were considered eradicated. Sclerotherapy complications included minor bleeding in 12 (10%) sessions, bleeding demanding erythrocyte transfusions after two sessions, mucosal ulceration of the sclerotherapy site in three patients, and one esophageal perforation which led to mediastinitis and death (the patient was listed for LT due to terminal liver failure and suffered from recurrent variceal bleedings).

Based on observations in study II, the protocol for endoscopic surveillance and prophylactic sclerotherapy of esophageal varices was updated. After failed PE, the surveillance is initiated at the age of six months and after successful PE if serum bilirubin concentration exceeds 40 μmol/L or clinical signs of advanced portal hypertension such as splenomegaly or low platelet count occur.

![Figure 12](image)

**Figure 12.** Native liver survival among 19 patients with a successful portoenterostomy, 10 with and 9 without esophageal varices.
Liver histology

In 30 liver biopsies obtained at PE, typical histological features of BA (cholestasis, fibrosis, portal area inflammation, hepatocellular multinucleation and swelling, and portal tract edema) were observed, Figure 13A. No correlation between the degree of liver fibrosis and age at PE was observed. No histological feature at PE differed between 19 patients who by two years’ age underwent LT and 11 who lived with their native liver.

After successful PE, in 23 native liver follow-up biopsies obtained at the median age of 4.2 (1.6-18.9) years, the most prominent histological feature was fibrosis. The median Metavir fibrosis score was 4 (1-4), and 12 (52%) biopsies were cirrhotic, Figure 13B. Cholestasis was visible in only 4 (17%) biopsies, and in 15 (65%) a predominantly lymphocytic portal cellular infiltrate was observed. In 14 (61%) biopsies, CK7 immunostaining of periportal hepatocytes was observed, and 20 (87%) showed bile duct proliferation; these features correlated with the Metavir score \( r=0.606, p=0.002 \) and \( r=0.657, p=0.001 \). Serum levels of conjugated bilirubin [median 4 (range 1-13 μmol/L)] correlated with CK7 positivity and Metavir score \( r=0.600, p=0.002 \) and \( r=0.601, p=0.002 \).

Of 19 patients with a successful PE, a liver biopsy taken both at PE and at follow-up was reviewed, allowing assessment of longitudinal changes in liver histology during follow-up. Portal inflammation diminished uniformly, and in one third of the patients all the inflammatory features disappeared. The individual Metavir scores at PE and at follow-up are shown in Figure 14. In ten (53%) isolated BA patients, the Metavir score increased during follow-up. Fibrosis progression was less severe among BASM patients when compared to patients with isolated BA \( p=0.007 \).

Fourteen (61%) patients had portal hypertension, defined as splenomegaly or presence of esophageal varices or both, with a platelet count below age-appropriate lower limits of normal. The patients with portal hypertension showed significantly higher Metavir scores than those with no portal hypertension [four (range 1-4) vs. two (range 1-4), \( p=0.011 \)]. Three patients, however, had splenomegaly, varices, and a low platelet count, but only had slight fibrosis (Metavir \( \leq 2 \)) in the follow-up biopsy. APRI \( \geq 1.70 \) discerned patients with esophageal varices with 83% positive and 100% negative predictive value.

Following a failed PE, 17 biopsies taken at LT at the median age of 1.1 years all were cholestatic and cirrhotic. Liver biopsies taken both at PE and at LT were available for seven patients after failed PE. Advancing trends of ductal cholestasis [1 (0-3) vs. 2 (1-3), \( p=0.034 \)] and appearance of lobular fibrosis [0 (0-1) vs. 2 (1-2), \( p=0.024 \)] occurred while fibrosis progressed to cirrhosis in all [Metavir 2 (1-4) vs. 4 (4-4), \( p=0.039 \)].
Figure 13. A. Liver histological findings typical for biliary atresia at the time of portoenterostomy. Intraductal (right arrow) and intracanalicular (left arrow) cholestasis, hepatocellular multinucleation (up arrow) and swelling (down arrow). B. Liver cirrhosis with fibrous septae (right arrows) at nineteen years after portoenterostomy.
Follow-up tools

Among 36 BA patients in study IV (12 were listed or died within one year of measurements, 24 had an uneventful follow-up), PELD/MELD score and Gal½ measurement showed both significant predictive value for LT listing or death. Of these two measures, PELD/MELD was more powerful with an AUROC of 0.947 (95% CI 0.877-1.000, p<0.001) while Gal½’s AUROC was 0.814 (95% CI 0.654-0.974, p=0.003). The most optimal cut-offs were PELD/MELD over 3, which gave 83% sensitivity and 84% specificity, and Gal½ over 12.0 minutes with 83% sensitivity and 64% specificity.

None of the BA patients who were listed or died in study IV had a combination of a normal (<20 μmol/L) serum bilirubin level and a Gal½ below 12.0 minutes. Half of the patients with uneventful follow-up, however, had either or both tests above the cut-off. The combination of normal bilirubin and Gal½ <12.0 minutes was highly sensitive for one year uneventful follow-up (no LT listing or death): 100% sensitivity, 50% specificity, 50% positive, and 100% negative predictive value, p= 0.003.
In study III, APRI appeared a useful non-invasive tool for predicting liver fibrosis and even more useful as a predictor of esophageal varices. The best cut-off for advanced liver fibrosis (Metavir ≥3) was APRI over 0.60, which gave 93% sensitivity and 67% specificity, p=0.005. APRI scores were calculated for the entire cohort of 23 native liver survivors (of whom 19 attended study III earlier); of these 11 (48%) had endoscopically verified varices. The AUROC of APRI for esophageal varices was 0.841 (95% CI 0.666-1.000, p=0.006), Figure 15. The most optimal cut-off was over 1.0 with 91% sensitivity, 83% specificity, 83% positive, and 91% negative predictive value.

Bilirubin levels, although normal or near normal [median 11 (2-35) μmol/L], associated with liver fibrosis progression in study III. Patients whose Metavir score increased or remained at 4 had significantly higher serum bilirubin levels than did patients whose Metavir score diminished or remained low (at score 2) [15 (3-35) vs. 5 (2-11) μmol/L, p=0.006]. In study II, patients with a combination of serum bilirubin below 20 μmol/L and no varices in surveillance endoscopies had the most favorable native liver survival (Figure 12).

Figure 15. Receiver operating characteristic curve of aspartate to platelet ratio index (APRI) score as a predictor for esophageal varices among 23 biliary atresia patients living with their native liver.
DISCUSSION

The BA incidence and treatment outcomes in Finland were previously unknown and as a result of this study, the 1987-2010 cohort is now thoroughly characterized. The most important finding was the improvement of treatment results with careful planning, even in such a small country like Finland where even the centralized treating centre remains, by international standards, a low-volume one. Importantly, the overall survival improved based on improved native liver survival. Extending the BA patients’ native liver survival period is of utmost importance because LT, although a tremendous possibility for BA patients, comes with many risks, the need of continuous immunosuppressive medications, and high costs (193). Moreover, nonadherence of adolescent LT recipients is an unfortunate cause of acute rejections and graft losses (194), and the liver allografts are not everlasting (195).

This study has several limitations, including the small number of patients and the retrospective nature of some of the data. No controls from the general child population exist for the galactose half-life measurements or liver biopsies, nor can they be obtained due to ethical considerations. Strengths include all-inclusive patient identification and complete access to follow-up data. The identified BA patients were cross-checked with the national Register of Congenital Malformations, which has a high standard and reliability (196-198). All native liver survivors were invited and participated in study III and no patient in the whole cohort was lost to follow-up.

BA is a very rare disorder. As expected, BA incidence in Finland was similar to other European countries and Canada (30, 31, 33, 34). In this study cohort, somewhat more BA patients were born during the autumn-winter season than in spring-summer. More BA births during the cold season were earlier observed in Atlanta, the US, and in the Niigata prefecture, Japan (28, 37). A study from eight states in the US reported that infants conceived during the spring and thus born during the winter had a higher risk of having BA when compared to infants conceived in winter (41). In contrast, another two prefectures of Japan reported no seasonality in BA births (36). An infectious pathogen with a seasonal pattern could explain the seasonal variation in BA births (41). So far the exact reason for the seasonal clustering, however, remains obscure. A robust visual analysis of the geographical distribution of BA cases in Finland revealed no clustering. A study from England and Wales reported significantly higher BA incidence in South-West England; the authors discuss the possible association with the racial distribution of the population living in the area (30).

By definition epidemiology includes, besides studying incidence and distribution of a disease, the search for means to diminish the incidence of the disease. Internationally, BA incidence has remained similar over the years (30, 33, 34, 38, 168, 199) as was the case in the Finnish material. In a recent report from Taiwan, a trend towards decreasing BA incidence in areas of high rotavirus vaccine coverage was observed (50). In Finland, rotavirus vaccine was given to approximately one third of newborns in 2008 and added to the national immunization protocol in September 2009. BA was diagnosed in four patients born in 2009 and in five patients born in 2010. As the median number of BA cases per
year during the study period 1987-2010 was three, we cannot speak of decreased incidence.

In 5-14% of patients, BA is combined with other structural abnormalities, such as BA splenic malformation (BASM) (30-33). In studies from the UK (51, 169) BASM patients had worse native liver survival than isolated BA patients but reports from North America (2, 52) failed to confirm the finding nor was it observed in this study. Interestingly, during the follow-up in study III, liver fibrosis progressed significantly less in BASM patients than in patients with isolated BA. The diagnostic criteria for BASM were defined in a UK report (200) but have varied from study to study (2, 52, 201). A recent large Canadian report of BA-associated structural abnormalities revealed a significant proportion of BA patients with only a heart defect and another cluster with intra-abdominal vascular abnormalities, either with or without splenic abnormality (52). The observed cardiac anomalies, rotation disorders, vascular abnormalities, and spleen anomalies all associate with disordered development of asymmetric laterality of the organs (202). In this study, 10 patients (14%) were classified as BA splenic malformation but altogether 17 (24%) had some combination of abnormalities associated with disordered laterality. It seems reasonable to support the Canadian group’s suggestion to change the meaning of the acronym BASM from BA splenic malformation to stand for BA structural malformation.

In order to improve survival, as many deaths as possible should be avoided at all phases of treatment. The survival figures during the first ten years of this series are rather depressing, 18 of 30 (60%) patients died before their second birthday and 9 of these died of liver failure without ever being listed for LT. These were the first years of the pediatric LT program. Obvious improvement has occurred, but still three patients died after centralization in 2005. One died of sepsis after being assessed for LT but declined because of severe lack of social support, one died of an inoperable heart defect, and one of a sudden midgut volvulus. Even in retrospective evaluation, it is hard to imagine how these deaths might have been avoided. Some mortality associated with associated anomalies, especially severe cardiac anomalies, is inevitable. A similar decrease in the proportion of patients who died without being assessed or accepted for LT was observed in a report from the years 1987-2008 from the Netherlands (199).

Age at PE is recognized as an important factor affecting PE success – the younger the better (164, 203). A report from the US found no difference in age at PE during 1997-2006 (172). In the UK, the median age at surgery decreased from 63 days in the early 1980s to 54 days in the 1999-2009 cohort (6, 204). In the current study, the treatment centralization caused no significant decrease in the median age at PE or the proportion of late referrals. Even in cases of late referral, PE seems worth an attempt: in a study of 35 BA patients aged ≥100 days at PE, the five-year native liver survival was 40% (171). To avoid late referrals, Taiwan (with manifold BA incidence compared to Europe) has started a stool color card screening among newborns with favorable results (173). Canadian economists recently calculated that with ≥75% stool color card utilization rate in a population of half a million new births screening would be cost-effective (205). In Finland no screening is currently offered. Of other European countries, Switzerland has started a pilot program of voluntary stool color card screening (57). In the UK, no screening is offered, but the matter is currently under review by the UK National Screening Committee (206).
Educating parents of newborns and health care professionals about signs of BA is another important approach in promotion of earlier referrals. Many countries have BA awareness and education campaigns, for example Yellow Alert in the UK (207). In October 2011, five thousand cyclists attended a BA awareness event in Jakarta, Indonesia (208).

Increasing the experience of the centre treating BA patients is important in improving results. France and Canada have reported similar clearance of jaundice rates achieved in both high and low volume centres (34, 167). In England and Wales, BA treatment centralization into three high volume centres led to a clear improvement in the clearance of jaundice rate (169). Denmark reported a similar observation of improved results after treatment centralization (209). Study I is the first report of the effects of a complete national centralization of BA treatment into one centre. In this study, a highly significant increase in clearance of jaundice rate and native liver and overall survival was observed after centralization into Helsinki in 2005. Since then, the Children’s Hospital has treated a median of four new BA cases per year, remaining a medium volume centre at the most. Actuarial four-year transplant-free survival was 73% and overall survival 83%. These figures are well comparable to results from high-volume centres in France (respective figures 48% and 90%) (34), Canada (45% and 83%) (167), and England and Wales (47% and 91%) (6).

The factors affecting the improvement of treatment results in Finland are most likely multiple. A group of pediatric surgeons perform all PE operations with two attending each operation in order to increase personal experience – a factor that is also recognized in other centres (7). Infants with suspected BA are prioritized for diagnostic procedures and operating rooms in order to minimize in-hospital delay and to provide rapid access to surgery for those in need. The postoperative adjuvant medical therapy and follow-up are standardized and appear as written protocols. A multidisciplinary team follows all Finnish BA patients, both with native liver and post-LT, providing a continuum of care and increased expertise, which will benefit both current and future BA patients. Besides increased centre caseload, general improvement in health care facilities and skills such as intensive care of infants may have contributed to the improvement in treatment results. All study limitations considered, BA treatment centralization undoubtedly directly benefited BA patients’ health and survival.

Increased clearance of jaundice rate and thus longer native liver survival after PE may translate into improved survival after LT because infants have a higher risk of dying both on the waiting list and soon after LT (210). A recent report from California described 134 BA LT recipients, of whom 22 underwent no PE operation prior to LT and 63 received LT after failed PE within the first year of life. The patients without a prior PE operation had similar survival rates with the over one-year-olds, and the under one-year-olds with a failed PE, experiencing the worst survival. The authors stress, however, that PE should remain the first line treatment of BA (211).

Portal hypertension is a common and significant complication of any chronic liver disease (100). Esophageal varices were observed in approximately half of the patients in the material studied here, but bleeding only occurred in patients with significant hyperbilirubinemia after failed PE. In BA, current evidence supports treating significant esophageal varices with red markings and gastric varices preemptively (110), the most
common treatment being endoscopic injection sclerotherapy. In adult patients, variceal band ligation has displaced sclerotherapy, but due to the large size of the multiband ligation devices, this treatment option is not suitable for children weighing less than 10 kg (212). Successful use of band ligation as primary prophylaxis of variceal bleeding among BA patients aged over three years has, however, been reported (112, 114). Endoscopic surveillance and primary prophylaxis of variceal bleeding are recommended for all BA patients (7), although the recommendations remain somewhat controversial (100, 213). Esophageal varices mostly appear in patients with advanced liver fibrosis. In study III, however, three patients with esophageal varices had spleen enlargement and lowered platelets but only mild fibrosis (Metavir ≤2) in their follow-up liver biopsies. In such cases the contractile portal myofibroblasts may be the culprits, causing increased intrahepatic vascular resistance (214).

The reasons why liver fibrosis progresses in BA also after successful PE are not completely understood (3, 81, 215). According to study III, BA native liver survivors with low serum bilirubin levels may still suffer from a hidden form of cholestasis that may contribute to the progression of liver fibrosis. Chronic cholestasis induces hepatocytes to transform into cholangiocytes – possibly the liver tries to maximize the surface of cholangiocytes in order to increase chole-hepatic cycling of bile acids (216). The proliferating cholangiocytes secrete numerous profibrogenic substances and function as the pacemaker of portal fibrosis (217). Another feature contributing to fibrosis accumulation may be inflammation. Increasing evidence supports that in BA, the biliary epithelium is destroyed by an autoimmune type of inflammatory process continuing after the immune system has cleared the initial, probably viral, trigger (43). During the follow-up in study III, the consistence of the portal cellular inflammatory infiltrate changed from acute inflammatory cells towards lymphocytic preponderance. The inflammation observed in the follow-up biopsies may also be a factor sustaining the fibrotization of the liver. In many organs, a fibrotizing process characterized by accumulation of activated myofibroblasts may be observed. The stimuli that activate the myofibroblasts differ from organ to organ - cholestasis and inflammation in the liver, proteinuria in the kidneys, oxidized low density lipoprotein-induced inflammation in the arterial intima – but the irreversible fibrotic change may have a molecular pathway common to all tissues (214).

The evidence for inflammatory disease progression in BA has evoked ideas of studying either prolonged or high dose postoperative anti-inflammatory treatment (44). The existing studies are on corticosteroids which have a choleretic effect, in addition to their anti-inflammatory features. The only currently available randomized control trial and 16 observational studies on postoperative steroids in BA were collected in a meta-analysis, which gave no support to steroid treatment (88). The BA treatment protocol in Helsinki includes a postoperative course of corticosteroids with a medium dosage.

Reliable severity of disease indicators for pediatric chronic liver diseases like BA are scarce and developing such tools is important for the clinical follow-up practice. Of the non-invasive tools to identify patients with varices, platelet count alone or in combination with spleen size or liver stiffness score measurement and the APRI score appear most promising (118, 119). Among our 23 native liver survivors, APRI over 1.0 gave 91% sensitivity and 83% specificity for varices. In a Korean study, APRI score over 1.0
predicted significant liver fibrosis (Metavir score ≥3) at the time of PE (218). Among patients at a median age of 4.2 years in study III the optimal APRI cutoff for significant fibrosis was 0.6. A healthy liver has a large unused functional reserve, while in chronic liver disease, a proportion of the liver’s functional reserve is in constant use and the evaluation of the available reserve is complicated. Study IV evaluated the usefulness of the PELD/MELD score and a Gal½ measurement in predicting the risk of being listed for LT or dying within one year of measurements. PELD/MELD score appeared especially useful among BA patients. A combination of normal serum bilirubin level and Gal½ below 12.0 minutes was present in no BA patient who was listed or died within one year. During the follow-up the patients and their families need continuous counseling regarding future prospects. Combining the results from studies I-IV, the patients with normal serum bilirubin level, no varices, and short Gal½ seem to have the most carefree prognosis. On the contrary, a rising trend of serum bilirubin level, prolonged Gal½, development of esophageal varices, and growth retardation (219) may be signs of an approaching end-stage liver disease.

After securing survival, the measures to improve health and quality of life arise. Chronic cholestatic liver disease causes decreased intestinal absorption of fat-soluble vitamins A, D, E, and K and if the liver function is severely compromised, the synthesis of 25-OH vitamin D may also be impaired (220). The target level of serum 25-OH vitamin D concentration for patients with chronic liver disease (220) and for optimal bone health in general (221) is over 80 nmol/L. Despite that 21 of 23 of our native liver survivors were prescribed vitamin D supplementation, only 11 (48%) reached the targeted serum concentration. The vitamin D dosage should be individually adjusted based on measured serum levels, a matter that has recently gained more attention in our clinic (221). Further studies regarding vitamin D metabolism and bone health among BA patients are well-founded. A study from Japan, where a peroral newborn vitamin K supplementation is in use, reported a high 8% incidence of intracerebral hemorrhage as the first symptom of BA (54). A vitamin K deficiency-associated hemorrhage causing the death of a BA patient was reported from the Netherlands (32). These serious and regrettable complications were absent in our cohort, possibly because newborns in Finland receive intramuscular vitamin K supplementation, which prevents vitamin K deficiency associated bleeding more efficiently than peroral prophylaxis (55).

Normal growth of the BA patient is important in many aspects: decreased growth velocity despite optimized nutritional therapy may be a sign of end-stage liver disease (219), and children with severe growth retardation have poorer survival after LT (96, 136, 203). In the Finnish material, the height scores at one and two years of age improved significantly after treatment centralization and at the end of the study all the native liver survivors grew within normal limits. In a French series of 63 adult native liver survivors, all but two had adult height above -1.5 SD (108).

Approaching the regulation of liver fibrosis progression at the molecular level is an interesting future study topic. Elastography as a clinical follow-up tool seems worth studying as a predictor of varices and liver fibrosis. In BA, early diagnosis is essential in order to further improve treatment results. An awareness campaign directed at primary health care nurses and/or parents of newborns might be beneficial.
CONCLUSIONS

The epidemiology and treatment results of a 24-year national cohort of BA patients born between 1987 and 2010 were assessed in this study, and the effects of treatment centralization from five centres to one in 2005 were evaluated. The surveillance and treatment protocol for esophageal varices was evaluated and prognostic markers for varices, significant liver fibrosis, and risk of dying or being listed for LT were sought. Longitudinal changes in liver histology following successful PE were described and native liver histological findings were correlated with clinical follow-up data.

The main conclusions of this study are:

1. The incidence of BA in Finland between 1987 and 2010 was 1: 20000, similar to other European and North American countries. BA cases showed no geographical clustering but births with BA were concentrated to autumn-winter season supporting viral etiology.

2. Centralization of BA treatment yielded significantly improved results in terms of clearance of jaundice, native liver survival and overall survival.

3. Esophageal varices were common among BA patients. No variceal bleeding occurred in patients with normal serum bilirubin levels and thus surveillance endoscopies could be allocated to patients with elevated serum bilirubin or clinical signs of portal hypertension.

4. Liver histology at the time of PE showed no association with native liver survival. In most patients portal hypertension associated with advanced liver fibrosis. BA native liver survivors had immunohistological signs of subtle, chronic cholestasis that may contribute to the progression of liver fibrosis.

5. Gal½ alone, or combined with serum bilirubin level, and the PELD/MELD score showed significant predictive value for LT listing within one year of measurements and are simple, useful tools in the long-term follow-up of BA native liver survivors. APRI showed significant potential as a noninvasive marker of esophageal varices and advanced liver fibrosis.
ACKNOWLEDGEMENTS

This study was carried out at the Department of Pediatric Surgery at the Children’s Hospital, University of Helsinki. Docent Jari Petäjä, Director of the Department of Gynecology and Pediatrics is acknowledged for providing the research facilities. I had the privilege to join the Pediatric Graduate School, which provided me with a valuable scientific education, travel grants, and excellent working facilities on the sixth floor of Biomedicum 2C, with networking opportunities on the side. I sincerely thank Professor Markku Heikinheimo (former Director of the Pediatric Graduate School and the National Graduate School of Clinical Investigation) and Docent Jussi Merenmies (present Director of the Pediatric Graduate School) for their valuable work, and especially for their genuine interest in the well-being of young clinical investigators.

I thank the financial supporters of this study: the Finnish Association of Pediatric Surgeons – Sulamaa Society, the Finnish Association of Transplant Surgeons, the Finnish Foundation for Pediatric Research, the Finnish Medical Society Duodecim, the Martti I. Turunen Fund, the Mary and George C. Ehrnrooth Foundation, the National Graduate School for Clinical Investigation, the Päivikki and Sakari Sohlberg Foundation, and the Sigrid Juselius Foundation.

I sincerely thank all the patients and their families for participating in this study.

I gratefully acknowledge my outstanding supervisor, Docent Mikko Pakarinen, for his support and guidance throughout this learning project, and for providing me the option of doing research full time right from the beginning. You always made time for my questions, sometimes with slightly frightening efficiency. Your genuine interest in the subject and humorous, straightforward approach to research and life in general have helped to keep this project moving.

Dear Silja, receiving a friendship like ours on top of doing a thesis is an extraordinary gift. Sharing all this work with you made it go so much more smoothly. Besides the work, together we have traveled, laughed our heads off, shed a few tears (though not over the research), sang and danced, and envisioned a refined health care system as well as a better world. You, the warm-hearted enthusiast, have taught me to celebrate successes both great and small. Thank you for everything thus far and I hope more is to come!

Professor Risto Rintala deserves my big thanks for encouraging us young researchers to travel and present our results at international meetings, and for tasty, relaxed dinners while abroad. Docent Jouni Lauronen and Jesper Kivelä, members of the research group, are acknowledged for their easy-going collaboration. Annika, Janne, Kristiina, Saara, Topi, and Tuomas, my research colleagues in Pediatric Surgery: thank you all for the great company while traveling.
I warmly thank Docents Päivi Heikkilä and Jouko Lohi for patiently introducing me to the fascinating world of liver histopathology. Hearing your laughter revealed your presence already from the other end of the corridor, and you both created a cheerful atmosphere during the many hours spent at the microscope. I am grateful to Annukka Ritvanen at the National Institute for Welfare and Health for her friendly collaboration and for letting me glimpse the potential of register studies.

I gratefully acknowledge the members of my thesis committee, Professor Hannu Jalanko and Docent Heikki Mäkisalo, for their friendly support and helpful comments, and Hannu for making available to me his invaluable expertise in pediatric hepatology. I warmly thank Professor Emeritus Christer Holmberg for his support in the beginning of this project and for chaperoning Silja and me on our first congress trip to Istanbul. I also thank Docents Paulina Salminen and Tarja Ruuska for their friendly and efficient review of this thesis, and for their valuable advice. I am grateful to Dr. Jennifer Rowland for her efficient language revision of this thesis.

Anita Kiianmies-Kaba is acknowledged for scheduling the hospital visits for all our patients. I thank Lisbet Björkstam and Ulla Sandholm for their kind help. I sincerely thank the nurses at the outpatient surgery units of Peijas and Children’s Hospitals, especially Mervi Mannonen, for their important help in collecting the control blood samples.

I warmly thank my roommate Eve, as well as Anne N, Anne S, Hanne, Juuso, Maija, Mervi, Satu, Sonja, Susann, and all my other research colleagues on the sixth floor of Biomedicum 2C for their cheerful company and shared feelings during these years.

Anne, Gisele, Hanna, Henna, Kaisa, Laura, Matti, Minna, Pia, Saana, Suvi, Taru, Tuire, and Ville, my fellow residents in gastrointestinal surgery: thank you for your company and the relaxed, joyful atmosphere during our working days and nights at the hospital. I wish to thank my senior GI surgeon colleagues for their support and teaching, Esko Kemppainen for providing me with study leave from my clinical work (even after reminding me that in the old days, research was done after hours), and Professor Pauli Puolakkainen for his friendly encouragement.

I warmly thank my dear bovine herd Katriina, Lea, Mari, and Tuomo for their durable friendship and shared hilarious May Days and other celebrations. I deeply apologize for undermining the founding principles of the anti-research program. I gratefully acknowledge Elina and Juha for their friendship, support, and joyful trips and evenings together, both at sea and ashore. I thank my neighbor Aiju for our numerous Nordic walking sessions, coupled with interesting discussions of work and life. Johanna, thank you for our friendship, which has already lasted for a quarter of a century. And I warmly thank all my other friends outside of work for being such an important source of joy.

I thank my parents-in-law Leea and Heikki for their thought-provoking discussions, support, and help with childcare. I’ve been privileged to join a family with many in-laws.
thank Anna, Iiro, Laura, Mikko, Jaakko, Emelie, and their children for the joyous moments together, and Anna and Laura for introducing me to the world of unforgettable cakes.

I wish to thank my dear parents Marketta and Jorma Ahola for their unconditional love, support, and letting me be me. Thank you also for always being there for my children. I warmly thank my grandmother Ringa for her constant praise and encouragement. I also thank my dependable brother Rami and his family for their support, and for humorously sharing the course of life.

To my children Eero, Armi, and Juha: thank you for bringing such wonderful amounts of music, laughter, sports, and love into my life! You have taught me to defend my opinions in discussions with your jaw-dropping arguments. You constantly provide fresh, new insights into the world, and indeed other worlds imagined. You truly are the new generation.

And to my beloved husband, Antti, thank you for your flexibility, support, patience, and humorous approach to my projects. Above all, thank you for sharing your life with me. I feel blessed to have such a beautiful family as ours.

Helsinki, January 2013

[Signature]
REFERENCES


161. Scandiatransplant transplantation and waiting list figures; accessed at www.scandiatransplant.org [Internet]; 2012.

69