Strategies to improve the outcome of biliary atresia

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Advances in diagnosis and treatment of biliary atresia

In preparation

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Chapter 1

1

ABSTRACT

Background

Biliary atresia (BA) is a cholestatic disease in infancy caused by obliteration of the biliary tract. In the past decades it has evolved from a uniformally lethal disease to a disease for which curative treatment is feasible. However, substantial morbidity and mortality still exists. Many controversies regarding diagnostic procedures and treatment have remained.

Data Sources

Based on available scientific literature, we reviewed the current knowledge regarding the diagnosis and management of BA. Articles were retrieved from PubMed using the terms: biliary atresia, neonatal jaundice, diagnosis, outcome, etiology. Articles from 1959 onwards were used, with a focus on the most recent 5 years.

Results

Much research has focused on the management of patients, clarifying the beneficial effects of early surgery and the importance of surgical expertise. Still, little is known about potential adjuvant treatment strategies. The pathophysiology is still poorly understood, hampering the development of strategies to prevent disease development and progression.

Conclusion

Screening tools to facilitate early diagnosis may further increase survival. More prospective research on the effects of nutritional support, ursodeoxycholic acid and prophylactic antibiotics is demanded. As long-term survival is now feasible, research should focus on developmental and psychological outcomes as well.

1. INTRODUCTION

Biliary atresia (BA) is a cholestatic disease presenting in infancy. The disease is characterized by obliteration of (parts of) the extrahepatic bile duct. The incidence varies from 1:17,000 to 1:19,000 life births in Western countries[33,111,147,151], to between 1:7,000 and 1:3,300 life births in Taiwan, Japan and French Polynesia.[20,128,179] Generally, three subtypes are distinguished (See figure 1).[62] In type I (5-10% of cases), obstruction is located in the common bile duct. In type II (2-5%), the common hepatic duct is obstructed and may be associated with the formation of a ductal or biliary cyst. In type III, the most common variant (>90%) the entire extrahepatic bile duct is obstructed including the porta hepatis (Figure 1).[60] The disease is not inheritable although case reports have described the concordant occurrence in twins, or mother and sib.[85] There are reports of monozygotic twins of which one child developed BA and the other child was unaffected.[73,168] Until now, there is no evidence for a higher incidence of BA in offspring of BA patients who have reached the childbearing age.[88,103]

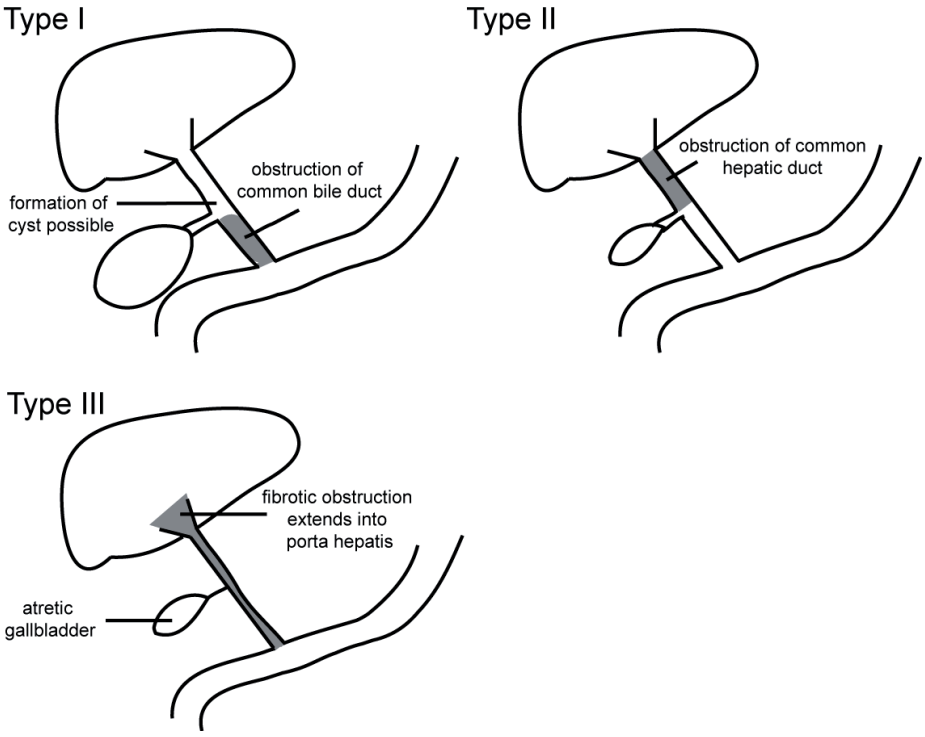

Figure 1: Three subtypes of biliary atresia are distinguished.

2. ETIOLOGY

2.1 Current theories

About 20% of the BA patients have associated malformations and in this subgroup a joint etiology is likely. Patients with BA and splenic malformations (asplenia or polysplenia) are considered a prognostic subgroup and the condition is termed Biliary Atresia Splenic Malformation syndrome (BASM). It occurs in up to 10% of BA patients.[31,80,192] These patients frequently have other malforma-

tions as well, such as situs inversus, intestinal malrotation, preduodenal portal vein, cardiac abnormalities, and absence of the inferior vena cava.[31]

The majority of BA patients however, have an isolated form. The current idea is that in these patients, a local immune reaction is triggered by an unknown cause, leading to the formation of fibrotic tissue with subsequent obstruction.[105,157] A corresponding picture is seen in histologic examination of resected specimens.[13,27] It is thought that the syndromic form develops early in pregnancy and is therefore termed the embryonic form. The isolated form is thought to develop shortly before or after birth and is called the perinatal form. Despite extensive research efforts, the exact etiopathogenesis of BA is currently still unknown. The cause is likely to be multifactorial and many factors have been suggested to be involved, such as genetic factors, perinatal infections, vascular insults, defective morphogenesis, disorders of immunity and toxic insults (Figure 2). The best evidenced factors will be discussed in the next sections.

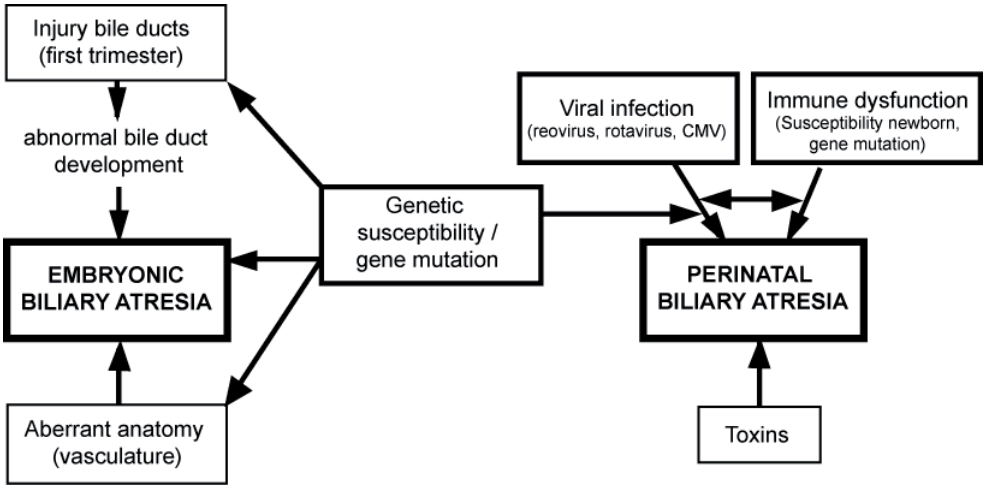

Figure 2: Proposed factors to be involved in the pathogenesis of embryonic and perinatal biliary atresia.

2.1.1 Perinatal infections

A viral infectious genesis was first proposed by Landing.[89] Moreover, some studies evidenced seasonal variation or time/space clustering of BA cases suggesting an infectious or toxic origin of the disease, whereas others could not demonstrate clustering.[67,167,197,210] A number of studies indeed showed evidence of reoviral RNA[182], cytomegalovirus DNA and IgM[46] and rotavirus RNA[138] in up to 50% of BA patients. Proving an infectious cause is difficult because tissue samples are obtained a substantial time after the suspected primary insult. One of the current theories is that autoimmunity is induced by viral proteins that show great similarity to proteins in bile duct epithelium, based on molecular mimicry. After clearance of the virus, the induced autoimmunity causes persistent inflammation and injury.[104,120]

2.1.2 Autoimmunity

The principle of BA being an autoimmune disease has been supported by two studies. First, T-cells

from BA mice are able to induce bile duct inflammation in naieve severe combined immunodeficiency mice.[105] Second, antibodies against the bile duct epithelial antigen α-enolase, derived from BA mice, have been shown to cross-react with proteins from RRV.[102] As of yet, it is unclear whether these auto-immune processes are the eliciting factor in BA, or that they develop as by-effects in the course of disease. It is striking that the recurrence of BA after liver transplantation has not been reported, which would plead against a primary auto-immune cause.

Other studies suggest that BA is rather a graft-versus-host-like disease, caused by the engraftment of maternal CD8+ T-lymphocytes that passed through the placenta. This is a naturally occurring phenomenon. However, without an adequate host immune-reaction, the maternal cells remain in the body of the offspring, causing maternal microchimerism. It has been shown that BA patients have higher levels of maternal chimeric cells in the liver compared to controls.[86,122,170]

2.1.3 Genetic factors

The susceptibility to BA has also been linked to immunogenetic factors. Several HLA-subtypes each have been shown to have a high frequency and linkage disequilibrium in distinct populations of BA patients. So far, however, the results have not been replicated in other cohorts.[2,161,214] The largest study (101 patients), also the first study to apply molecular genotyping, focused on previously associated HLA alleles and could not confirm an association with BA.[41] Genome-wide association studies in two Chinese cohorts revealed a susceptibility locus on 10q24.2, a gene involved in the metabolism of inflammatory mediators.[50]

Furthermore, epigenetic modifications may also play a role in the development of BA. In zebrafish, hypomethylation appeared to cause biliary defects and activation of interferon-γ responsive genes, which are features of human BA. Moreover, DNA methylation was reduced in bile duct cells from BA patients, compared to patients with other infantile cholestatic diseases.[107]

2.2 Animal models for BA

The mouse model for BA was first described by Riepenhof-Talty and the model was further explored by Petersen *et. al.*[137,139] Inbred Balb/c mouse pups are inoculated intraperitoneally with a specific viral strain, rhesus rotavirus MMU18006. Subsequently, depending on the dose utilized, 60-100% of the mice will develop a disease with clinical and pathological symptoms similar to human BA. It is intriguing that, for unknown reasons, not all mouse strains are equally susceptible for the disease. Furthermore, BA is only inducible in mice within one day after birth, suggesting a temporary window in immunity. In BA mice, the viral infection is typically eliminated within a few days, leaving an ongoing inflammatory response targeted at the bile duct epithelia. The BA mouse model thus supports the viral hypothesis. Until now, the mouse model has mainly been utilized to study elements of the immune cascade elicited by BA. These studies clarified the involvement of both the adaptive and innate immune system.[105,157]

3. CLINICAL ASPECTS

3.1 Presentation

BA patients present at a median age of 4-6 weeks of age. The most observed presenting symptoms include jaundice of skin and sclerae, and acholic, and thus discoloured stools, typically in term infants with normal birth weights. The first presentation may also be growth failure, or bleeding due to vitamin K deficiency. The latter may occur typically in breastfed infants who received no or low

dosages of vitamin K prophylaxis. In a study of 30 breastfed infants receiving 25 µg vitamin K daily, 83% experienced vitamin K deficiency bleeding, and 43% had an intracranial hemorrhage.[187] The first symptoms of BA are often not recognized by the parents and primary care givers, as jaundice is difficult to detect or is not considered alarming. The incidence of physiological jaundice and breast-milk jaundice after birth is high, and it is very difficult to distinguish among those infants the few cases with underlying pathology requiring specific treatment. The paleness of stools may not be noted or regarded normal in (breastfed) newborns.

Other diagnoses which need to be considered in the diagnostic work-up of presumed BA are, among others, alpha-1 antitrypsin deficiency, Alagille syndrome, forms of progressive familial intrahepatic cholestasis, (idiopathic) neonatal hepatitis, metabolic disorders, storage diseases and choledochal cysts.[163]

A stool color card has been successfully introduced in Taiwan and Japan as a screening tool, aiding parents in recognizing abnormal stool color and thereby improving both the referral of cases to hospital and the 5-year transplant-free and overall survival rates.[71,98] This simple but promising screening tool might be practicable in many health care systems.

In some cases BA may be diagnosed in the antenatal period by ultrasound. In cystic BA the choledochal cyst can be visualized, or the absence of a gallbladder may be noted. Measurement of amniotic gamma-glutamyl transferase (GGT) levels may then help to distinguish BA from congenital absence of the gallbladder, but amnioscentesis is then required, and the reference values are currently not well enough defined to allow for adequate discrimination.[9]

3.2 Diagnostic work-up

Ultrasonographic examination[72], hepatobiliary scintigraphy, endoscopic retrograde cholangiopancreatography (ERCP)[83,136,193], magnetic resonance cholangiopancreato-graphy (MRCP)[95], duodenal tube tests (continuous aspiration via a nasoduodenal tube, to assess presence of bile)[90], liver biopsy and laboratory investigations may aid in the diagnostic work-up. Yet, the gold standard for the diagnosis of BA is a peroperative cholangiography. However, ultrasonography may yield high accuracy (98%) in diagnosis upon careful evaluation of multiple features (gallbladder morphology, triangular cord sign, presence of common bile duct, liver size and echotexture, splenic appearance, vascular anatomy) by an experienced operator. It may therefore be a pre-selection tool before invasive diagnostic modalities are applied, but only in those centers where the case-load of infants with suspected BA is sufficiently large to become experienced.[72]

The histological findings of liver biopsies from BA patients include bile ductular proliferation, portal and periportal inflammation, fibrosis and bile plugs.[79] Biopsies from patients with alpha-1 antitrypsin deficiency, Alagille syndrome and cystic fibrosis may show an identical picture. In early presenting BA patients, there may be paucity of bile ducts rather than ductular proliferation, the former being a typical finding in Alagille's syndrome. Serial biopsies may then aid in differentiation between the two diseases.[8] Periodic acid-Schiff staining preceded by diastase digestion may visualize alpha-1 antitrypsin deposits, but those are difficult to recognize in early infancy.[97] Giant cell transformation is found in at least 25% of BA patients, particularly in early presenting patients, which makes differentiation with other causes of neonatal hepatitis difficult.[8,79,163]

3.3 Primary treatment and prognostic factors

BA is uniformally lethal when untreated. In 1959 the Japanese surgeon Morio Kasai described a novel

technique termed portoenterostomy, in which the fibrotic bile ducts are removed with transection of the porta hepatis to expose the remaining ductules. Subsequently, a Roux-en-Y bowel loop is constructed which is attached to the porta hepatis (Figure 3).[82] Variants called choledochojejunostomy or hepaticojejunostomy can be utilized to treat type I and type II BA, respectively.

The development of Kasai portoenterostomy would prove to be a major breakthrough in BA treatment and has markedly improved the prognosis of the disease, with 4-year transplant-free survival rates of 40-50% nowadays in most Western countries.[32,151,203] The success of treatment is measured by the rates of clearance of jaundice (normalization of bilirubin levels within 6 months post-surgery) and transplant-free survival. Complete clearance of jaundice and full restoration of excretory and synthetic liver functions are feasible, although inflammation and liver damage may continue despite adequate bile drainage.[124]

The most important potentially influenceable parameters determining the outcome of Kasai portoenterostomy have been shown to be age at surgery and center case-load. Most large series show concordantly that early surgery leads to better short-term survival.[147,152,203] A true age threshold has not been determined, but most studies find better survival when surgery is performed before the age of 60 days. Some studies detected additional benefits when surgery is

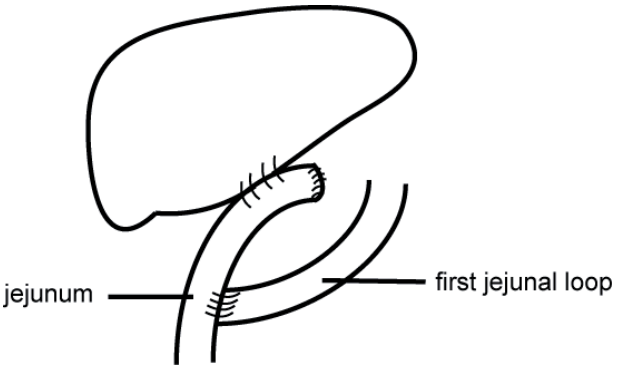

Figure 3: Schematic representation of the anatomical situation after the Kasai portoenterostomy procedure.

performed before 45 days, with a reported 4-year transplant-free survival of 75%, versus 33% when surgery is performed at 47-70 days.[152] Even surgery performed before 30 days has been shown to be beneficial, with a 5-year transplant-free survival rate of 58% compared to 41% when surgery is performed at 31-45 days of age.[152] In another study, a 4-year transplant-free survival rate of 49% was achieved when surgery was performed before 30 days, compared to 36% when surgery was performed between 31 and 90 days of age.[147] However, other studies do not show a clear benefit of early surgery. In a study of 225 patients, the effect of age at surgery on clearance of jaundice and transplant-free survival was not significant in isolated BA, whereas increased age had significant negative effects in BASM and cystic BA (type I BA with cyst formation).[26] In a study from Hong Kong, the one-year transplant-free survival rate was higher in patients operated on between 61 and 80 days (81%) when compared to surgery performed before 60 days (54%).[205]

It has been proposed that late presenting patients (>90 days) should be subjected to primary OLT rather than Kasai portoenterostomy. However, transplant-free survival rates of 20-40% after 10 years, and of 4% beyond 20 years are reported in children operated on >90 days. Based on

these numbers, it is advised to perform Kasai surgery first, even on late presenting patients.
The effect of centralization on the outcome after Kasai portoenterostomy was investigated in two studies (see Table 1). In England and Wales it was shown that centers performing at least five Kasai procedures a year had a higher 5-year transplant-free survival. Therefore, a government-induced centralization policy was instituted resulting in three BA treatment centers.[32] In France, a decentralized policy was introduced, where large treatment centers serve as a source of expertise for the smaller centers. From those two studies it was concluded that surgical treatment for BA should be centralized. Center size effects have also been studied in Canada, where beneficial outcomes in the large treatment center (>5 cases/year) were only demonstrated in the BA patients operated on between 45 and 90 days of age, and not before 45 days.[146]

Patients with BA subtypes I and II tend to have better survival rates compared to those with subtype III.[128,151] Mortality of patients with BASM was higher than that of isolated BA patients in three large cohorts, which has been speculated to relate with intrinsically worse liver disease, susceptibility to infections and increased progression to pulmonary hypertension.[26,29,151,159,178] In smaller series, no differences could be detected between isolated BA and BASM.[191,192]

3.4 Liver transplantation

The second surgical breakthrough in the treatment of BA was the advent of orthotopic liver transplantation (OLT), which could at first only be performed in older children but can now be performed successfully even in small infants. One-year patient survival rates after OLT for BA have risen to ~90%, and 10-year survival to ~80%.[10,47,184,195] OLT is indicated in BA patients with decompensated liver cirrhosis, failure to thrive, frequent cholangitis or hepatopulmonary syndrome. BA is the most common indication for OLT in childhood, accounting for ~50% of the cases.

Novel transplant techniques such as reduced and split liver grafts and living donor grafts have increased the availability of donor organs, to counteract the waiting list. The outcome after use of these alternative allograft types has become similar compared to the use of whole grafts.[12,38,117] The timing of OLT is very important. The pediatric end-stage liver disease (PELD) score (calculated from growth parameters, serum bilirubin and albumin levels, and the International Normalized Ratio) has been developed as an objective measure for the need for OLT in children with end-stage liver disease.[108] The score has been shown to adequately predict waiting list mortality and post-transplant mortality, and implementation of this scoring system has been shown to improve survival rates.[10,109,184]

The success of Kasai portoenterostomy and OLT as treatment modalities was facilitated by many advances in general medical treatment. These allowed for good quality of care in the pre-operative and post-operative phase, contributing to the ultimate outcome after Kasai and OLT.

3.5 Outcome of BA

Currently, the 20-year transplant-free survival of BA patients is ~25-30%. In a subset of these patients, the achieved restoration of bile flow may persist for many years thereafter. Only prolonged follow-up will reveal whether Kasai will be a definitive cure in these patients. However, the majority is expected to need OLT at some point.[103] It is not known how the incidence of malignancies in BA patients relates to that of other causes of fibrosis and cirrhosis. Therefore, lifelong specialized follow-up is of importance for BA patients. There are indications that liver function may deteriorate substantially during pregnancy in female BA patients.[88]

Table 1: Outcomes of BA patients according to annual case load per treatment center

Case load (cases/center/year)	4-year transplant-free survival	4-year overall survival	4-year transplant-free survival	4-year overall survival
	Before centralization		*After centralization*	
England, Wales [32] 1999-2002	n=91	n=91	n=148	n=148
< 5	14%*	91%*		
>5	61%*	75%*		
≥20			51%	89%
France [151] 1986-2002	n=438	n=465	n=252	n=270
≤2	34%	63%	33%	79%
3-5	31%	61%	45%	87%
≥20	48%	81%	48%	90%
	No centralization			
Canada [146] 1999-2002	n=299	n=299		
>5	44%	83%		
<5	36%	84%		

*5-year survival rates reported

Generally, it is assumed that most BA patients manage to lead normal lives, but detailed analyses concerning course of life and health-related quality of life are scarce. One study compared the quality of life of adolescent transplant-free BA patients from Japan and the UK with their respective reference groups and concluded that BA patients experience a normal quality of life.[70]

Studies in young (aged <2 years) BA patients revealed that language and gross motor skills are impaired in OLT candidates.[19] One third of 40 studied BA patients was considered developmentally delayed one year post-OLT.[198] Even after an average post-OLT follow-up of 9.2 years, the working memory and visuo-spatial functions appeared to be deficient in a group of thirteen BA patients.[213] Thus, the disease seems to have major developmental consequences. Currently, there are no detailed studies on the achievement of developmental milestones in BA patients on the longer term.

4. FUTURE PERSPECTIVES

4.1 Screening

The major gain in the prognosis of BA may be achieved by getting the child under specialized medical attention early in order to assure timely surgery. Until now, there is no suitable mass screening tool. Conjugated bile acids in dried blood spots are elevated in children with BA.[123] However, the considerable overlap with healthy subjects makes the test unfit for mass screening. Sulfated bile acids measured in urine are not useful as a screening tool because of the high false positive rate, but may be used as a noninvasive diagnostic tool in jaundiced infants.[121] As BA is a rare disease, it is important

that primary care givers are made aware of the importance of avoidance of diagnostic delay. Tools such as the stool color card may be very helpful.

4.2 Adjuvant therapies

Problems encountered in a child with insufficient or borderline sufficient bile flow are fat malabsorption and fat-soluble vitamin deficiency. This may lead to growth failure, particularly when combined with an increased energy expenditure. Growth failure is related to poor outcome of BA and is therefore used as a selection criterium for OLT candidates.[36,108] Furthermore, growth failure is correlated with developmental delay in BA patients.[19,198] Early aggressive nutritional support seems warranted in patients with poor growth, but further studies should detail whether enhancement of growth and outcome is feasible. Plasma levels of fat soluble vitamins should be monitored regularly, and supplemented if necessary.[158]

Disturbances in essential fatty acid and cholesterol metabolism have been described even in BA patients with normal bilirubin levels after the Kasai procedure, but the clinical implications remain to be established.[132,169]

BA patients are susceptible to cholangitis, particularly after Roux-en-Y enterostomy, caused by ascending gut flora. Cholangitis is thought to accelerate liver damage and can be life-threatening in patients with end-stage liver disease. Prophylactic antibiotics are therefore often used post-Kasai, although the effects on outcome of different antibiotic regimens have never been studied in a randomized fashion.

It is thought that the hydrophilic choleretic bile acid ursodeoxycholic acid (UDCA) may promote bile flow in BA patients. A study in 16 BA patients with successful portoenterostomies showed that cessation of UDCA therapy resulted in a deterioration of liver biochemistry parameters.[204] The effects on outcome and effects in patients with insufficient bile flow have barely been studied.

The use of steroids post-Kasai as inhibitors of inflammation and bile flow promoting agents has been studied quite extensively, with conflicting results. In the single randomized, double-blind placebo controlled trial performed, with a low dosage prednisolone (2 mg/kg/day), effects on clearance of jaundice and transplant-free survival were not detected.[30] The same was true for an open-labeled study of high-dose methylprednisolone (10 mg/kg/day).[135] However, due to the relative rarity of BA, these studies are typically underpowered and subtle effects can not be detected. A systematic review of 20 studies containing 1820 infants (1175 receiving steroids) showed an improvement of clearance of jaundice and survival with steroid therapy.[207] Only large-scale studies can provide a definite answer.

5. CONCLUSIONS

Despite extensive research efforts, the cause of BA remains largely unknown. More knowledge regarding the pathophysiological processes may provide new targets for screening, intervention and eventually disease prevention, for which the recent advances in the animal models may be of great utility. Due to the development of Kasai's portoenterostomy and liver transplantation, this previously lethal disease has now reasonable survival rates. The focus has shifted towards improving transplant-free survival and the developmental consequences. Early detection of the disease is expected to improve the survival further. By current lack of a biochemical screening tool this may best be achieved by stool color cards. Furthermore, adjuvant therapies such as post-surgical

administration of prophylactic antibiotics and UDCA, and intensive nutritional support deserve more research to assess the potential benefits. As BA is a rare disease, studies should be performed on an (inter)national basis.

AIM OF THE THESIS

The aim of the thesis was twofold. First, to evaluate the treatment of BA in the Netherlands by the aid of the national NeSBAR (Netherlands Study group on Biliary Atresia Registry) database. This is a detailed database spanning two decades. As detailed data concerning outcomes in adulthood are scarce, we also studied the outcomes of a cohort of BA patients originally described in 1989, before NeSBAR was established.[68] The Dutch treatment results were compared to those reported from other Western countries. Second, by the aid of these data we aimed to find strategies to further improve the outcome of BA. In this respect, we did not only focus on (transplant-free) survival parameters, but also studied functioning of BA patients who have reached adult age. Finally, we adopted the murine BA mouse model in order to study disease mechanisms and potential therapeutical targets.

In the first three chapters, we focused on outcomes and prognostic factors in infancy and early childhood. In **chapter 2**, we examined the occurrence of pre-transplant mortality in Dutch BA patients and investigated how pre-transplant mortality might be prevented. As it was known from a previous study that breast-fed cholestatic infants in the Netherlands had a high incidence of vitamin K deficiency-related bleeding, we examined the incidence of vitamin K deficiency-related bleeding in cholestatic infants fed with hydrolyzed formula in **chapter 3**.[187] Finally, an overall analysis of the clearance of jaundice, transplant-free survival and overall survival of BA patients in the Netherlands was performed in **chapter 4**. There is still considerable debate on when Kasai portoenterostomy should be performed, and very few studies have assessed the effects of adjuvant medicaments. Therefore, we set out to explore the effects of (among others) age at Kasai portoenterostomy, post-surgical antibiotic prophylaxis administration and post-surgical UDCA administration on the prognosis of BA patients by the aid of multivariate analyses.

In the next three chapters, outcomes and prognostic factors of adult non-transplanted and transplanted BA patients were studied. The outcomes at 20 years post-surgery were analyzed in **chapter 5**. As little is known on the long-term course of BA after Kasai, we delineated the clinical condition of the transplant-free patients at the age of 20. Because long-term survival is now possible in a major part of BA patients, the impact of disease on patients' lives become an important outcome measure as well. A detailed study on the health-related quality of life of young adult BA patients, both transplant-free and transplanted, was performed in **chapter 6**. By the aid of the RAND-36 questionnaire, the results of BA patients were compared to those of a Dutch age-matched comparison group. We aimed to identify patient groups at risk for health-related quality of life problems, and correlated quality of life to disease state. Very little is known about the impact of chronic liver disease with very early onset on course of life, i.e. the achievement of developmental milestones. As BA is a life-threatening disease, requiring major surgery, one might expect that it has major consequences on course of life. Therefore, we studied the course of life of young adult transplanted and non-transplanted BA patients in detail in **chapter 7**, and aimed to examine whether additional support is required in BA patients to normalize the course of life. For this purpose, the Course of Life questionnaire was used, and the results of the BA patients were compared to a Dutch age-matched comparison group.

In other cholestatic diseases, it has been shown (both in several animal models and human liver tissue specimens) that the Hedgehog signaling cascade might be an important mechanism in the induction of liver fibrogenesis. In a pilot study described in **chapter 8**, we aimed to assess the presence of this signaling pathway in the disease process of the murine BA model, and to characterize the changes in biliary epithelial cells which may be linked to Hedgehog signaling.

For this purpose, we examined components of the Hedgehog signaling cascade and epithelial markers by mRNA expression levels and immunohistochemistry. Studies were performed in liver tissues of BA mice at different disease stages, and control mice.