



**BRAZILIAN JOURNAL**  
OF MEDICAL AND BIOLOGICAL RESEARCH

[www.bjournal.com.br](http://www.bjournal.com.br)

ISSN 0100-879X

Volume 43 (6) 522-599 June 2010

CLINICAL INVESTIGATION

**Braz J Med Biol Res, June 2010, Volume 43(6) 522-527**

doi: 10.1590/S0100-879X2010007500035

## Advances in biliary atresia: from patient care to research

J.L. Santos, E. Carvalho and J.A. Bezerra

The Brazilian Journal of Medical and Biological Research is partially financed by



Ministério  
da Ciência e Tecnologia



Ministério  
da Educação



### *Institutional Sponsors*



GE Healthcare

Hotsite of proteomics metabolomics  
developed by:



# Advances in biliary atresia: from patient care to research

J.L. Santos<sup>1</sup>, E. Carvalho<sup>2</sup> and J.A. Bezerra<sup>3</sup>

<sup>1</sup>Laboratório Experimental de Hepatologia e Gastroenterologia, Hospital de Clínicas, Universidade Federal of Rio Grande do Sul, Porto Alegre, RS, Brasil

<sup>2</sup>Unidade de Pediatria, Hospital de Base do Distrito Federal, Brasília, DF, Brasil

<sup>3</sup>Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

## Abstract

Biliary atresia, the most common cause of liver transplantation in children, remains a challenge for clinicians and investigators. The development of new therapeutic options, besides the typical hepatoportoenterostomy, depends on a greater understanding of its pathogenesis and how it relates to the clinical phenotypes at diagnosis and the rate of disease progression. In this review, we present a perspective of how recent research has advanced the understanding of the disease and has improved clinical care protocols. Molecular and morphological analyses at diagnosis point to the potential contributions of polymorphism in the *CFC1* and *VEGF* genes, to the pathogenesis of the disease, and to an association between the degree of bile duct proliferation and long-term outcome. In experimental models, cholangiocytes do not appear to have antigen-presenting properties despite a substantial innate and adaptive immune response that targets the biliary epithelium and produces duct obstruction. Initial clinical trials assessing the efficacy of corticosteroids in decreasing the inflammation and improving outcome do not show a superior effect of corticosteroids as an adjuvant treatment following hepatoportoenterostomy. The best outcome still remains linked to an early diagnosis and surgical treatment. In this regard, the Yellow Alert campaign by the Sociedade Brasileira de Pediatria and the inclusion of the Stool Color Card in the health booklet given to every neonate in Brazil have the potential to decrease the age of diagnosis, shorten the time between diagnosis and surgical treatment, and improve the long-term outcome of children with this devastating disease.

Key words: Biliary atresia; Etiology; Prognosis; Therapeutics

## Introduction

Biliary atresia (BA) is an infantile disorder characterized by the complete obstruction of a portion or the entire length of the extrahepatic bile duct caused by a fibro-inflammatory process that disrupts the flow of bile from the liver to the duodenum. Clinically, infants present with hepatomegaly, cholestatic jaundice beginning soon after birth or in the first few weeks of life, acholic stools, and choloria. There are two clinical forms of BA, congenital and perinatal. In the congenital form, the onset of jaundice begins soon after birth and associated with non-hepatic congenital anomalies, including the biliary atresia splenic malformation (BASM) syndrome. This contrasts to the perinatal form, in which signs of cholestasis appear during or beyond the second week of life, and infants have no congenital anomalies.

Although the liver histopathology of biliary atresia varies, expanded portal tracts with edema and bile duct prolifera-

tion with bile plugs in neoductules are the most frequent findings at diagnosis (1,2). Different levels of fibrosis and inflammation may be related to staging of the disease or to predominant biological processes producing the atresia phenotype (3,4). The histological features are more uniform in extrahepatic bile ducts, with concentric fibrosis and no residual epithelium. The best chances to restore bile flow is surgical portoenterostomy, a procedure that removes the remnants of extrahepatic bile ducts and sews a jejunal segment onto the porta hepatis in a roux-en-Y fashion. However, even when portoenterostomy is performed in a timely fashion, most patients experience progressive derangement of the intrahepatic biliary system, leading to fibrosis and eventually end-stage cirrhosis. At this time, liver transplantation is the only option for long-term survival, and represents the main indication for liver transplantation in

Correspondence: J.L. Santos, Hospital de Clínicas, Universidade Federal of Rio Grande do Sul, Rua Ramiro Barcelos, 2350, 90035-903 Porto Alegre, RS, Brasil. E-mail: kapars@terra.com.br

Received January 26, 2010. Accepted April 19, 2010. Available online April 30, 2010. Published June 11, 2010.

children worldwide (5). Although we are currently unable to interrupt the progression of liver disease or to improve long-term outcome with the native liver, research on biliary atresia has attracted the attention of clinicians and scientists alike. In this review, we discuss what they have reported

**Table 1.** Putative factors involved in the etiology of biliary atresia.

A - Genetic factors	
Genetic alterations	
	CFC1 mutation
	ICAM G241R polymorphism
	NFkappaB/c mutation
	VEGF A gene polymorphism
Numerical chromosomal abnormalities	
	Trisomies of chromosomes 10q, 17, 18, and 21
B - Environmental factors	
Drugs used during gestation	
	Amphetamines
	Alcohol
Maternal/neonatal infections	
	Cytomegalovirus
	Reovirus type 3
	Papillomavirus
	Rotavirus
Phytotoxins, mycotoxins	
Industrial toxins	
	DAPM
C - Others	
Structural anomalies	
	BASM
	Choledocopancreatic junction anomalies
	Ductal plate malformation
	Hepatic arterial anomalies
Metabolic abnormalities	
	Abnormal bile acids
	L-proline
Immunologic abnormalities	
	Maternal serologic auto-immunity
Immunogenetic alterations	
	Oligoclonal expansion of T lymphocytes
	NK lymphocytes
	Biliary aberrant MHC class expression
	Microchimerism
Maternal diabetes	
	Gestational diabetes
Maternal age	

VEGF = vascular endothelial growth factor; ICAM = inter-cellular adhesion molecule; BASM = biliary atresia splenic malformation; NK = natural killer; MHC = major histocompatibility complex.

recently. Their contribution increases our knowledge of the clinical phenotypes of disease, pathogenic mechanisms of biliary injury, and the effect of adjuvant medical therapy on the clinical course of children with biliary atresia.

## Broadening the phenotypes of disease

Although most infants with BA can be classified into the embryonic or perinatal forms, others have unique anatomical features that suggest the existence of other clinical subsets or variants. More recently, a review of 225 infants with BA from a single center identified a subset of 23 infants who had cystic formations in the biliary remnants (6). These infants were younger at diagnosis than those with the perinatal form, and as young as those with BASM. Notably, there was no apparent influence of age, of clearance of jaundice or 2-year survival with the native liver on infants with the acquired form, but there was a marked detrimental effect of age at Kasai operation for both the BASM and cystic forms. This report should lead to new studies examining the frequency of this anatomical variant and its relationship to clinical outcome. The existence of such variant raises the possibility that the pathogenesis of BA includes biological processes that may differ according to individual phenotypes, perhaps linked to genetic predisposition or influenced by environmental triggers (Table 1) (7).

## Developmental and genetic factors for biliary atresia

During human embryogenesis, the septum transversum takes on a ventral position where an endodermal projection of the ventral portion of the primitive gut (the hepatic diverticulum) gradually develops and gives rise to the liver. From the caudal segment of the hepatic diverticulum, cells differentiate to form the gallbladder and the cystic, hepatic, and common bile ducts. Adjacent endoderm cells undergo pancreatic specification and form the endocrine and exocrine pancreas. Despite the physical proximity of these cells, several genes have been mechanistically linked to the specification of the hepatobiliary or pancreatic organs (8) although the possibility exists that the two organ lineages may share common molecular pathways.

Recent evidence shows that the extrahepatic biliary system and the ventral pancreas may share common progenitor cells that are distinct from those that form the liver. Sox 17, a protein involved in the formation of endodermal organs, controls specification of the liver and bile ducts as demonstrated by the expression of pancreatic markers in the liver bud and by the presence of ectopic pancreatic tissue in the common duct in mouse embryos lacking Sox 17. Thus, Sox 17 is necessary to induce a ductal fate in endoderm cells, with the separation into the biliary or pancreatic lineages being regulated by Hes1, a protein belonging to the Notch signal that may act in a feedback loop with Sox 17 (9). Although

the extrahepatic biliary tract forms a single anatomic tract, another study showed that anatomically different segments may have unique molecular underpinnings. In this study, the embryogenesis of the gallbladder and cystic duct was shown to depend on the expression of the *Lgr4* gene, a member of the family of leucine-rich repeat-containing G protein-coupled receptors (10). In mice with mutant *Lgr4*, the hepatic and common bile ducts were normal, but there was complete absence of the gallbladder and cystic duct. The independent molecular circuits governing embryogenesis of segments of the biliary system raises the possibility that mutations in members of these circuits may constitute a genetic factor in the pathogenesis of BA.

Polymorphisms in other genes have already been linked to the pathogenesis of BA. Heterozygous mutations in the CFC1 gene, which encodes the CRYPTIC protein, have been reported in children with BASM syndrome (11). In a different set of patients, the vascular endothelial growth factor (VEGF) +936 C/T gene polymorphism, particularly the C allele, was associated with BA, possibly conferring increased susceptibility to the disease (12). VEGF is an angiogenic growth factor associated with cell-mediated inflammatory reactions. The angiogenic properties of VEGF are particularly relevant in view of the putative role of hypoxia in the pathogenesis of BA (13).

## Environmental triggers

Environmental factors potentially related to BA pathogenesis include maternal and/or neonatal infection, drugs used during pregnancy, toxic agricultural products, industrial toxins, as well as phyto- and mycotoxins. The hypothesis of a viral etiology has been reinforced by numerous contributions from studies using the rhesus rotavirus (RRV)-induced mouse model of BA (14). There is an intrinsic hyper-responsiveness of cellular immunity that renders the neonate more susceptible to virus- and lipopolysaccharide-induced morbidity. This hyper-response is temporally restricted to the early neonatal period and functionally linked to a lower T-cell number (15). Even if lower in number, neonatal T cells can activate a broad pro-inflammatory program in response to tissue infection, as demonstrated by the effective clearance of RRV from the liver and biliary tract by neonatal CD8<sup>+</sup> cells (16). A prominent pro-inflammatory footprint and an enriched population of CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes have been reported in the murine model, with evidence of an antigenic oligoclonal expansion (17-20). The loss of interferon-gamma (IFN- $\gamma$ ) or CD8<sup>+</sup> lymphocytes prevents obstruction of bile ducts, suppressing the disease phenotype, and, when virus-primed T cells are transferred to RRV-naïve recipients, they home to bile ducts and induce cholangitis. While clearing the virus, however, CD8<sup>+</sup> cells can secondarily injure the epithelium and produce the obstructive phenotype typical of experimental BA (16).

Some recent findings provide further insight into the

pathological mechanisms of RRV infection in relation to BA. In a series of experiments, investigators showed that RRV-infected cholangiocytes express both major histocompatibility complex (MHC) class I and aberrant MHC class II (21). Although they had all necessary markers for effective antigen presentation, cultured cholangiocytes were not shown to function as antigen-presenting cells in T-cell proliferation assays. Instead, cholangiocytes appeared to modulate the immune response by producing pro-inflammatory cytokines and chemokines. In another study, the susceptibility of cholangiocytes to RRV infection was found to be dependent on the interactions between RRV and cholangiocytes through  $\alpha 2\beta 1$  integrin (22). Notably, blockade of this molecule resulted in a significant reduction in virus attachment and virus yield in RRV-infected cells, and pretreatment of newborn mice with anti- $\alpha 2\beta 1$  antibody reduced the ability of RRV to produce the full phenotype of BA in neonatal mice.

Examining the biological fate of cholangiocytes following RRV infection, another study found an increased number of cholangiocytes displaying markers of apoptosis in bile ducts of infected neonatal mice (23). Apoptosis was also induced when cholangiocytes were exposed to IFN- $\gamma$  and tumor necrosis factor-alpha (TNF- $\alpha$ ) simultaneously, but not individually. These findings suggest that apoptosis of the biliary epithelium results from at least two separate mechanisms: 1) direct consequences of a viral infection, and 2) increased concentration of pro-inflammatory cytokines (such as IFN- $\gamma$  and TNF- $\alpha$ ) in the biliary microenvironment. The additional finding that the administration of a caspase inhibitor decreased apoptosis and the extent of epithelial injury is consistent with the potential role of this therapeutic approach to ameliorate the disease phenotype or to slow down the progression of liver disease following portoenterostomy.

## Innate immunity and pathogenesis of the disease

Recent studies examined how the neonatal innate immune system contributes to the injury of the duct epithelium in BA. First, analyzing the livers of infants at the time of diagnosis, investigators found that natural killer (NK) cells populate the vicinity of intrahepatic bile ducts and over-express several genes involved in cytotoxicity (24). Activated NK cells also populated neonatal murine livers in the RRV-induced mouse model and were the most abundant cells in extrahepatic bile ducts at the time of obstruction. To investigate how NK cells might be involved in the experimental model, the authors used cell culture systems and *in vivo* manipulation assays. In cell cultures, they found that RRV-primed hepatic NK cells lysed cholangiocytes in a contact- and NKG2D receptor-dependent fashion. *In vivo*, the investigators used antibodies to deplete NK cells or to block NKG2D. Both types of antibodies prevented

injury of the duct epithelium after RRV infection as well as the obstruction of the duct lumen. As a consequence, bile ducts remained patent and maintained continuity between the liver and duodenum despite the presence of virus in the tissue. These findings point to epithelial integrity as a key factor in the pathogenesis of experimental BA, and to the critical role of NK cells, and not necessarily of RRV, in targeting cholangiocytes.

Cholangiocytes actively participate in innate immunity by expressing antimicrobial molecules such as defensin and lysozyme, as well as Toll-like receptors (TLR), specifically TLR3, which recognize pathogen-associated molecular patterns (PAMPs), such as the RRV double-stranded RNA (dsRNA). Repeated signaling through TLR in response to PAMPs usually induces TLR tolerance. To examine the contribution of this molecular circuit to BA, investigators exposed cultured biliary epithelial cells to poly(I:C), a synthetic analogue of viral dsRNA, and found that they did not induce TLR tolerance (25). However, they maintained significant up-regulation of molecules involved in biliary injury and apoptosis, even after the end of poly(I:C) stimulation. This unique biliary innate immune response to dsRNA may be related to the progressive obstructive cholangiopathy occurring in BA patients.

### Epithelial-mesenchymal transition in the pathogenesis of the disease

Proliferation of intrahepatic bile ducts is a consistent feature of the liver at diagnosis. Although this has been traditionally linked to a reactive hyperplasia secondary to the obstruction of bile flow, recent studies uncovered a potential relationship with clinical outcome and a change in cellular plasticity to a mesenchymal phenotype that may be related to the excessive fibrosis typically seen in BA. In the first study, the investigators quantified the proliferation of bile ducts by morphometric analysis of the percentage of CK7-positivity (PCK7) in livers of patients with BA at the time of portoenterostomy. PCK7 correlated with a poor postoperative prognosis, suggesting that it might serve as a histological predictor of clinical course (26). There is increasing evidence that cholangiocytes may be an important source of fibrogenesis either indirectly by activating fibrogenic cells through cytokine release, or directly by tilting the balance toward matrix accumulation. In studies using human tissues, investigators reported that cells lining small- and medium-sized bile ducts and those of proliferated bile ducts from patients with BA and other liver diseases undergo epithelial-mesenchymal transition (EMT) and form invasive myofibroblasts (27). In another study, experiments using cultured human cholangiocytes revealed that stimulation with the viral dsRNA analogue poly(I:C) increased the expression of EMT inducers, mesenchymal markers and transcriptional factors associated with EMT, while decreasing the expression of epithelial markers (28). In tissue

culture, treatment of cholangiocytes with the EMT-inducers basic fibroblast growth factor (bFGF) and TGF- $\beta$ 1 evoked the typical EMT response, with features of transformation into myofibroblasts. Other data suggesting a potential role for EMT in the sclerosing cholangiopathy of BA include the lack of epithelial markers and the aberrant expression of the mesenchymal marker vimentin by epithelial cells lining extrahepatic bile ducts and peribiliary glands, as well as the expression of bFGF by the biliary epithelium affected by the sclerosing injury.

### Early diagnosis and clinical outcome

The contemporary management of BA includes portoenterostomy and its variants in the first weeks of life as the primary surgical therapy, aiming at restoring bile flow. When bile flow is not successful, the timely use of liver transplantation offers the best chance for long-term survival. Liver transplantation should be delayed as long as possible to permit maximum growth and development, but must be pursued promptly at the onset of substantial complications of end-stage cirrhosis. Survival with the native liver of patients demonstrating evidence of improved bile flow for 20 years after portoenterostomy has been reported in up to 20% of patients, and for 30 years in up to 10%, with a good quality of life (5,29). Complications of biliary atresia in survivors included ascending bacterial cholangitis, mostly in the first months of life; portal hypertension, present in about two thirds of patients; intrahepatic biliary cysts, which may become infected or compress the portal vein; hepatopulmonary syndrome or pulmonary hypertension, and malignancies.

Some clinical factors add substantial challenges to the initial operation and to the postoperative medical treatment of infants with BA, such as the presence of severe cardiovascular malformations. Their presence often leads to a poor outcome. Other factors, such as the experience of the center in the management of patients with BA and the accessibility to liver transplantation, influence the outcome and constitute opportunities for intervention. Another factor is age at the time of portoenterostomy, which is thought to influence the postoperative course. In a recent review of the outcome of a large patient population, investigators showed that increased age at portoenterostomy had a progressive and deleterious effect on the surgical outcome, with survival rates decreasing with increasing age at surgery (30). The authors estimated that if portoenterostomy were performed before 46 days of life, up to 5.7% of the liver transplantations performed in France in patients younger than 16 years of age could be avoided. A delay in referral and surgery remains a problem worldwide because of difficulties in differentiating BA from other causes of prolonged jaundice in the young infant (31). In Taiwan, a universal screening system was established using an infant stool color card previously tested in other countries. By this method, the sensitivity of

detecting BA before 60 days of age reached 97.1%, and both the national rate of portoenterostomies before 60 days of life and the jaundice-free rate at 3 months after surgery increased significantly (32).

### Adjuvant therapy following portoenterostomy

Different drugs have been proposed for the management of BA following portoenterostomy. In this patient population, some of the aims of adjuvant therapies are to reduce the inflammatory process and to increase bile flow. Both effects are presumed to be promoted by corticosteroids. Different regimens of corticosteroids have been reported to improve clinical outcome in a number of retrospective, uncontrolled reports. To more adequately examine the relationship between corticosteroids and clinical outcome of infants with BA, two large centers in the United Kingdom performed the first prospective, placebo-controlled, double-blind, randomized clinical trial, using 2 mg·kg<sup>-1</sup>·day<sup>-1</sup> oral prednisolone starting 1 week postoperatively (33). The study did not show a significant improvement in long-term survival with the native liver in the corticosteroid-treated group when compared to placebo controls. Another European prospective, open-label, non-randomized study reported that high-dose corticosteroids did not improve survival with the native liver over historical controls not treated with corticosteroids (34). While these studies do not support the previous reports of the benefits produced by the use of corticosteroids, more definitive studies must be done with the inclusion of a larger, statistically powered patient population to conclusively answer the question of whether corticosteroids are beneficial to infants when administered during the immediate postoperative period.

Ursodeoxycholic acid, a hydrophilic bile acid used in

the treatment of some adult and pediatric liver diseases, is frequently used as an adjuvant therapy for children with BA. In an "on-off-on" study, investigators found that the use of ursodeoxycholic acid was associated with improved markers of bile flow (35). Despite the lack of randomization or placebo control, this is an important report supporting a potential benefit provided by ursodeoxycholic acid when used in infants following portoenterostomy for biliary atresia.

### Conclusion

Biliary atresia remains a challenge for investigators and clinicians. Investigators are continually challenged by the need to decipher the cellular and molecular events that initiate biliary injury, promote duct obstruction, and facilitate progression to atresia. Their work holds the promise for new therapies to stop progression of the disease, and perhaps novel ways to prevent the disease altogether. Clinicians face a dual challenge. First, they seek to establish an early diagnosis permitting patients to undergo a timely portoenterostomy and to receive early management of complications of liver disease. Second, they are also challenged to conduct studies to critically evaluate the efficacy of current practice protocols. In Brazil, we are facing the dawn of a new era in the management of BA. We have chosen to start with a focus on improving the timely diagnosis of the condition. Our strategy is simple: the addition of the stool color card to the health booklet given to every neonate by means of the Yellow Alert campaign launched by the Sociedade Brasileira de Pediatria and the creation of a Brazilian Biliary Atresia Study Group. In addition to improving the outcome of children by an early diagnosis of BA, we also aim to explore prospective multicenter studies on the etiology and treatment of this devastating disease.

### References

- Zerbini MC, Gallucci SD, Maezono R, Ueno CM, Porta G, Maksoud JG, et al. Liver biopsy in neonatal cholestasis: a review on statistical grounds. *Mod Pathol* 1997; 10: 793-799.
- Santos JL, Almeida H, Cerski CT, Silveira TR. Histopathological diagnosis of intra- and extrahepatic neonatal cholestasis. *Braz J Med Biol Res* 1998; 31: 911-919.
- Desmet V. Pathology of paediatric cholestasis. In: Anonymous, *Falk Symposium 63. Paediatric Cholestasis. Novel Approaches to Treatment*. London: Kluwer Academic Publishers; 1992. p 49-54.
- Pacheco MC, Campbell KM, Bove KE. Ductal plate malformation-like arrays in early explants after a Kasai procedure are independent of splenic malformation complex (heterotaxy). *Pediatr Dev Pathol* 2009; 12: 355-360.
- Lykavieris P, Chardot C, Sokhn M, Gauthier F, Valayer J, Bernard O. Outcome in adulthood of biliary atresia: a study of 63 patients who survived for over 20 years with their native liver. *Hepatology* 2005; 41: 366-371.
- Davenport M, Caponcelli E, Livesey E, Hadzic N, Howard E. Surgical outcome in biliary atresia: etiology affects the influence of age at surgery. *Ann Surg* 2008; 247: 694-698.
- Bezerra JA. The next challenge in pediatric cholestasis: deciphering the pathogenesis of biliary atresia. *J Pediatr Gastroenterol Nutr* 2006; 43 (Suppl 1): S23-S29.
- Bezerra JA. Liver development: a paradigm for hepatobiliary disease in later life. *Semin Liver Dis* 1998; 18: 203-216.
- Spence JR, Lange AW, Lin SC, Kaestner KH, Lowy AM, Kim I, et al. Sox17 regulates organ lineage segregation of ventral foregut progenitor cells. *Dev Cell* 2009; 17: 62-74.
- Yamashita R, Takegawa Y, Sakumoto M, Nakahara M, Kawazu H, Hoshii T, et al. Defective development of the gall bladder and cystic duct in Lgr4- hypomorphic mice. *Dev Dyn* 2009; 238: 993-1000.
- Davit-Spraul A, Baussan C, Hermeziu B, Bernard O, Jacquemin E. CFC1 gene involvement in biliary atresia with polysplenia syndrome. *J Pediatr Gastroenterol Nutr* 2008; 46: 111-112.

12. Lee HC, Chang TY, Yeung CY, Chan WT, Jiang CB, Chen WF, et al. Genetic variation in the vascular endothelial growth factor gene is associated with biliary atresia. *J Clin Gastroenterol* 2010; 44: 135-139.
13. dos Santos JL, da Silveira TR, da Silva V, Cerski CT, Wagner MB. Medial thickening of hepatic artery branches in biliary atresia. A morphometric study. *J Pediatr Surg* 2005; 40: 637-642.
14. Mack CL, Tucker RM, Lu BR, Sokol RJ, Fontenot AP, Ueno Y, et al. Cellular and humoral autoimmunity directed at bile duct epithelia in murine biliary atresia. *Hepatology* 2006; 44: 1231-1239.
15. Adkins B. Development of neonatal Th1/Th2 function. *Int Rev Immunol* 2000; 19: 157-171.
16. Shivakumar P, Sabla G, Mohanty S, McNeal M, Ward R, Stringer K, et al. Effector role of neonatal hepatic CD8+ lymphocytes in epithelial injury and autoimmunity in experimental biliary atresia. *Gastroenterology* 2007; 133: 268-277.
17. Bezerra JA, Tiao G, Ryckman FC, Alonso M, Sabla GE, Shneider B, et al. Genetic induction of proinflammatory immunity in children with biliary atresia. *Lancet* 2002; 360: 1653-1659.
18. Mack CL, Falta MT, Sullivan AK, Karrer F, Sokol RJ, Freed BM, et al. Oligoclonal expansions of CD4+ and CD8+ T-cells in the target organ of patients with biliary atresia. *Gastroenterology* 2007; 133: 278-287.
19. Mack CL, Tucker RM, Sokol RJ, Karrer FM, Kotzin BL, Whittington PF, et al. Biliary atresia is associated with CD4+ Th1 cell-mediated portal tract inflammation. *Pediatr Res* 2004; 56: 79-87.
20. Shinkai M, Shinkai T, Puri P, Stringer MD. Increased CXCR3 expression associated with CD3-positive lymphocytes in the liver and biliary remnant in biliary atresia. *J Pediatr Surg* 2006; 41: 950-954.
21. Barnes BH, Tucker RM, Wehrmann F, Mack DG, Ueno Y, Mack CL. Cholangiocytes as immune modulators in rotavirus-induced murine biliary atresia. *Liver Int* 2009; 29: 1253-1261.
22. Jafri M, Donnelly B, Allen S, Bondoc A, McNeal M, Rennert PD, et al. Cholangiocyte expression of alpha2beta1-integrin confers susceptibility to rotavirus-induced experimental biliary atresia. *Am J Physiol Gastrointest Liver Physiol* 2008; 295: G16-G26.
23. Erickson N, Mohanty SK, Shivakumar P, Sabla G, Chakraborty R, Bezerra JA. Temporal-spatial activation of apoptosis and epithelial injury in murine experimental biliary atresia. *Hepatology* 2008; 47: 1567-1577.
24. Shivakumar P, Sabla GE, Whittington P, Chougnet CA, Bezerra JA. Neonatal NK cells target the mouse duct epithelium via Nkg2d and drive tissue-specific injury in experimental biliary atresia. *J Clin Invest* 2009; 119: 2281-2290.
25. Harada K, Sato Y, Isse K, Ikeda H, Nakanuma Y. Induction of innate immune response and absence of subsequent tolerance to dsRNA in biliary epithelial cells relate to the pathogenesis of biliary atresia. *Liver Int* 2008; 28: 614-621.
26. Santos JL, Kieling CO, Meurer L, Vieira S, Ferreira CT, Lorentz A, et al. The extent of biliary proliferation in liver biopsies from patients with biliary atresia at portoenterostomy is associated with the postoperative prognosis. *J Pediatr Surg* 2009; 44: 695-701.
27. Diaz R, Kim JW, Hui JJ, Li Z, Swain GP, Fong KS, et al. Evidence for the epithelial to mesenchymal transition in biliary atresia fibrosis. *Hum Pathol* 2008; 39: 102-115.
28. Harada K, Sato Y, Ikeda H, Isse K, Ozaki S, Enomae M, et al. Epithelial-mesenchymal transition induced by biliary innate immunity contributes to the sclerosing cholangiopathy of biliary atresia. *J Pathol* 2009; 217: 654-664.
29. Howard ER, MacLean G, Nio M, Donaldson N, Singer J, Ohi R. Survival patterns in biliary atresia and comparison of quality of life of long-term survivors in Japan and England. *J Pediatr Surg* 2001; 36: 892-897.
30. Serinet MO, Wildhaber BE, Broue P, Lachaux A, Sarles J, Jacquemin E, et al. Impact of age at Kasai operation on its results in late childhood and adolescence: a rational basis for biliary atresia screening. *Pediatrics* 2009; 123: 1280-1286.
31. Kieling CO, Santos JL, Vieira SM, Ferreira CT, Linhares AR, Lorentz AL, et al. Biliary atresia: we still operate too late. *J Pediatr* 2008; 84: 436-441.
32. Hsiao CH, Chang MH, Chen HL, Lee HC, Wu TC, Lin CC, et al. Universal screening for biliary atresia using an infant stool color card in Taiwan. *Hepatology* 2008; 47: 1233-1240.
33. Davenport M, Stringer MD, Tizzard SA, McClean P, Mieli-Vergani G, Hadzic N. Randomized, double-blind, placebo-controlled trial of corticosteroids after Kasai portoenterostomy for biliary atresia. *Hepatology* 2007; 46: 1821-1827.
34. Petersen C, Harder D, Melter M, Becker T, Wasielewski RV, Leonhardt J, et al. Postoperative high-dose steroids do not improve mid-term survival with native liver in biliary atresia. *Am J Gastroenterol* 2008; 103: 712-719.
35. Willot S, Uhlen S, Michaud L, Briand G, Bonneville M, Sfeir R, et al. Effect of ursodeoxycholic acid on liver function in children after successful surgery for biliary atresia. *Pediatrics* 2008; 122: e1236-e1241.