Screening and Outcomes in Biliary Atresia: Summary of a National Institutes of Health Workshop

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Biliary atresia is the most common cause of end-stage liver disease in the infant and is the leading pediatric indication for liver transplantation in the United States. Earlier diagnosis (<30-45 days of life) is associated with improved outcomes following the Kasai portoenterostomy and longer survival with the native liver. However, establishing this diagnosis is problematic because of its rarity, the much more common indirect hyperbilirubinemia that occurs in the newborn period, and the schedule for routine infant health care visits in the United States. The pathogenesis of biliary atresia appears to involve immune-mediated fibro-obliteration of the extrahepatic and intrahepatic biliary tree in most patients and defective morphogenesis of the biliary system in the remainder. The determinants of the outcome of portoenterostomy include the age at surgery, the center's experience, the presence of associated congenital anomalies, and the postoperative occurrence of cholangitis. A number of screening strategies in infants have been studied. The most promising are early measurements of serum conjugated bilirubin and a stool color card given to new parents that alerts them and their primary care provider to acholic stools. This report summarizes a National Institutes of Health workshop held on September 12 and 13, 2006, in Bethesda, MD, that addressed the issues of outcomes, screening, and pathogenesis of biliary atresia. (HEPATOLOGY **2007;46:566-581.**)

Biliary atresia (BA) is the most common pediatric cause of cirrhosis, end-stage liver disease, and indication for liver transplantation in children. It occurs in 1 in 5000 to 1 in 18,000 live births and is characterized by persistent jaundice and progressive cholestasis developing within weeks of birth, which are caused by a progressive fibro-obliterative obstruction of extrahepatic and intrahepatic bile ducts.[1-4] The disease is difficult to identify during the perinatal period. Early diagnosis is important, however, because hepatoportoenterostomy (HPE; the Kasai procedure), if performed in the first 2-3 months of life, can restore bile flow and help prevent worsening of the liver disease. Yet, even with a successful HPE, more than 70% of children eventually develop cirrhosis and require liver transplantation before adulthood. Recent data suggest that long-term survival without transplantation can be achieved in a higher proportion of children if HPE is performed very early, within the first 30-45 days of life. However, establishing the diagnosis this early is not common, and there are no convenient means of screening newborns. For these reasons, the diagnosis is often delayed, and treatment is inadequate. Several approaches to routine screening for BA have been developed and are under evaluation. Because of the importance of BA and the opportunities for improved outcomes with earlier diagnosis, the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health, in collaboration with the Office of Rare Diseases of the National Institutes of Health, the Human Resources Services Administration, and the American Liver Foundation, sponsored a 2-day meeting

Abbreviations: AAP, American Academy of Pediatrics; BA, biliary atresia; BARC, Biliary Atresia Research Consortium; BASM, biliary atresia splenic malformation; BDE, bile duct epithelia; BSEP, bile salt export pump; CMV, cytomegalovirus; Flc1, familial intrahepatic cholestasis 1 gene; HPE, hepatoportoenterostomy; IL-2, interleukin-2; NO, nitric oxide; PAI-1, plasminogen activator inhibitor-1.

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held on September 12 and 13, 2006, in Bethesda, MD, focusing on new information on pathogenesis and outcomes and approaches to screening and early diagnosis. This report is a summary of that meeting.

Clinical Course of BA

Dr. Jeffrey Maisels (William Beaumont Hospital, Royal Oak, MI) described the current understanding of the frequency and significance of jaundice in the newborn. Neonatal jaundice is the most common problem in the management of newborns, approximately two-thirds of whom develop jaundice during the first week of life.[5] Jaundice at this age is almost always caused by elevations in indirect (unconjugated) bilirubin, which result from increased bilirubin production, decreased clearance, and excessive enterohepatic recirculation.[5] Although most jaundiced infants are healthy, rare infants with extreme hyperbilirubinemia develop kernicterus because of the direct toxicity of unconjugated bilirubin to the central nervous system. This problem is made difficult because newborns are usually discharged from the hospital before 48 hours, well before bilirubin levels peak (usually after 96 hours).[5,6] For these reasons, the American Academy of Pediatrics (AAP) recommends that infants discharged before 72 hours be seen by their primary care provider within 2 days of discharge.[6]

Pathological causes of indirect hyperbilirubinemia in the newborn include hemolytic disease due to isoimmunization, glucose-6-phosphatase dehydrogenase deficiency, red cell membrane defects, and unstable hemoglobins. Other causes include cephalohematomas, bruising, and rare inborn errors of bilirubin metabolism.[5] In most infants, however, no definite cause is identified other than breast feeding.[7] The association of jaundice with breast feeding has not been completely explained but appears due to a decrease in caloric intake and increase in the enterohepatic circulation of bilirubin.[5,8,9] Gilbert's disease may contribute to hyperbilirubinemia in some infants: a recent study showed that 27% of breastfed infants with a bilirubin level greater than 5.8 mg/dL on day 28 were homozygous for the (TA)7 mutation in the promoter of the UDP glucuronosyltransferase 1 family polypeptide A1 gene, as seen in Gilbert's disease.[10] In other instances, mutations in the organic anion transporter protein gene may be important. In Taiwanese newborns, breastfeeding and mutations in the UDP glucuronosyltransferase 1 family polypeptide A1 gene and organic anion transporter protein 2 gene were identified as important risk factors for hyperbilirubinemia,[11] and if all 3 factors were present, the odds ratio for an infant developing a bilirubin level greater than 20 mg/dL was 88.

Because BA and neonatal cholestasis are generally not recognized before 2-3 weeks of age, the AAP currently recommends that infants who are jaundiced at 3 weeks should have a measurement of total and direct bilirubin.[6] Compliance with this recommendation in practice has never been evaluated. More than 95% of newborns who have jaundice at age 3 weeks are healthy, breast-feeding infants, and at least 20% of breast-fed infants are jaundiced for more than 2-3 weeks. Nevertheless, tests for fractionated bilirubin are inexpensive and widely available. Unfortunately, the current schedule for newborn visits in the United States does not include a visit between 2 and 4 weeks, so persistent jaundice may not be detected until much later. If all serum bilirubin measurements included a direct bilirubin, particularly after discharge from the nursery, infants with cholestasis might be identified at an earlier age.

Dr. Barbara Haber (Children's Hospital, Philadelphia, PA) discussed the clinical and epidemiologic features of BA. The anatomic understanding of BA came with the careful pathologic descriptions of John Thompson in 1891.[12] Infants typically appeared healthy at birth but developed jaundice, pale stools, and dark urine by the first month of life, thereafter becoming progressively ill and dying by the age of 2 or 3 years. Before 1900, BA was considered a uniformly fatal disease. Shortly thereafter, Holmes at Johns Hopkins University introduced the concept of "correctable" BA, noting that, in some cases, a partially patent proximal biliary tree could be anastomosed directly to the bowel. The critical advance came in the 1950s, when Morio Kasai, a Japanese surgeon, pioneered the HPE procedure, in which the diseased biliary tree is excised, the hepatic porta is carefully dissected, and a roux-en-Y loop of jejunum is anastomosed directly to the liver.[13] With this procedure, bile drainage was achieved in the majority of patients. Despite this breakthrough, considerable morbidity accompanied survival, and many patients eventually succumbed to biliary cirrhosis later in childhood. With subsequent improvements in surgical and postoperative care and the advent of liver transplantation, children rarely died of BA; however, 50% still required liver transplantation by 2 years of age,[14] and more than 70% underwent liver transplantation by adulthood.[15-19]

The epidemiology of BA has revealed few clues to its etiology. In a study of 30 cases of BA presenting between 1972 and 1980, a higher frequency was found in rural areas, with clustering of cases and seasonal variation.[20] Twenty-three cases presented during 3 years, usually during the last 6 months of the year, whereas the remaining 7 presented in the other 6 years. This pattern suggested nonrandom, time-space clustering indicative of a viral,

Table 1. Differential Diagnosis of Neonatal Cholestasis

I. Extrahepatic Causes
A. Biliary atresia
B. Choledochal cyst
C. Bile duct stenosis, strictures, or cholelithiasis
D. Spontaneous perforation of the common bile duct
E. Tumors or masses (extrinsic or intrinsic compression of bile ducts)
II. Intrahepatic Causes
A. Infectious: cytomegalovirus, rubella, herpes simplex, human herpesvirus 6, varicella zoster, adenovirus, enterovirus, parvovirus B19, hepatitis B virus, human immunodeficiency virus, toxoplasmosis, syphilis, tuberculosis, listeriosis, bacterial sepsis, and urinary tract infection
B. Metabolic: alpha-1 antitrypsin deficiency, cystic fibrosis, galactosemia, hereditary tyrosinemia, hereditary fructose intolerance, glycogen storage disease type IV, Niemann-Pick type C, Gaucher's disease, Wolman's disease, cholesterol ester storage disease, panhypopituitarism, hypothyroidism, bile acid synthesis defects, peroxisomal disorders, arginase deficiency, and mitochondrial respiratory chain deficiencies
C. Genetic: Alagille syndrome, Turner syndrome, trisomy 21, arthrogryposis–renal dysfunction–cholestasis syndrome, Aagenaes syndrome (cholestasis with lymphedema), progressive familial intrahepatic cholestasis (*FIC1*, *BSEP*, and multiple drug resistance 3 gene deficiencies), North American Indian childhood cirrhosis (cirhin deficiency), congenital hepatic fibrosis/autosomal recessive polycystic kidney disease, Caroli's disease, and neonatal Dubin-Johnson syndrome
D. Toxic: total parenteral nutrition–associated, endotoxin from gram-negative infection, choral hydrate and other medications, and aluminum
E. Cholangiopathies: nonsyndromic paucity of interlobular bile ducts and neonatal sclerosing cholangitis
F. Miscellaneous: idiopathic neonatal hepatitis, congenital lupus, ischemia-reperfusion injury, histiocytosis X, erythrophagocytic lymphohistiocytosis, veno-occlusive disease, erythroblastosis fetalis (inspissated bile syndrome), and neonatal iron storage disease

toxin, or environmental etiology. Outbreaks of a BA-like syndrome in lambs in Australia in 1964 and 1988 also suggested an environmental factor.[21] No specific infectious agent was identified, but all cases occurred during periods of drought in which grazing patterns were altered and the mother animals fed on grasses watered from a single source.

BA has been reported from all countries and racial populations.[22-26] The disease appears to be more common in infants of Asian or African descent, in females, and in premature infants.[27,28] In a Swedish national database[27] representing 1.2 million births and 99% of the population born between 1987 and 1997, 4 independent factors were associated with BA: low birth weight, increased maternal age, parity greater than 4, and prematurity. Other less strongly linked factors included maternal smoking, asthma, and rural residence.[27]

Two clinical patterns of BA have been described. Infants with the perinatal (or acquired) form of BA, which accounts for approximately 80% of affected infants, are asymptomatic and anicteric at birth and develop jaundice in the first postnatal weeks. These infants are otherwise healthy and appear to suffer from a perinatal insult that leads to biliary obstruction. Infants with the embryonic form have no jaundice-free interval and suffer from 1 or more congenital anomalies, such as interruption of the suprarenal segment of the inferior vena cava with azygous continuation, preduodenal portal vein, midline symmetric liver, intestinal malrotation, situs anomalies (inversus and ambiguous), bronchial anomalies, and polysplenia or asplenia.[29,30] The embryonic form thus appears to be caused by a developmental abnormality of the biliary tree and includes those infants with the biliary atresia splenic malformation (BASM) syndrome.

Medical therapy for BA includes supporting nutrition and growth and anticipating nutritional inadequacies that are common even following HPE.[31,32] Prophylactic antibiotics (to prevent cholangitis) and choleretic agents are commonly prescribed, although definitive evidence supporting their use is lacking.[14,33] Despite these therapies, biliary cirrhosis develops by age 20 in more than 70% of patients, and liver transplantation remains the ultimate therapy for the large majority of patients.

Dr. Peter F. Whitington (Children's Memorial Hospital, Chicago, IL) summarized the current paradigm for diagnosing BA. The differential diagnosis for an infant presenting with cholestasis includes intrahepatic and extrahepatic causes (Table 1). The North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition recently published evidence-based guidelines for the optimal evaluation of the cholestatic infant,[34] which provide a guide for rapid evaluation by the primary care physician and appropriate referral to a pediatric gastroenterologist. The first step in diagnosis is the identification of conjugated hyperbilirubinemia in an infant with prolonged jaundice (beyond 2 weeks of age), pale stools, or dark urine. A conjugated bilirubin that is greater than 20% of an elevated total serum bilirubin is diagnostic of cholestasis. An examination of the color of a fresh stool specimen is also useful in differentiating cholestasis (pale stools) from indirect hyperbilirubinemia (bright yellow stools). The history and physical examination may guide diagnostic studies to identify specific causes of intrahepatic cholestasis (Table 1). For example, clinical features

of Alagille syndrome may preclude the need for invasive procedures. In addition, infectious and metabolic causes of cholestasis need to be considered and treated promptly. Routine liver tests generally do not differentiate among causes of cholestasis; however, it is quite unusual to have a gamma-glutamyl transpeptidase level lower than 200 U/L in BA.

An evaluation should include testing for alpha-1 antitrypsin deficiency, hypothyroidism, hereditary tyrosinemia, and other relevant and treatable disorders. Ultrasonography is used to exclude choledochal cysts and evaluate for congenital anomalies associated with BA such as polysplenia or preduodenal portal vein. The triangular cord sign and gall bladder length, as shown by ultrasound, have been reported to be predictive of BA,[35] but the accuracy of these measurements is operator-dependent and not very reliable. Although hepatic scintigraphy showing definite biliary excretion excludes BA, the absence of excretion has poor predictive value because any form of severe cholestasis may show similar findings.[36] Liver biopsy can correctly predict extrahepatic biliary obstruction in more than 90% of cases, directing the evaluation toward cholangiography.[1,34] The definitive diagnosis is made when cholangiography fails to show a patent biliary tree and surgical exploration confirms the diagnosis. The current challenge is to establish means of making this diagnosis at an earlier age.

Dr. Pierre Russo (Children's Hospital, Philadelphia, PA) described the role of hepatic and biliary histology in the diagnosis of BA. The interpretation of the liver biopsy plays a key role in the diagnostic algorithm of neonatal cholestasis. The histological features suggesting extrahepatic obstruction of bile ducts (and hence the need for cholangiography) are characteristic although not specific, and they evolve as the disease matures. The expansion of portal tracts with various degrees of fibrosis, proliferation of bile ductules, inflammatory cell infiltration, and bile plugs in portal tracts are features suggesting bile duct obstruction (Fig. 1). Parenchymal changes are usually not marked and may be indistinguishable from other causes of neonatal cholestasis, and they include lobular disarray, extramedullary hematopoiesis, and syncytial giant cell formation. Fibrosis is variable, and its severity may be predictive of the long-term success of HPE.[37] The differential diagnosis (Table 2) of a biliary obstructive pattern in a liver biopsy of a cholestatic infant includes choledochal cysts, bile duct strictures, alpha-1 antitrypsin deficiency, total parenteral nutrition–associated cholestasis, cystic fibrosis, multiple drug resistance 3 deficiency (progressive familial intrahepatic cholestasis type III), North American Indian childhood cirrhosis (cirhin deficiency), and Alagille syndrome. Thus, differential diagnosis relies on clinical and laboratory test results in addition to the histology. Standardized histological scoring systems for BA are needed and are the focus of a pathology working group of the Biliary Atresia Research Consortium (BARC).

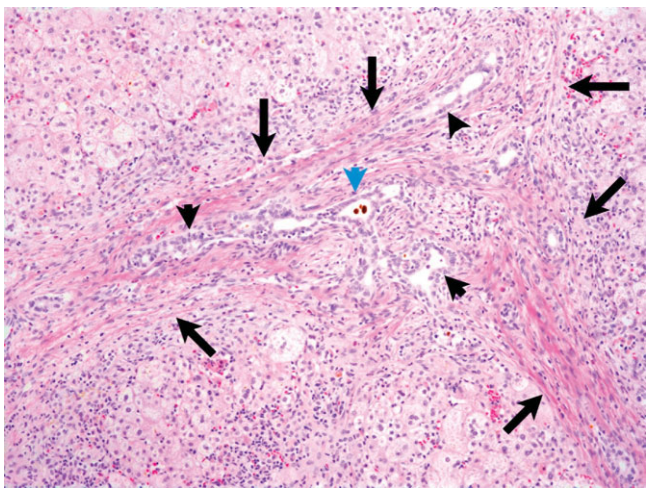

Fig. 1. Liver histology of a patient with biliary atresia at 8 weeks of age. The biopsy shows an expanded and fibrotic portal tract (outlined by the arrows), with inflammation, biliary proliferation (outlined by the black arrowheads), and bilirubinostasis within a ductal structure (indicated by a blue arrowhead). Hematoxylin and eosin were used; the original magnification was ×100.

Table 2. Diseases Associated with Liver Histology that Mimics Biliary Atresia

Choledochal cyst
Bile duct strictures and stones
Total parenteral nutrition–associated cholestasis
Alpha-1 antitrypsin deficiency
Cystic fibrosis
Multiple drug resistance 3 deficiency
North American Indian childhood cirrhosis (cirhin deficiency)
Alagille syndrome

The bile duct remnant, which is commonly resected at the time of HPE, shows fibrosis and obstruction of the bile duct lumen, variable degrees of periductal inflammation, and apoptosis and degeneration of residual bile duct epithelia. Cystic variants of BA, in which a bile ductal cyst is present, may prove to have etiologic and prognostic implications.[38] Similarly, the potential value of the histological findings of ductal plate malformation in predicting the outcome is controversial and requires further investigation. Hepatic histology does not differentiate patients with the embryonic and perinatal forms of BA.[39]

HPE and Outcomes for BA

Dr. Riccardo Superina (Children's Memorial Hospital, Chicago, IL) presented a perspective on the surgical

Table 3. Contemporary Outcome of Biliary Atresia Following Kasai Hepatoportoenterostomy (HPE) and Liver Transplantation

Country, Year, and Number of Centers	Number of Patients	Age at HPE	Survival with Native Liver	Survival After Liver Transplantation	Overall Survival of Patients
Japan, 1989-1999, 93 centers[18]	1381	Median of 61-70 days	5 years: 59.7% (actual)	–	5 years: 75.5% (actual)
United Kingdom and Ireland, 1993-1995, 15 centers[26]	93	Median of 54 days	5 years: 30.1% (actuarial)	2.4 years: 89% (actual)	5 years: 85% (actuarial)
United States, 1997-2000, 9 centers[14]	104	Mean of 61 days	2 years: 55.8% (actual)	2 years: 88% (actual)	2 years: 91.3% (actual)
France, 1997-2002, 22 centers[53]	271	Median of 57 days	4 years: 42.7% (actuarial)	4 years: 88.8% (actuarial)	4 years: 87.1% (actuarial)
England and Wales, 1999-2002, 3 centers[92]	148	Median of 54 days	4 years: 51% (actuarial)	2 years: 89% (actuarial)	4 years: 89% (actuarial)

Adapted from Chardot C, Serinot M-O. Prognosis of biliary atresia: what can be further improved? J Pediatr 2006;148:432-435.

management of BA. The Kasai procedure[40] was slow to find supporters in the West[41] but eventually became the standard of care all over the world.[42-45] The HPE has 3 components: (1) abdominal exploration and cholangiography to confirm the diagnosis, (2) dissection of the porta hepatis and transection of biliary tissue remnants at the portal plate, and (3) establishment of biliary drainage through the construction of a 40-50–cm roux-en-Y jejunal conduit. Variants of the operation, designed to minimize cholangitis, have been described,[46,47] including the creation of a stoma to decompress the bile drainage and the construction of a valve in the jejunal conduit to prevent reflux proximally.[48,49] Both stoma formation and valve formation have fallen out of favor as they have not improved the outcome or reduced the incidence of cholangitis.[50]

The experience of the last 40 years of BA surgery has led to several widely held beliefs: (1) success in obtaining bile flow is better if HPE is done before 60-90 days of age, (2) cholangitis has an adverse effect on bile flow, (3) the establishment of bile flow results in better survival, and (4) progressive fibrosis and liver dysfunction occur in many children whose jaundice resolves after the HPE procedure. The sequential use of HPE followed by liver transplantation is the standard surgical paradigm followed around the world.[37,51,52] Future trends that will bear watching include the growing laparoscopic experience with the HPE procedure.

Dr. Richard Schreiber (British Columbia Children's Hospital, Vancouver, Canada) presented a retrospective analysis of 349 Canadian patients treated over 17 years at 12 academic centers. The mean age at HPE was 68 days, but 7% had HPE at less than 30 days of age and 14% had HPE at greater than 90 days of age. Patients benefited from early HPE. Survival with the native liver was significantly higher for those with HPE at less than 30 days of age compared with those with HPE at greater than 90 days of age (52% versus 21%, $P < 0.006$). Of those with HPE at less than 30 days of age, 48% had a liver transplant versus 71% when HPE was performed at greater than 100 days of age. Overall, the 2-year, 5-year, and 10-year survival rates with the native liver after HPE were 48%, 36%, and 33%. Twenty-seven children (8%) had BASM.

Dr. Mark Davenport (King's College Hospital, London, United Kingdom) presented data prospectively collected in the United Kingdom between 1999 and 2005 from the 3 centers to which all children with BA are referred (Table 3). Among 280 children, the mean age at HPE was 52 days, and 11 underwent a primary liver transplant. More than half (55%) of the infants became anicteric, and the 2-year and 4-year survival rates with the native liver were 55% and 47%, with an overall 4-year survival rate of 91%. In the 42 (15%) children with the BASM syndrome, the outcomes were significantly poorer, with a 4-year survival rate of 77% and a survival rate with the native liver of 32%.

Dr. Christophe Chardot (Cantonal University Hospital, Geneva, Switzerland) presented the French experience with the management of BA (Table 3).[53] A total of 743 patients were studied: 472 who were seen between 1986 and 1996 and 271 who were seen between 1997 and 2005 at 45 centers throughout France and French territories. The mean age at HPE was 60 days. The survival rates with the native liver after HPE were 57%, 38%, and 32% 2, 5, and 10 years after surgery, respectively. There were 55 patients with the BASM syndrome (8%), among whom the 2-year and 5-year survival rates were 44% and 16%, significantly worse than the 59% and 41% survival rates in those without BASM. Survival with the native liver was worse in children undergoing HPE at greater than 90 days of age (42% and 27% 2-year and 5-year survival rates, respectively). Interestingly, the use of postoperative steroids was associated with a modest but not significant decrease in survival with the native liver. There was a modest center effect on survival in the earlier cohort

Table 4. Candidate Genes and Biliary Phenotype in Mice with Gene Mutations

Gene	Intrahepatic Bile Ducts	Extrahepatic Bile Duct	Gallbladder
Inversin	Normal	Obstruction	Normal
Jagged-notch circuit	Abnormal	Unaffected	Unaffected
Hairy and enhancer of split 1 (Hes 1)	Unaffected	Hypoplasia	Agenesis
Hepatocyte nuclear factor 6	Ductal plate malformation Intrahepatic biliary cysts	Abnormal	Agenesis
Hepatocyte nuclear factor 1 α	Paucity of small intrahepatic bile ducts Dysplasia of larger intrahepatic bile ducts	?	Abnormal epithelium Dilated cystic duct
Forkhead box F1	Normal	?	Small or absent No epithelial cells
Forkhead box M1b	Agenesis	?	?

Adapted from HEPATOLOGY 2005;42:222-235.

of patients but not in the later cohort, and this was ascribed to better education among smaller centers in the later cohort. The resolution of jaundice after the HPE operation was 39% in the second cohort of patients.

Dr. Benjamin Shneider (Mount Sinai Medical Center, New York, NY) presented the US experience of treating BA (Table 3).[14] A retrospective analysis was conducted on 104 patients seen at the 9 BARC centers over a 4-year period. Failure of the HPE was defined as death or liver transplantation, and a good outcome was defined as survival with the native liver and a bilirubin level less than 2.0 mg/dL at 24 months of age. The average age at presentation was 53 days, and the average age at HPE was 61 days. Overall, 56% of patients were alive at 2 years with the native liver, and 52% were alive with a serum bilirubin level lower than 2.0 mg/dL. Patients with a good outcome had HPE at an earlier average age (57 days) than those with a poor outcome (64 days), but the difference was not statistically significant. The 11 patients (11%) with BASM had poorer survival with the native liver at 24 months (18%). The total serum bilirubin at 3 months after HPE was an excellent predictor of the 24-month outcome: 84% with a bilirubin level lower than 2 mg/dL had a good outcome versus only 16% with a bilirubin level greater than 6 mg/dL. The presence of ascites predicted a poor outcome.

A summary (Table 3) of the published contemporary series shows that the overall 2-5–year survival following HPE ranges from 85%-91%. The largest North American long-term experience with HPE demonstrated survival with the native liver of 35% at 10 years and 21% at 20 years.[54] A recent series of 755 BA patients listed for liver transplantation from North America reported a 3-year graft survival rate of 88% and a patient survival rate of 80%.[55] Thus, the current use of HPE followed by liver transplantation in children who subsequently develop cirrhosis provides excellent long-term survival for a disease that is uniformly fatal without surgery.

New Insights into the Etiology and Pathogenesis

Dr. Michael Pack (University of Pennsylvania, Philadelphia, PA) reviewed the embryogenesis of the biliary tract. The formation of the intrahepatic and extrahepatic bile ducts is closely linked to liver development. The first indication of the developing human liver during embryogenesis appears in the ventral foregut at ~4 weeks of gestation. Thereafter, liver development follows a hierarchical activation of molecular networks that act upon the thickening of the ventral endodermal foregut, which sends rows of hepatoblasts into the neighboring septum transversum to form the liver bud.[56] The hepatoblasts surrounding the portal venules begin expressing high levels of biliary cytokeratins and become arranged as a monolayer and then as a bilayer to form the ductal plate. The ductal plate undergoes remodeling, first with tubular dilatation of the slitlike lumen of the double-layered plate and subsequently with the disappearance of most of the nontubular portions of the ductal plate. The remaining tubular structures become surrounded by mesenchyme to form intrahepatic bile ducts. These ducts merge to form larger ducts toward the hilum, where they establish continuity with extrahepatic bile ducts later in embryogenesis. The development of extrahepatic ductular structures begins as an outpouching from the caudal portion of the liver bud, giving rise to a diverticulum that later forms the gallbladder, cystic duct, and other major extrahepatic bile ducts. The disruption of molecular and cellular networks that regulate these developmental steps has been proposed as a contributor to the pathogenesis of BA (Table 4).

Model organisms provide a highly valuable approach to the analysis of developmental biology and the role of specific genes in congenital malformations. One such approach is zebrafish-based screening, in which the use of fluorescent biologicals that are excreted by the biliary system enables the rapid identification of abnormal biliary

morphogenesis induced by random or gene-specific mutations. Similar approaches have the potential to identify novel molecular networks that are critical to hepatobiliary development and may broaden our knowledge of how genes modulate the pathogenesis of BA.

Dr. Cara Mack (University of Colorado School of Medicine, Denver, CO) reviewed the role of viral infection and immunologic factors in the etiology of BA. Three agents have been consistently associated with BA: cytomegalovirus (CMV), reovirus, and rotavirus. CMV infection was identified in 4 of 10 infants with BA,[57] and this was consistent with previous reports that found the virus in 24%-38% of affected infants[58,59]; however, at least 1 report failed to identify CMV.[60] Reovirus infection has been identified in the liver in up to 55% of patients with BA and in 78% with choledochal cysts[61-65] versus 10%-20% in control groups. In addition to these patient-based studies, reovirus has been shown to induce edema and fibrosis of extrahepatic bile ducts when inoculated into weaning mice.[66,67] To explore whether reovirus could trigger a similar lesion in newborn mice, investigators inoculated reovirus in the first 2 days after birth and observed the development of intrahepatic cholangitis and extrahepatic bile duct dilation, but without the obstruction of extrahepatic bile ducts typical of BA.[68]

Evidence of rotavirus infection in infants with BA has been reported; however, the presence of rotavirus in livers of affected patients has not been a consistent finding.[69,70] A phenotype similar to human BA develops following the inoculation of rhesus rotavirus in newborn mice, which triggers an inflammatory obstruction of extrahepatic bile ducts with features similar to those found in BA.[71,72] In summary, the identification of viruses in children with BA has been inconsistent across studies, but several relevant viruses have provided animal models that may be valuable in assessing the pathogenesis and treatment of this disease.

The immune response has received the most attention in human-based studies of BA pathogenesis. The infiltration of CD4+ and CD8+ T lymphocytes and macrophages has been consistently observed in the periductal space or along the duct epithelium in conjunction with increased expression of cytokines and receptors commonly seen when these cells are activated.[2] More recently, 2 studies carried out comprehensive molecular and cellular surveys of liver biopsies and found a proinflammatory gene expression signature, with increased activation of interferon-γ, osteopontin, tumor necrosis factor-α, and other inflammatory mediators.[73,74] In studies using the rotavirus-induced newborn mouse model of biliary injury, interferon-γ was shown to regulate the tropism of lymphocytes to the duct epithelium and luminal obstruction.[75] In addition, T lymphocytes from the liver and spleen of these mice produced interferon-γ in response to stimulation by bile duct epithelial antigens, and the adoptive transfer of primed T lymphocytes triggered periductal inflammation in adult mouse recipients. Thus, both immune and possible autoimmune mechanisms appear to mediate bile duct injury in this mouse model. These types of patient-based and animal-based studies may prove to be instrumental in dissecting the molecular networks responsible for the proinflammatory response and autoimmunity in BA (Fig. 2).

Dr. David Perlmutter (University of Pittsburgh, Pittsburgh, PA) reviewed the hypothesis that genetic defects play a role in BA. In 1991, investigators from Brazil reported finding nonhepatic congenital anomalies in 20% (47/237) of patients with BA,[30] including situs anomalies in 8%. The reported incidence of congenital anomalies in BA has varied in published series but may be as high as 33%.[14,22,29,76] The occurrence of situs abnormalities suggests that laterality genes may contribute to the phenotype. An initial candidate gene was inversin on the basis of the developmental defects and biliary obstruction described in the *Inv*(−/−)mouse.[77,78] However, studies failed to identify mutations in the inversin gene that segregated with the BA phenotype in humans.[79] Further studies identified abnormalities in laterality genes in a small number of subjects with BA, including the transcription factors *ZIC3* and *CFC*.[80,81] Future studies in larger patient populations, using state-of-the-art methodologies such as screening for microsatellite deletions, high-resolution chromosomal footprinting, and high-throughput gene sequencing, may prove instrumental in determining the incidence of these mutations in affected patients.

Finally, other nonlaterality genes that regulate the embryonic development of the biliary tree may play a role in the pathogenesis of BA (Table 4). Thus, a high incidence of polymorphic variants in the jagged 1 (*JAG1*) and keratin 8 and keratin 18 genes has been reported in a series of 18 children with BA.[82,83] Taken together, the increased incidence of nonhepatic anomalies in children with BA and the genetic mutations reported in subsets of patients with laterality defects suggest that multiple genes are involved, each affecting a small number of patients.

Dr. Robert Heuckeroth (Washington University School of Medicine, St. Louis, MO) discussed the use of large-scale gene expression and proteomic profiling in the search for pathogenic mechanisms of BA. An initial study compared large-scale gene expression signatures in livers of patients with BA and those from age-matched disease controls with intrahepatic cholestasis.[73] Infants with BA had high levels of intrahepatic expression of proinflammatory genes, particularly interferon-γ. A second study of

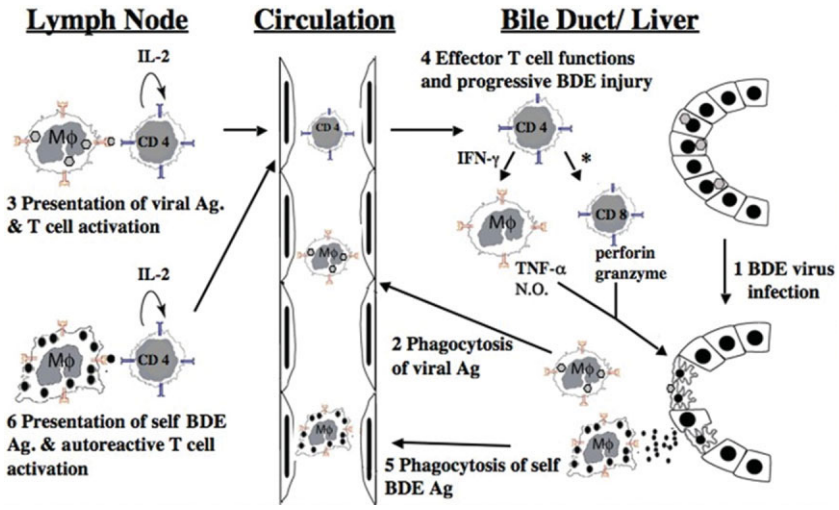

Fig. 2. Proposed model of virus-induced, T cell–mediated autoreactivity leading to bile duct epithelial injury in biliary atresia. The viral infection of the bile duct epithelia (BDE) (1) results in an initial injury to the cells. Virus particles phagocytosed by macrophages or dendritic cells (2) are presented to naive T cells in local lymph nodes, in which the activation and interleukin-2 (IL-2)–stimulated proliferation of virus-specific $CD4^+$ T cells ensues (3). These activated $CD4^+$ T cells travel back to the original site of exposure and elicit T cell effector functions (4), including interferon-γ–induced macrophage stimulation and activation of cytotoxic $CD8^+$ T cells. The macrophages release TNF-α, nitric oxide (NO), and reactive oxygen species, whereas the $CD8^+$ T cells release granzyme and perforin; together, they result in further BDE injury through apoptotic or necrotic pathways. Previously sequestered or altered BDE antigens released from this initial injury are now phagocytosed by macrophages or dendritic cells and presented to autoreactive T cells (5), causing further activation of this T cell–mediated immune cascade (6) and progressive destruction of BDE. Adapted from Sokol RJ, Mack C. Etiopathogenesis of biliary atresia. Sem Liv Dis 2001;21:S17-S124. Reproduced by permission of the authors.

liver samples obtained at transplantation reported increased expression of genes involved in morphogenesis, fibrogenesis, tissue remodeling, transcription regulation, and cell signaling.[84] In both studies, the gene expression profile for many genes provided a distinct signature for BA.

In the mouse model of rotavirus-induced biliary tract injury,[85] proinflammatory genes (including interferon γ) and markers of apoptosis and the complement system were predominantly expressed at early and intermediate stages of injury. In separate experiments, 2 other groups of investigators studied liver gene expression profiles in mouse models of bile duct ligation. In 1 study, the functional classification of genes differentially expressed at different times after the ligation of extrahepatic bile ducts identified a transcriptional reprogramming that correlated with predominant physiologic consequences of bile flow.[86] In the second study, the liver overexpressed plasminogen activator inhibitor-1 (PAI-1) following duct ligation.[87] The investigators then subjected PAI-1–deficient mice to bile duct ligation and showed that the loss of PAI-1 protected the liver from cholestasis-induced liver cell necrosis. Future studies will be necessary to investigate whether PAI-1 and/or other genes that are expressed in chronic cholestasis contribute to the pathogenesis of BA.

More recently, expression profiling distinguished between the 2 main clinical forms of BA (perinatal and embryonic),[88] with the overexpression of 5 imprinted genes (the insulin-like growth factor 2, paternally expressed gene 3, paternally expressed gene 10, maternally expressed gene 3, and imprinted in Prader-Willi syndrome gene) in children with the embryonic form. The simultaneous expression of these genes was in keeping with a biological setting in which epigenetic forces may regulate the phenotypic features of the embryonic form of BA.

Proteomic approaches are now needed to complement gene microarray studies of pathogenesis, to search for biomarkers of disease, and to identify therapeutic targets. In this respect, the availability of samples and clinical data prospectively collected from infants with BA by BARC will generate unmatched opportunities.

Improving Outcomes of BA

Dr. Ronald J. Sokol (University of Colorado School of Medicine, Denver, CO) summarized the obstacles to making an early diagnosis of BA, which are particularly challenging in the United States. Despite the need for early surgical intervention in this disease,[89] the diagnosis is rare, and there is a general lack of understanding of the importance of early identification among U.S. health care providers. Few primary care physicians see more than 1 or 2 cases of BA during their careers, whereas neonatal jaundice (unconjugated hyperbilirubinemia) is extremely

common, particularly among breast-fed infants. Studies from the United Kingdom suggest that BA accounts for only 1 in 500 instances of jaundice in children 2-3 weeks old, the age at which this diagnosis might most appropriately be made.[90]

A second obstacle to early diagnosis is the lack of convenient methods of screening. Currently, the efficacy of stool color cards and conjugated bilirubin testing is being evaluated in Europe and Asia,[91] but these approaches are not widely available and may not be easily applicable in the United States.

A third obstacle to early diagnosis is the recommended postnatal routine care visit schedule in the United States, which calls for infant visits at 8 rather than 4 weeks of age, as is common elsewhere. Consequently, the jaundiced infant may not be seen at the optimal time for identification of BA. Moreover, the conjugated hyperbilirubinemia of BA is usually relatively mild, making it difficult to differentiate it from the moderate unconjugated hyperbilirubinemia frequent in breast-fed infants. Importantly, the unconjugated hyperbilirubinemia due to breast feeding in the first 2-3 weeks of life may obscure the conjugated hyperbilirubinemia of BA, making it appear that jaundice is actually improving (Fig. 3). Although assessing stool color may be helpful in distinguishing between these 2 causes of jaundice, it is not part of the recommended routine postnatal care in the United States.

Finally, the management of BA, once the diagnosis is established, is decentralized and varies by locale in the United States. Studies from both the United Kingdom[92] and France[53] suggest that outcomes are better at centers that perform at least 5 HPE procedures per year, yet only rare U.S. centers perform this many. Moreover, governmental action to restrict HPE to certain centers is unlikely in the U.S. healthcare system. More data are needed that compare the outcomes of HPE surgery at US centers of differing experience.

Thus, any recommendations regarding screening for BA in the United States will have to take into account the current structure of the healthcare system and the obstacles to early diagnosis. More widespread use of conjugated bilirubin testing and increased attention to stool color are appropriate means of improving early detection of BA in the United States. The adoption of a universal approach to screening must await the development of more practical means of screening.

Dr. Masaki Nio (Miyagi Children's and Tohoku University Hospital, Sendai, Japan) summarized the results on the use of corticosteroid therapy following HPE in Japan.[93] There has been extensive experience with HPE at the Tohoku University Hospital in Japan, where this operation was first developed by Professor Kasai in the 1950s. Corticosteroid therapy was introduced in the 1980s as a means of improving bile flow after HPE. The typical regimen used in Japan relies on intravenous prednisolone (10 mg twice daily) for postoperative days 1-7 followed by oral dosing starting at 20 mg daily for 4 days and tapering doses thereafter. Once the serum bilirubin level is less than 2.0 mg/dL for 3 months, corticosteroids are discontinued. Other adjunctive therapies include intravenous bile acids followed by orally administered ursodiol (10-20 mg/kg/day) once oral intake is established. These combined medical and surgical developments have been associated with improved outcomes, achieving clearance of jaundice in 80% of children and, since 1991, a 10-year survival rate of 60% with the native liver.[93]

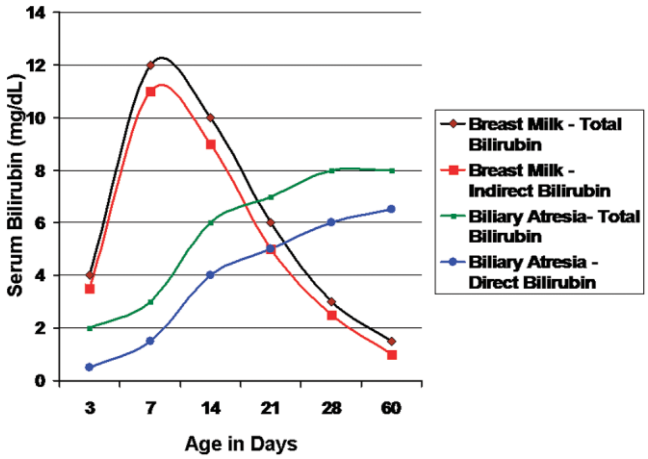

Fig. 3. Comparison of serum bilirubin concentrations in infants with breast milk–associated jaundice (primarily indirect hyperbilirubinemia) and biliary atresia (primarily direct hyperbilirubinemia). As the indirect bilirubin falls during the first month of life in an infant with biliary atresia who is also breast-fed, it may appear that there is an overall improvement in jaundice. Thus, the diagnosis may be delayed, and the delay may lead to late referral for the evaluation of cholestasis.

The Japanese Biliary Atresia Registry was established in 1989 and includes standardized information gathered on 1930 patients. This registry shows that almost all Japanese children with BA receive postoperative corticosteroids and ursodiol. The overall rate of clearance of jaundice after HPE is 60%, the 10-year survival rate with the native liver is 50%, and the overall 10-year survival rate (with or without transplantation) is more than 90%.[18] The best outcomes were achieved when HPE was performed before 30 days of age. Children treated with corticosteroids had a higher survival rate than those who were not, but fewer than 8% of patients undergoing HPE were untreated. A prospective study is now underway, focusing on the optimal dosing of corticosteroids. Children are randomly assigned to either high-dose prednisolone (starting at 4 mg/kg/day on day 7 after HPE) or low-dose prednisolone

(starting at 2 mg/kg/day); thereafter, the dose is reduced weekly for a total of 30-60 days of treatment.

Dr. Ross Shepherd (Washington University School of Medicine, St. Louis, MO) discussed the importance of nutrition in the course and outcome of BA. BA and its associated cholestasis affect the oral intake, intestinal absorption, and metabolic processing and utilization of nutrients. Malnutrition and growth failure are common and serious consequences of BA. Furthermore, poor preoperative nutrition is associated with poor outcomes after HPE.[94] Nutritional status also has a major impact on the success of liver transplantation; underweight and growth failure are major predictors of waiting list mortality and posttransplant morbidity and mortality.[55] Importantly, poor nutrition is potentially preventable and reversible and should be considered a modifiable risk factor.[55,95,96] However, whether interventions to improve nutritional status will also improve outcomes after HPE or liver transplantation for BA has yet to be shown in prospective trials.

Efforts to optimize nutritional support by providing adequate calories and protein, avoiding fasting, and using specialized amino acid and vitamin formulations can improve nutritional status, as shown in at least 1 controlled study[97] and a number of open label studies. In particular, the achievement of at least 125% of the recommended daily energy intake by the use of supplemental nocturnal enteral feedings, the use of semielemental formulas with added branched chain amino acids, and the administration of fat-soluble vitamin supplements have been shown to be beneficial in BA. Major challenges include integrating nutritional support into the overall management of BA from the time of diagnosis through surgery or transplantation, validating the accuracy of methods for assessing nutritional status, and providing evidence that nutritional interventions improve outcomes.

Dr. Estella Alonso (Children's Memorial Hospital, Chicago, IL) discussed neurodevelopmental outcomes in BA. Children with BA are at significant risk for neurological deficits and cognitive delay because the disease has its onset during a critical phase of brain growth and neurological maturation. Unfortunately, current means of assessing neurodevelopment in early infancy are imprecise, relying on motor function, social and environmental interactions, and early language development, which are more difficult to measure accurately than the typical neurocognitive functions used in older children.

Several studies have shown that older infants with advanced BA have low-to-average developmental scores, and the deficits are more pronounced in children with malnutrition, lower serum vitamin E levels, and worse liver dysfunction.[98,99] Older children awaiting liver transplantation for BA also have low-to-average neurocognitive functioning, and the deficits are most severe in children with prolonged illness and malnutrition. A catch-up in neurodevelopment occurs after transplantation (as assessed by testing at 1 year), but deficits may persist, particularly in children who had long hospitalizations and greater nutritional impairment. Medium-term studies (2-10 years after transplantation) have shown that 10%-15% of children with BA have significant neurocognitive deficits (intelligence quotient < 70), approximately 26% have learning disabilities, and up to 40% require special educational services.[99]

Long-term studies of neurological development and cognitive function in children with BA are needed, with a focus on identifying modifiable factors that might prevent or help restore residual deficits. In the meantime, an analysis of resource utilization for educational support services for this growing population of children might help improve access to these necessary aspects of long-term care.

Screening for BA

Dr. Brad Therrell (University of Texas Health Sciences Center, San Antonio, TX) provided an overview of the current status of newborn screening in the United States. There is no national policy regarding newborn screening, and mandates differ from state to state. An effort has been made to standardize newborn screening through working groups of both the AAP and the American College of Medical Genetics.[100,101]

Newborn screening[100,102] is not simply the application of a screening test but is rather a coordinated program with multiple components: (1) obtaining the sample and sending it to a laboratory for testing; (2) obtaining the result, repeating it if necessary, and informing the local physicians and parents; (3) a medical evaluation of the child, including diagnostic testing and confirmation; (4) case management, which can be long-term and complex; (5) the evaluation and quality control of the screening program; and (6) the education of both medical and lay participants. All components are needed for a successful screening program.[102] The criteria for a condition appropriate for universal newborn screening are as follows: it is an important health problem; it has a recognizable latent period or early symptomatic period in which intervention may be beneficial; there are suitable screening tests or examinations that are acceptable, reliable, and available; and there are accepted treatments that are available and beneficial when applied early.[90,102]

The American College of Medical Genetics recently conducted a major survey of newborn conditions for their appropriateness for screening.[101] A total of 84 conditions, including BA, were considered, and 29 core conditions were identified as appropriate for newborn screening

(http://genes-r-us.uthscsa.edu). As of 2006, 2 of the 50 states in the United States have requirements for the screening of 1-10 disorders, 10 states have requirements for the screening of 10-19 disorders, 2 states have requirements for the screening of 20-29 disorders, 12 states have requirements for the screening of 30-39 disorders, 15 states have requirements for the screening of 40-49 disorders, and 9 states have requirements for the screening of more than 50 disorders. Most commonly, screening is performed for phenylketonuria, inborn metabolic disorders (aminoacidurias and urea cycle disorders), hypothyroidism, and, increasingly, hearing loss. Currently, no state requires screening for BA.

BA fulfills many but not all of the criteria for newborn screening. It is an important medical condition; although not frequent, it has major implications for the affected child and family. BA is also amenable to early diagnosis, which can result in improved outcomes. Furthermore, there are currently reliable algorithms for the diagnosis of BA and medical centers with expertise in its management. What is lacking is the final element, which is a convenient screening test for BA that can be incorporated into the routine neonatal screening regimens. These regimens generally allow for a blood sample or physical examination to be done before the newborn is discharged from the hospital. Until such a test is available, screening for BA will be difficult to incorporate into routine newborn screening in the United States.

Dr. James Heubi (University of Cincinnati College of Medicine, Cincinnati, OH) discussed the possibility of using bile acid measurements to screen for BA. During the first postnatal weeks, the enterohepatic circulation of bile acids is immature, producing a condition termed physiological cholestasis.[103] Serum bile acid levels (both cholic and chenodeoxycholic acid) are increased after birth and remain high for the first month of life, thereafter slowly declining to normal childhood levels by 1 year of age. Serum and urinary bile acids are increased further in children with cholestatic liver disease, but the absolute level of abnormality during the neonatal period has not been well defined.[104] Furthermore, the pattern of bile acid elevations in BA is not different from that of other neonatal cholestatic liver diseases, except for the inborn errors of bile acid metabolism.[104-107] Current evidence suggests that the cholestasis of BA is not fully evident at birth but worsens thereafter, so pathological elevations of serum and urinary bile acids may not be present until 2-4 weeks of age. The large-scale application of screening using serum bile acids with tandem mass spectrometry has shown that this approach is feasible with dried blood spots, but there is overlap in the levels between controls and infants with cholestatic liver diseases, including BA.[108] Smaller studies using isotopic dilution mass spectrometry,[109] high-performance liquid chromatography,[110] and enzymatic methods[111-114] have also shown that urinary and serum bile acids are elevated in children with BA, but the levels overlap with those of control newborns during the critical first several weeks of life. Thus, bile acid levels may be helpful in differentiating breast milk jaundice or prolonged physiologic jaundice from jaundice caused by liver disease but cannot be used alone for the screening and early detection of BA.

Dr. Deirdre Kelly (Birmingham Children's Hospital, Birmingham, United Kingdom) discussed the use of conjugated bilirubin levels as a means of screening for BA. An investigation of the feasibility of screening for neonatal liver disease using measurements of conjugated bilirubin in blood was undertaken as a pilot study in 1995.[115] Conjugated bilirubin was determined by spectrophotometry after the enzymatic measurement of total bilirubin on liquid blood collected by a heel prick during routine newborn home visits by public health nurses between 6 and10 days after delivery. Among the 1157 specimens analyzed, the total serum bilirubin levels averaged 103 μmol/L (6 mg/dL) and ranged from 9-428 μmol/L ($\sim$0.5-25 mg/dL). Conjugated bilirubin levels were far lower, averaging 5 μmol/L (range = 0-176 μmol/L), which represented 5% (range = 0%-57%) of the total. These data provided a reference range for normal conjugated bilirubin of 0-18 μmol/L (0-1.1 mg/dL) and a reference range for the percentage of conjugated bilirubin of 0%-20%. The conjugated bilirubin concentration was little affected by the age at specimen collection (ranging from 4-14 days) or gestational age.

A full-scale population-based screening observational study using conjugated bilirubin levels was subsequently conducted between August 1995 and August 1997 as an addition to an existing program.[90] A total of 31,832 specimens were received, and 26,358 were analyzed (83%), the untested samples being hemolyzed, insufficient in volume, or from children whose parents refused participation. Two hundred seventeen children (0.8%) had abnormal conjugated bilirubin values, which upon repeat were still abnormal in 15 of 129 children (12%) in the community and 54 of 88 children (61%) who were inpatients when tested. Causes in the hospitalized children were often acquired liver conditions, such as parenteral nutrition, cardiac surgery, Rh incompatibility, prematurity, or sepsis; only 8 children had neonatal hepatitis or metabolic liver disease, and none had BA. Causes of conjugated hyperbilirubinemia detected in the community by home visits included 6 cases of neonatal hepatitis, 2 of BA, 2 of hypopituitarism, and individual cases of Alagille syndrome, progressive familial intrahepatic cholestasis, al-

pha-1 antitrypsin deficiency, alpha-1 antitrypsin carrier state, and congenital heart disease. During this same period, 7 children with cholestatic liver disease who had not been detected previously were referred to their center: 6 had not been screened, and 1 had levels below the cutoff, but none of these had BA. Thus, with a conjugated bilirubin cutoff level of 18 μmol/L, the sensitivity of the testing in the community was 100%, and the specificity was 99.6%. These data were obtained in liquid blood specimens. To date, efforts to measure conjugated bilirubin using tandem mass spectrometry on dried blood spots have been unsuccessful.

Dr. Akira Matsui (Jichi University, Japan) discussed an evolving program using stool color cards to screen for BA in Japan. The concept of routine screening of newborns for BA using a stool color card was initiated in the early 1990s.[116,117] A card with color photographs of 7 different stool colors was found to be most reliable and easiest to use. Mothers were asked to compare the color of their infants' stool to that of the card and fill in the corresponding number just before the routine 1-month health check. The stool color was confirmed by the physician, and the cards were mailed to a mass screening center.

A large-scale program using color stool cards was initiated in the Tochigi Prefecture.[118] Among 147,337 children screened (85% of live births), 86 (0.06%) reported a pale stool color at 1 month, of whom 10 were found to have BA. At the same time, 5 other children who had been screened were found to have BA. Thus, the sensitivity of screening was 67%, and the specificity was 99.9%. For children in the screening program, HPE was performed between 40 and 109 days of life (mean = 53 days, all but 1 <60 days), whereas among the 5 children who were not in the screening program, surgery was performed between 25 and 138 days of life (mean = 84 days, only 1 less than <60 days). Nevertheless, the screened and unscreened children had similar rates of survival with the native liver after HPE (both 50% at 5 years) and rates of liver transplantation or death (44% and 40%). Thus, stool card screening programs can reduce the average age of HPE for BA. Currently, stool color card screening for BA is being expanded to other areas of Japan that will prospectively analyze the average age of HPE and survival.

Dr. Mei-Hwei Chang (National Taiwan University Hospital, Taipei, Taiwan) discussed a stool color card screening program that has been adopted in Taiwan. Nationwide universal screening was initiated in January 2004.[91] The program currently uses cards with 6 colored photographs of stools of different colors that are attached to the child health booklet. Parents are asked to observe the infant's stool color and report if it is abnormal within 24 hours. The color is checked by medical staff during routine health checkups at the age of 1 month. Between 29 (in the regional study) and 40 cases (in the national study) of BA have been identified each year in Taiwan with stool color cards. The rate of HPE before the age of 60 days increased gradually from 2002-2003 to 2005. These rates are far higher than historic rates, reported to be 23% in 1976-1989 and 36% in 1976-2000.[119] Rates of sensitivity and specificity have been estimated on the basis of whether detection is made before 60 days of age. The sensitivity was 72%-97%, and the specificity was 99.9%. This universal screening program has effectively increased the rate of HPE before the age of 60 days.

Dr. Margarita Ramonet (Hospital Nacional Alejandro Posadas, Buenos Aires, Argentina) discussed a pilot screening program in Argentina. From August 1999 to August 2002, 12,484 children were delivered at the Hospital Nacional Alejandro Posadas, of whom 4239 (39%) were monitored and screened with a Spanish-language stool color card based on the model developed by Matsui et al.[116,117] Eighteen infants were identified, of whom only 4 were subsequently proven to have liver disease (Alagille syndrome, syphilitic hepatitis, idiopathic neonatal hepatitis, and biliary lithiasis).[120] No case of BA was identified.[121] This program is being expanded to be included as a part of the routine screening of newborns in a larger area in Argentina.

Summary Recommendations for Future Research

I. Improving Outcomes

1. A younger age at the time of HPE consistently has been found to be associated with better outcomes. These findings justify further efforts to improve early diagnosis and prompt referral and treatment of BA.

2. In other countries, surgical results of HPE have been optimized by the limitation of the procedure to centers of proven expertise. Attempts should be made to gather data from US centers assessing outcomes with respect to known risk factors and the experience of the center and surgeon (cases per year). This analysis might be done through an Internet-based registry organized by the academic societies of pediatric gastroenterology (North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition) and surgery (American Pediatric Surgical Association).

3. International working groups should be formulated to develop standardized definitions for assessing outcomes. For example, "resolution of jaundice" might be defined as a total serum bilirubin level below 2 mg/dL (34 μM) at 3 months after HPE. "Survival with the native liver" should be reported at fixed and agreed-on times,

such as 2, 5, and 10 years of age. "Successful outcome of HPE" might be defined as survival without transplantation and without listing at specific times. Furthermore, children with the native liver could be further categorized according to the degree of liver dysfunction, serum bilirubin levels, or signs of chronic liver disease and portal hypertension.

4. A meta-analysis should be conducted combining the raw data of a number of large reported contemporary series of patients who have undergone HPE in different countries, with the goal of achieving a definitive analysis of the effects of the age at HPE, the center experience, the anatomic pattern of bile duct atresia, the presence of other anomalies, and the histological features on surgical and other outcomes. Such an analysis would be most helpful in defining the optimal age for HPE and at what age the success rates of the procedure begin to decline.

5. A prospective trial of aggressive nutritional support and its effects on pretransplant and posttransplant outcomes is justified. Practice guidelines should be developed for integrating nutritional support into the overall management of BA.

6. A longitudinal and long-term analysis of neurodevelopmental outcome and its predictors and modifiers should be conducted in a cohort of BA patients, including the posttransplantation period.

7. National and international efforts should be coordinated for the investigation of the potential benefits and risks of corticosteroid (or any other) therapy during the perioperative period. The utilization of standardized treatment and follow-up protocols and data sharing by different groups of investigators would enhance these efforts.

II. Screening and Early Diagnosis

1. The ideal screening method for BA would be applicable at birth, highly sensitive, adequately specific, inexpensive, and feasible in routine practice. Although no method currently satisfies these criteria, 2 promising methodologies include newborn testing for conjugated bilirubin levels and stool color card programs. Despite their limitations, these 2 screening modalities deserve prospective assessment in the United States and Canada.

2. New serum or urine biomarkers for the presence of BA applicable to the first few weeks of life should be sought with state-of-the-art technologies, such as genomics, gene arrays, proteomics, and metabolomics.

3. Pediatricians and primary care providers should be educated about the role of routine testing for conjugated and total serum bilirubin levels during the assessment of jaundiced infants more than 1 week old. A finding of an elevated conjugated bilirubin level that represents more than 20% of the total bilirubin concentration should be considered abnormal and prompt further evaluation and referral. The AAP is encouraged to include these recommendations in guidelines for the management of the jaundiced infant.

4. Educational efforts aimed at primary care providers should stress the importance of vigilance in following infants with prolonged jaundice beyond 2 weeks of age, even in the breast-fed infant.

III. Etiology and Pathogenesis

1. Because understanding the etiology of BA will likely be the best strategy for the development of novel and effective therapies and preventative measures, efforts should continue to be directed toward understanding its etiology and the cellular, molecular, and immunologic processes involved in its pathogenesis.[121]

2. Basic and clinical research should focus on a better categorization of subtypes of BA at the molecular, gene transcription, proteomic, and phenotypic levels.

3. A comprehensive survey of the role of candidate genes in the pathogenesis of BA, including the laterality genes and those involved in biliary development, and of epigenetic factors that modify gene expression should be conducted.

4. The potential role of triggering viruses or other infectious agents should be thoroughly investigated in human cases of BA with molecular, genomic, and proteomic techniques.

5. An investigation of the role of the innate and adaptive immune system in initiating and perpetuating extrahepatic and intrahepatic bile duct injury should be conducted in both human cases and animal models of BA.

6. Combinations of transgenic, gene-knockout, and gene-silencing technologies should be applied to model organisms and mouse models focusing on the developmental biology of the biliary tract and genes that affect developmental abnormalities.

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