Neonatal cholestatic disorders are a group of hepatobiliary diseases occurring within the first 3 months of life. Bile flow is impaired, and patients have conjugated hyperbilirubinemia, acholic stools, and hepatomegaly. Overall, 1 in 2,500 live births is affected with a neonatal cholestatic disorder (1). The two most common causes of neonatal cholestasis are biliary atresia and idiopathic neonatal hepatitis, accounting for up to 50% to 70% of cases. Other causes include a variety of neonatal infections (viral, toxoplasmosis, syphilis, bacterial), metabolic and genetic diseases, progressive familial intrahepatic cholestatic disorders (PFIC), paucity of interlobular bile duct disorders (e.g., Alagille syndrome), choledochal cyst, ischemia–reperfusion injury, association with parenteral nutrition administration, and other conditions (Table 1). Despite clinical improvement after the portoenterostomy procedure, approximately 70% to 80% of children with biliary atresia will eventually require liver transplantation; thus, biliary atresia alone accounts for almost 50% of all liver transplants performed in children (1). It should be noted that $77 million is spent each year in the United States on liver transplantation for children and the ensuing hospitalizations (2). This sum of money covers 0.2% of total health care expenditures related to children, even though these children represent 0.0006% of the total pediatric population. Importantly, this disproportionate expenditure for liver transplantation in children could be cut in half if improved therapies for biliary atresia were developed that could abrogate or further delay the need for liver transplantation. Remarkably, little is known about the etiopathogenesis of biliary atresia; consequently, there has been slow progress in developing improved therapies or preventative strategies during the past decade. The purpose of this review is to summarize recent advances in the diagnosis and management of biliary atresia, examine the clinical outcome, describe the evolving theories of the etiology and pathogenesis of this disorder, and highlight gaps in our current knowledge.

DEFINITIONS AND CLINICAL FEATURES

Biliary atresia is the most common neonatal cholestatic disorder, occurring in approximately 1 of 8,000 (Asian countries) to 1 of 18,000 (European countries) live births, with a female preponderance, characterized by complete fibrotic obliteration of the lumen of all or part of the extrahepatic biliary tree within 3 months of life (1). Fibrous obliteration may involve the entire extrahepatic biliary system or any part of the system, with concomitant injury and fibrosis of intrahepatic bile ducts (hence, the term extrahepatic has been dropped from the name of this disorder in recent years). In a prospective study conducted between 1968 through 1993 in Atlanta, Georgia, the calculated incidence of biliary atresia was 0.73 cases per 10,000 live births, with a higher prevalence in African-American children than in white children (3). There was also a considerable seasonal clustering of cases, with the incidence three times higher in infants born between December and March. In contrast, a recent study using the Swedish National Health Database found no seasonal occurrence of biliary atresia between 1987 and 1997, with an incidence of 1 in 14,000 live births (4).

Biliary atresia is most likely a clinical phenotype resulting from a number of prenatal or perinatal insults to the hepatobiliary tree, although the etiologic factors and pathogenesis of the obliteration of the biliary tree are poorly understood (5). In approximately 20% of patients with biliary atresia, the presence of at least one other major congenital anomaly suggests that defective development of the bile duct apparatus played a role in these cases (5,6). In particular, polysplenia syndrome (polysplenia, midline liver, interrupted inferior vena cava, situs inversus, preduodenal portal vein, and malrotation of the intestine) is present to some degree in 8% to 12% of
of biliary atresia is not associated with other congenital anomalies of thoracic and abdominal organ development (associations with polysplenia syndrome) (11). Alternatively, an intrauterine insult may interrupt normal development of duct development, such as those that determine laterality of thoracic and abdominal organ development (association with polyplasia syndrome) (11). Alternatively, an intrauterine insult may interrupt normal development of multiple organs, including the biliary tree.

The more common form (found in 80% of patients) of biliary atresia is not associated with other congenital anomalies and has been termed the perinatal or acquired form, in which it is believed that various perinatal or postnatal events trigger progressive injury and fibrosis of a normally developed biliary tree during a critical period in the first 3 months of life (5,9,12). Clinically, the fetal form of biliary atresia is associated with early-onset jaundice and acholic stools (within the first 3 weeks of life without a jaundice-free period), whereas the acquired form of biliary atresia generally has onset of jaundice and acholic stools in the 2nd to 4th weeks of life after a period of normally pigmented stools. Despite these potential disparate etiologies, the clinical phenotype of these two forms of biliary atresia may appear identical unless other congenital anomalies (suggesting the fetal form) are discovered on clinical evaluation.

Idiopathic neonatal hepatitis is a descriptive term used for neonatal intrahepatic cholestasis in which the characteristic “giant cell hepatitis” lesion is present on liver biopsy, and for which no other infectious, genetic, metabolic, or anatomic cause is identified (13). In older series, idiopathic neonatal hepatitis comprised up to 30% to 40% of all neonatal cholestasis cases. However, during the past two decades, infants believed to have idiopathic neonatal hepatitis were later found to have newly discovered metabolic and genetic diseases, such as α-1 antitrypsin deficiency, PFIC, neonatal iron storage disease, inborn errors of bile acid synthesis (14), type 2 citrullinemia (15), Niemann–Pick type C disease, and infections with newly described viral agents (e.g., parvovirus and human herpes type 6 [HHV-6]). Up to 20% of cases of idiopathic neonatal hepatitis are progressive, appear to be familial, and hold a worse prognosis. These cases may be caused by novel genetic or metabolic disorders, some of which are now classified as PFIC, and others yet to be defined. Thus, the term idiopathic neonatal hepatitis now defines an ever-shrinking percentage of cases of intrahepatic cholestasis (perhaps 10–20%) for which an etiology has not been discovered.

The clinical presentation of idiopathic neonatal hepatitis and biliary atresia is similar, although patients with biliary atresia tend to be female and appear well nourished, whereas those with idiopathic neonatal hepatitis are frequently male, small for gestational age, and failing to thrive. It has been stated that the well-nourished appearance of infants with biliary atresia may result in a delay of diagnosis. However, when careful anthropometric evaluation is performed, infants with biliary atresia have significantly decreased fat stores and lean body mass (16). The added mass of an enlarged liver and spleen and the occasional finding of subclinical ascites may account for the appearance of a well-nourished infant (a relatively normal weight for age and weight for length on standardized growth curves) (16). In both conditions, jaundice, hypopigmented or frankly acholic (white or gray) stools, dark urine, and hepatosplenomegaly develop within the first 3 months of life. Although there are no other defining physical features of biliary atresia or neonatal hepatitis, findings that may lead to diagnosis of other causes of neonatal cholestasis include heart murmur and dysmorphic facial features (Alagille syndrome), hypotonia and long forehead (Zellweger syndrome), and lymphedema (Aagenaes syndrome). Laboratory evaluation typically shows conjugated hyperbilirubinemia (>20% of total serum bilirubin) with elevation of serum concentrations of hepatocellular (aspartate aminotransferase [AST], alanine aminotransferase [ALT]) and canalicular (alkaline phosphatase, γ-glutamyl transpeptidase [GGT]) enzymes (17). Although patients with biliary atresia tend to have higher GGT concentrations than those with intrahepatic forms of cholestasis.

### TABLE 1. Differential diagnosis of neonatal cholestasis

<table>
<thead>
<tr>
<th>Intrahepatic causes</th>
<th>Extrahepatic causes</th>
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<tr>
<td>Congenital infections—viral, protozoan, spirochetal, bacterial sepis</td>
<td>Biliary atresia</td>
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<tr>
<td>Metabolic disorders—galactosemia, tyrosinemia, hereditary fructose intolerance, alpha-1-antitrypsin deficiency, cystic fibrosis, hypopituitarism, bile acid synthesis defects, citrin deficiency, respiratory chain disorders</td>
<td>Choledochal cyst</td>
</tr>
<tr>
<td>Storage diseases—neonatal iron storage disease, Niemann-Pick type C, Gaucher’s disease, Wolman’s disease, glycogen storage disease type 4</td>
<td>Spontaneous perforation of the common bile duct</td>
</tr>
<tr>
<td>Genetic syndromes—Alagille syndrome, Turner syndrome, Down Syndrome, Aagenaes syndrome, Zellweger syndrome, arthrogryposis/cholestasis syndrome</td>
<td>Cholechochal cyst</td>
</tr>
<tr>
<td>Progressive familial intrahepatic cholestasis—FIC1 deficiency, BSEP deficiency, MDR3 deficiency, Byler syndrome</td>
<td>Toxins and drugs—endotoxemia, total parenteral nutrition-associated cholestasis, chloral hydrate, antibiotics, other drugs</td>
</tr>
<tr>
<td>Idiopathic disorders—idiopathic neonatal hepatitis, non-syndromic paucity of interlobular bile ducts</td>
<td>Miscellaneous—ischemia-reperfusion injury, neonatal lupus, congenital hepatic fibrosis, Caroli’s syndrome, inspissated bile syndrome, histiocytosis X</td>
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</table>

all children with biliary atresia (7,8). Biliary atresia that is associated with these and other congenital anomalies has been termed the fetal or embryonic form, although it may be a common phenotype of multiple prenatal etiologies (9,10). For some patients, it has been proposed that these anomalies are caused by abnormal expression of genes (somatic or inherited mutations) that regulate bile duct development, such as those that determine laterality of thoracic and abdominal organ development (association with polyplasia syndrome) (11). Alternatively, an intrauterine insult may interrupt normal development of multiple organs, including the biliary tree.

(ids)
(18), overlap in values precludes the differential value of this test. Surprisingly, in patients with biliary atresia, serum total bilirubin is rarely more than 12 mg/dL (and may be as low as 5–8 mg/dL), and conjugated bilirubin is usually less than 8 mg/dL despite complete bile duct obstruction, whereas bilirubin levels may exceed 20 mg/dL in patients with idiopathic neonatal hepatitis. Consequently, infants with biliary atresia may appear to only have mild scleral icterus without cutaneous jaundice, particularly if they have dark skin. Elevated serum bile acid concentrations are universal for patients with these disorders and do not differentiate between intrahepatic and extrahepatic obstruction. However, unexpectedly low or normal serum bile acid concentrations suggest a defect in synthesis of bile acids and should prompt evaluation for these disorders (14).

Although biliary atresia and idiopathic neonatal hepatitis are the two most common forms of cholestatic liver disease that occur in neonates, there are many other disorders that must be considered in the differential diagnosis (Table 1) (17,19). Treatable causes (primarily infectious and metabolic) must be identified promptly to prevent progressive liver injury and irreversible damage to other organs. Recent discovery of the genetic and molecular causes of several forms of PFIC (e.g., mutations in genes coding for BSEP, FIC1, and MDR3) (20) has not only provided an explanation for the etiology of these rare but important disorders but also has led to the discovery of new bile acid and phospholipid membrane transporters. This new knowledge has revolutionized our understanding of mechanisms of bile formation, predisposition to gallstone disease, and the role of heterozygote carriage of these and other genes in modifying or causing other hepatobiliary diseases (21,22). The discovery of the genetic basis of Alagille syndrome, mutations in the \textit{JAGGED-1} gene that codes for a ligand to the Notch receptor (23), has invigorated investigation into the molecular control of differentiation and morphogenesis of the intrahepatic bile ducts and the cardiovascular system.

**CURRENT THEORIES OF ETOLOGY OF BILIARY ATRESIA**

Our understanding of the etiology and pathogenesis of liver and bile duct injury in patients with biliary atresia and idiopathic neonatal hepatitis has remained essentially unchanged until recent years. Continued investigation in this area is essential to provide a rational scientific basis for the development of novel therapeutic and preventative strategies. Currently, biliary atresia is believed to be a common phenotypic response of the neonatal liver and bile ducts to a variety of prenatal and perinatal factors, including genetic and environmental insults (viral, metabolic, vascular) that perturb the normal development or maturation of the biliary tree and that occur during a specific period (prenatally to before 3 months of age) amid the milieu of a genetic or immunologic susceptibility to this disease (Fig. 1). Biliary atresia and idiopathic neonatal hepatitis are not believed to be inherited disorders (except for the 10–20% of familial cases of idiopathic neonatal hepatitis and some cases of polysplenia syndrome-associated biliary atresia) because human leukocyte antigen (HLA)-identical twins discordant for biliary atresia have been described, and recurrence of biliary atresia within the same family is exceedingly rare (24,25). Nevertheless, this does not exclude the possibility that during fetal development somatic mutations of key genes regulating morphogenesis of these structures may be involved, or that there is a genetic predisposition to an aberrant immune response that is only expressed on exposure to an exogenous agent during a critical period.

An additional factor leading to progressive sclerosis of the extrahepatic bile ducts, regardless of the etiology of the initial injury and disruption to the ductal epithelium, may be the extravasation of bile with its detergent constituents into the submucosa, eliciting a secondary inflammatory sclerosing process. The potential role of this mechanism was first described in 1901 by Rolleston and Hayne (26), who coined the term descending cholangitis.

**FIG. 1.** Proposed interaction of four factors that result in the phenotypic expression of biliary atresia.
Tan et al. (27) have demonstrated the presence of extravasated bile in bile duct remnants of patients with biliary atresia, perhaps originating from breaks in the biliary mucosa at the porta hepatitis. This group further proposed that there was a vulnerable stage of human biliary development between 11 and 13 weeks of gestation, when failure of remodeling of the ductal plate structures could lead to disturbances in the normal formation of an adequate mesenchymal cuff around developing biliary bile ducts, which could potentially be prone to rupture at the initiation of bile flow at 12 to 13 weeks of gestation. Extravasated bile in adjacent periductal tissues would then lead to protracted inflammation and fibrosis, causing secondary obliteration and obstruction of the more distal extrahepatic bile duct.

The pathogenesis of ongoing injury and sclerosis of intrahepatic bile ducts after portoenterostomy is also not well understood. Is it continued ductal obstruction to bile flow even after a “successful” portoenterostomy that eventually leads to biliary cirrhosis, albeit at a slower pace if initial bile flow is established? Is it an ongoing immune or autoimmune process that targets intrahepatic bile ducts? Is it asymptomatic bacterial cholangitis or absorption of bacterial cell wall products and lipopolysaccharides that continue to drive the intrahepatic inflammation? Clearly, bile duct epithelial cells are fully capable of secreting a number of cytokines and growth factors that can recruit inflammatory and hepatic stellate cells and activate synthesis of extracellular matrix (28). In addition, cholestasis leads to the retention of hydrophobic bile acids in hepatocytes, which stimulate the generation of considerable oxidative stress, mitochondrial dysfunction, and cell pathways involved in hepatocellular injury and cell death (29). In this way, hepatocytes may participate in the induction of fibrosis in patients with biliary atresia through secretion of cytokines and lipid peroxide products that regulate collagen synthesis in hepatic stellate cells (30).

The following summarizes the six current theories of the etiology of biliary atresia (Table 2).

<table>
<thead>
<tr>
<th>TABLE 2. Proposed etiologies of biliary atresia</th>
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<tr>
<td>Infectious—reovirus, rotavirus, retrovirus, cytomegalovirus, human papilloma virus, other agents</td>
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<tr>
<td>Immune dysregulation</td>
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<tr>
<td>Autoimmune mechanism</td>
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<tr>
<td>Vascular lesion/arteriopathy</td>
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<tr>
<td>Defective morphogenesis</td>
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<tr>
<td>Inherited mutations</td>
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<tr>
<td>Laterality genes (presumed), associated with polysplenia and asplenia syndromes (e.g., CFCI)</td>
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<tr>
<td>Ductal plate malformation (e.g., HNF6)</td>
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<tr>
<td>Jagged 1</td>
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<tr>
<td>Other genes</td>
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<tr>
<td>Somatic mutations</td>
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<td>Modifier genes</td>
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<td>Toxin exposure</td>
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</table>

Viral infection

Epidemiologic studies support a possible infectious etiology to biliary atresia and idiopathic neonatal hepatitis. There has been continued demonstration of seasonal clustering of cases, suggesting environmental exposure to an infectious agent (3). In addition, several models of viral infection in newborn mice produce lesions similar to biliary atresia (5), as described below. In 1974, Benjamin Landing, a pediatric pathologist, proposed that biliary atresia, idiopathic neonatal hepatitis, and choledochal cyst represented the result of different primary sites of injury to the hepatobiliary tree by a common insult, and coined the term infantile obstructive cholangiopathies (31). Although Landing proposed involvement of hepatitis B virus, subsequent studies have shown no association between the common hepatotropic viruses (hepatitis A, B, and C) and biliary atresia. More recent attention has focused on the possible role of five viruses.

For many years, cytomegalovirus (CMV) has been proposed as a possible etiologic agent because a modest proportion of infants with biliary atresia and idiopathic neonatal hepatitis have been infected with CMV, as are healthy infants (32). Although a recent study from Sweden (33) showed a higher prevalence of CMV antibodies in mothers of patients with biliary atresia, and CMV DNA was present in livers from 50% of infants with biliary atresia, a Canadian group (34) could not demonstrate CMV in bile duct remnants from 12 children with biliary atresia. The role of CMV has not been explored in a large prospective multicentered study with proper controls.

The two viruses most commonly implicated are reovirus and rotavirus. Interest in reovirus stemmed from the observation that infection in weanling mice caused pathologic features of the intrahepatic and extrahepatic bile ducts and the liver similar to those of biliary atresia (35). These lesions persisted even after infectious virus or viral antigens could no longer be detected. One group detected reovirus antigens in bile duct remnants from infants with biliary atresia (36,37) and in an infant rhesus monkey with biliary atresia (38); however, others did not replicate these findings in a study of infants with biliary atresia (39). Serologic studies of reovirus antibodies in infants with biliary atresia have likewise been inconclusive (36,39–41). The high incidence of passively transferred maternal antireovirus immunoglobulin (Ig) G may have confounded these studies. Two groups of investigators have examined hepatobiliary tissues removed from infants with biliary atresia for reovirus RNA. Steele et al. (42) failed to detect reovirus RNA in archived, formalin-fixed preserved hepatic tissues from 14 biliary atresia patients, 20 idiopathic neonatal hepatitis patients, and 16 control subjects using a nested reverse transcriptase–polymerase chain reaction (RT-PCR) assay. In contrast, Tyler et al. (43) reported nested RT-PCR evidence of reovirus infection in snap frozen liver or bile duct...
from 55% of patients with the acquired/perinatal form of biliary atresia and in only 8% to 15% of autopsy control samples and infants aged less than 1 year with other liver diseases. Reovirus RNA was not detected in three patients with the fetal form of biliary atresia. The discrepancies between these two studies may lie in the methods of preparation of the tissue, different methods of RNA isolation, and the use of PCR primers for different reovirus genes. If reovirus is shown to be involved, potential antiviral strategies may be entertained. In the future, the potential role of this virus can only be definitively evaluated using large numbers of well-characterized patients and appropriate disease and healthy control subjects.

Recent interest has also focused on group C rotavirus (another virus of the Reoviridae family) in the etiology of biliary atresia. Group A rotavirus infection was shown to produce extrahepatic bile duct obstruction in newborn mice with hepatic histology similar to biliary atresia (44). Czech-Schmidt et al. (45) further characterized this model and demonstrated that the optimal intraperitoneal infecting dose of rhesus rotavirus (RRV) serogroup 3 in newborn BALB/c mice was 10^{10} to 10^{8} plaque-forming units administered 12 hours after birth, resulting in appearance of cholestasis and biliary atresia in 38% to 86% of infected mice. Intrauterine infection did not cause cholestasis or biliary atresia despite transplacental infection of the fetal mice. Immunity induced by previous infection with RRV in dams appeared to protect subsequent newborn mice from developing hepatobiliary disease when infected postnatally with RRV, whereas maternal milk was not protective. Petersen et al. (46) reported that the administration of interferon-α before RRV infection prevented the biliary disease, and Qiao et al. (47) reported an increase in the incidence of bile duct obstruction in normal newborn BALB/c mice compared with SCID (immunodeficient) mice infected with rotavirus, indicating that the role of the immune system is critical in this mouse model. Riepenhoff-Talty et al. (48) examined hepatobiliary tissues from human patients for RT-PCR evidence of group C rotavirus infection. Ten of 18 patients with biliary atresia and 0 of 12 liver disease control subjects showed evidence of rotavirus RNA. In contrast, Bobo et al. (49) failed to detect RNA evidence for rotavirus groups A, B, or C in tissues from 10 patients with biliary atresia and 14 liver disease control patients using a nested RT-PCR enzyme immunoadassay; however, almost half the patients were aged more than 12 months when tissues were obtained. Thus, there is suggestive, but inconclusive, evidence that rotavirus infection may be involved in up to 50% of cases of biliary atresia, similar to the prevalence of reovirus infection in the study of Tyler et al. (43).

The possible role of other viruses has recently been investigated. Human papilloma virus (HPV) was detected using PCR in archived liver tissue from 16 of 18 patients with biliary atresia and from 0 control patients from Argentina (50,51). However, Domiati-Saad et al. (52) failed to demonstrate evidence of HPV DNA in 19 patients with biliary atresia or idiopathic neonatal hepatitis from the United States, although they did detect HHV-6 DNA in several patients with neonatal hepatitis and biliary atresia. The possible role of HPV and HHV-6 in biliary atresia is unsettled and requires further investigation.

Finally, Mason et al. (53,54) recently described immunoreactivity and PCR evidence of retroviral infection in the liver of patients with primary biliary cirrhosis, and serum immunoreactivity against a retrovirus in a limited number of patients with biliary atresia. They attributed this finding to an autoimmune response to antigenically related cellular proteins or to an immune response to uncharacterized viral proteins. Further work in this potentially important area of adult and pediatric biliary disorders is warranted.

Immune injury in biliary atresia

Schreiber and Kleinman (55) proposed that biliary atresia was the result of a "multihit" pathologic process, in which a viral or toxic insult to biliary epithelium leads to newly expressed or altered antigens on the surface of bile duct epithelia, which, in the proper genetically determined immunologic milieu, are presented by macrophages to T lymphocytes. Cytotoxic T cells then elicit a Th1 cellular response causing bile duct epithelial injury, eventually resulting in fibrosis and occlusion of the extrahepatic bile duct (Fig. 2). Unique aspects of innate and acquired immunity that are present in neonates may also play an important role in determining why this disorder only occurs within the first 3 months of life and in a presumably small percentage of infants infected with the putative agent. In addition, passively acquired maternal factors could potentially affect presentation and immune recognition of antigens and T-cell activation in neonates, causing liver injury as it does in the neonatal lupus syndrome (56).

Genes that encode a variety of immune regulatory proteins, in part, control the susceptibility of immune or autoimmune injury to biliary epithelium. A number of immune-mediated liver diseases, including autoimmune hepatitis, primary sclerosing cholangitis, and primary biliary cirrhosis, are associated with specific HLA antigens (57). For this reason, several investigators have sought HLA associations in biliary atresia. Silviera et al. (58) initially reported that HLA-B12 (49% biliary atresia patients vs. 23% control subjects) and haplotypes A9-B5 and A28-B35 were associated with biliary atresia in a European population. Other groups could not replicate these findings but reported a relationship of biliary atresia with HLA class I (59) and, in Japan, with A33, B44, and DR6 (60). However, a Spanish group of investigators could not detect any HLA association in 48 patients with biliary atresia (61). More recently, Donaldson et al. (62)
reported molecular genotyping of 101 European children with biliary atresia for selected HLA-A, HLA-B, DRB1, DQA1, DQB1, and DPB1 alleles that had previously been implicated in the pathogenesis of biliary atresia (58). No relationship could be demonstrated between these alleles and biliary atresia, with a statistical power of 75% at a type 1 error rate of 5%. Thus, a weak association may not have been detected by this study. It should also be noted that the complete MHC I and II genomes were not investigated in this study (there are more than 100 HLA genes and 1,000 sequenced alleles in the human MHC genome); therefore, it is possible that other HLA relationships may be present. Most recently, A-Kader et al. (63) reported a significantly increased frequency of HLA-B8 (83% patients vs. 6.5% of general population) and DR3 (94% patients vs. 15%) in 18 Egyptian children with biliary atresia, 10 of whom harbored the B8/DR3 haplotype. These findings are of particular interest because the HLA-B8 and DR3 haplotypes are frequently found in patients with primary sclerosing cholangitis and inflammatory bowel disease (64). The hypothesis that MHC class I and class II genotypes may predispose persons to biliary atresia or idiopathic neonatal hepatitis needs to be investigated in larger multiethnic cohorts of patients.

There is substantial evidence to support the role of a cytotoxic T-cell response in the pathogenesis of biliary atresia. In 1977, Gosseye et al. (65) demonstrated lymphocytes in the connective tissue of the portahepatis in patients with biliary atresia, and Bill et al. (66) noted the relationship of intramural mononuclear inflammatory cells with epithelial cell necrosis in bile duct remnants. In
specimens from patients with biliary atresia. Davenport aberrantly expressed by bile duct epithelium in liver (69) showed that HLA-DR (MHC class II molecules) was by bile duct epithelium. However, several groups (60,61, class I antigens, but not class II antigens, are expressed as APCs in patients with biliary atresia. Normally, MHC have proposed that bile duct epithelial cells may function in the context of self-MHC class I molecules. Based on this paradigm, several investigators have proposed that bile duct epithelial cells may function as APCs in patients with biliary atresia. Normally, MHC class I antigens, but not class II antigens, are expressed by bile duct epithelium. However, several groups (60,61,69) showed that HLA-DR (MHC class II molecules) was aberrantly expressed by bile duct epithelium in liver specimens from patients with biliary atresia. Davenport et al. (70) further demonstrated that CD4+ lymphocytes and natural killer (CD56+) cells predominated in the liver and extrahepatic bile duct of patients with biliary atresia, that the cellular infiltrate was activated and proliferating, and that ICAM-1 was expressed in sinusoidal endothelium. These data are consistent with the hypothesis that lymphocyte adhesion and T-cell activation and cytotoxicity, at least in part, mediate the extrahepatic bile duct damage and obliteration in patients with biliary atresia.

The Kupffer cell (resident liver macrophage) also functions as an APC in the liver. A recent study from Japan demonstrated increased numbers and size of Kupffer cells in liver tissue from patients with biliary atresia at the time of diagnosis (71). Davenport (70) also showed that an increase in CD68+ macrophage infiltration in portal tracts and biliary remnant tissue from patients with biliary atresia predicted a poor outcome after the portoenterostomy procedure, consistent with the function of activated macrophages to release cytokines, reactive oxygen intermediates, and growth factors that may signal hepatic stellate cells to synthesize and secrete collagen, thereby promoting fibrogenesis and cirrhosis. One other important feature of macrophages is the capacity to secrete tumor necrosis factor-α (TNF-α), reactive oxygen species, and nitric oxide, which may be involved in the induction of apoptotic and necrotic intracellular pathways. Along these lines, Funaki et al. (72) have shown that apoptosis of intrahepatic bile duct epithelial cells is highly prevalent in the liver of patients with biliary atresia compared with normal liver or that from patients with choledochal cyst. Moreover, Liu et al. (73) reported a relationship between Fas ligand (FasL) mRNA expression in bile duct epithelia and the presence of apoptosis in patients with biliary atresia. Because FasL is not normally expressed in bile duct epithelial cells, this finding appeared to be specific to biliary atresia. Bile drainage after the portoenterostomy procedure was significantly better in patients with negative signals for FasL on bile duct epithelium than in patients with positive signals (73). This observation suggests that up-regulation of FasL may result in apoptotic fratricide in which bile duct epithelial cells actually injure other similar cells, or perhaps bile duct epithelia are resisting attack from infiltrating lymphocytes by posing a counterattack against Fas-expressing lymphocytes (68).

Bezerra et al. (74) took a different approach to investigating the immune pathogenesis of biliary atresia. DNA microarrays, using the Affymetrix U95Av2 gene microchip (Santa Clara, CA) containing 12,651 gene products, were assessed on liver biopsies obtained from 14 infants with biliary atresia and compared with 6 taken from patients with intrahepatic neonatal cholestasis. A T_{H1} immune response in the patients with biliary atresia was suggested by the upregulation of a number of genes encoding products involved in lymphocyte differentiation, and several that regulate the T_{H1} response (osteopontin, γ-interferon) combined with downregulation of genes encoding immunoglobulin domains, consistent with suppression of a T_{H2} response. These provocative results emphasize complex interactions among macrophages, T lymphocytes, bile duct epithelial cells, hepatocytes, and other cells in the liver that should form the basis for future investigations.

**Autoimmunity in biliary atresia**

Biliary atresia shares features with several autoimmune diseases, such as the female predominance, apparent triggering by viral infection, and aberrant MHC expression in bile duct epithelium. Consequently, it has been proposed that tissue injury in patients with biliary atresia may represent an autoimmune-mediated process. Two studies have examined circulating autoantibodies in patients with biliary atresia. Vasiliauskas et al. (75) have reported that 10 of 11 patients with biliary atresia were positive for serum IgG and IgM antineutrophil cytoplasmic antibodies (ANCA), with higher levels of the IgM-ANCA in patients with biliary atresia compared with children and adults with other liver diseases. At our center, Burch et al. (56) studied autoantibodies in mothers of children with biliary atresia and idiopathic neonatal hepatitis to test the hypothesis that maternal transfer of autoantibodies might be involved in liver and bile duct injury. Low titer anti-Rho antibodies were more prevalent in mothers of infants with biliary atresia and idiopathic neonatal hepatitis than in control subjects, and low titer antinuclear antibodies were more common in mothers of infants with liver disease. The possible influence
of maternal factors on immune-mediated bile duct injury is a provocative, yet unconfirmed, observation.

An exciting advance in understanding risk factors for autoimmunity has been the demonstration of polymorphisms in genes that predict susceptibility of persons to autoimmune disorders. Recent reports of Bernal et al. (76) and Mitchell et al. (77) have shown that TNF-α gene polymorphisms are associated with susceptibility to primary sclerosing cholangitis (PSC; 58% of PSC patients vs. 29% of control subjects in Bernal’s study). Polymorphisms in the IL-1 gene family have recently been associated with susceptibility and disease progression in patients with primary biliary cirrhosis (78). Similarly, IL-10 polymorphisms have been linked to disease progression in patients with hepatitis C viral and alcoholic hepatitis (79,80). These studies raised the possibility that polymorphisms in genes that regulate immune function and the inflammatory response could potentially predispose persons to biliary atresia. In this regard, Davidson et al. (82) demonstrated the development of bile duct strictures, as observed after liver transplantation. Intrahepatic and extrahepatic bile ducts receive their blood supply exclusively from the hepatic arterial circulation; thus, hepatic arterial ischemia causes bile duct strictures, as observed after liver transplantation. Pickett et al. (82) demonstrated the development of biliary obstruction after ligation of hepatic vessels in fetal sheep. Several investigators have demonstrated an arteriopathy in branches of the hepatic artery in the extrahepatic biliary tree of patients with biliary atresia (83). It has been proposed that the vasculopathy may be the primary lesion in patients with biliary atresia; however, the causative or consequential nature of these lesions remains unclear.

**Defective morphogenesis**

Several lines of evidence suggest that certain cases of biliary atresia (fetal form) are caused by defective morphogenesis of the biliary tree. Because anomalies of visceral organ symmetry (the polysplenia syndrome) are associated with biliary atresia, it is of interest that a recessive insertion mutation in the proximal region of mouse chromosome 4 or complete deletion of the inversion (inv) gene in the mouse leads to anomalous development of the hepatobiliary system in this model (11,84). Schon et al. (85) recently mapped the human INV gene and examined 64 patients with heteroxia. No consistent mutations in this gene were identified; specifically, there were no mutations in seven patients with biliary atresia and various congenital laterality defects, making it unlikely that the INV gene is involved in the majority of “fetal” cases of biliary atresia.

Other human genes that determine laterality (ZIC3, LEFTB, and ACVR2B) have been associated with a small percentage of human situs defects. Bamford et al. (86) recently searched for mutations of the CFC1 gene (which encodes the human CRYPTIC protein) in genomic DNA from 144 patients with familial and sporadic cases of laterality defects. Nine of the patients had heterozygous mutations of this gene, including one with biliary atresia and the polysplenia syndrome. Jacquemin et al. (87) extended this observation by identifying heterozygous gene mutations in two brothers with laterality defects, one who had biliary atresia, which were inherited from their healthy mother. It appears that these heterozygous mutations of CFC1 may not be solely responsible for this phenotype but may provide for a predisposition that then requires a second genetic or environmental factor to produce the disease phenotype. Although the precise function of CRYPTIC protein is not known, it appears to act as a cofactor in the Nodal pathway that determines left–right axis development. Further investigations of this and other related genes that determine laterality may shed light in the coming years on whether inherited or somatic mutations or deletions are responsible for individual cases of biliary atresia.

Intrahepatic bile duct development depends on interactions between mesenchyme and portal venous radicals. Primitive hepatic precursor cells differentiate into a single layer of cells that soon form a double layer (the ductal plate) as the primitive intrahepatic bile ductule anlage. Cells then scatter and remodel as a single layer around the lumen to form the portal tract bile ducts (12). Abnormal remodeling leads to the ductal plate malformation that is believed to be responsible for the liver lesion of congenital hepatic fibrosis and other bile duct dysplasias. However, a number of infants with biliary
Bile duct development.

Interactions between vascular structures and intrahepatic artery and its branches (90), demonstrating the intricate malformation associated with anomalies of the hepatic extrahepatic bile ducts.

Duct epithelial layer into the submucosa of the damaged effects of bile that has extravasated through the injured bile (91). There are also possible toxic and inflammatory effects to a fungal or other environmental toxin exposure (92). Neonatal hepatitis in humans. Two outbreaks of biliary atresia or idiopathic neonatal hepatitis have clearly associated with biliary atresia or paucity of interlobular bile duct disorders. Recently, Kohsaka et al. (89) identified JAGGED1 missense mutations in 9 of 102 patients with biliary atresia. These patients had no phenotypic features of Alagille syndrome. Remarkably, prognosis was worse for the group with mutations, suggesting that JAGGED1 could be a modifying factor in patients with biliary atresia. Alternatively, it is possible that certain patients with biliary atresia have a unique new presentation of Alagille syndrome limited to the biliary tree. The hnf6 knockout mouse or lack of hnf1β in the mouse causes a ductal plate malformation associated with anomalies of the hepatic artery and its branches (90), demonstrating the intricate interactions between vascular structures and intrahepatic bile duct development.

Toxin exposure

Time and space clustering of cases of biliary atresia have led to the proposal that an environmental toxin could be involved in its pathogenesis. Currently, other than infectious agents, no environmental agent has been clearly associated with biliary atresia or idiopathic neonatal hepatitis in humans. Two outbreaks of biliary atresia in lambs and calves in Australia may have been related to a fungal or other environmental toxin exposure (91). There are also possible toxic and inflammatory effects of bile that has extravasated through the injured bile duct epithelial layer into the submucosa of the damaged extrahepatic bile ducts.

Diagnosis of Neonatal Cholestatic Disorders

It is important to identify the small subgroup of infants with jaundice who have direct (conjugated) hyperbilirubinemia and to establish the underlying diagnosis as soon as possible to afford the benefit of specific medical therapies and to allow for optimal outcome after portoenterostomy for biliary atresia. For this reason, it is recommended that a fractionated bilirubin be obtained for all infants who remain visibly jaundiced beyond 2 to 3 weeks of age, or who develop acholic stools or hepatomegaly. Clinical differentiation among the common causes of cholestasis is limited. In both biliary atresia and idiopathic neonatal hepatitis, infants present with jaundice in the first 8 weeks of life, progressive loss of pigmentation in their stools, and development of hepatomegaly and splenomegaly (17). Biliary atresia is more commonly found in infant girls who were appropriate for gestational age at birth and appear to be thriving, whereas idiopathic neonatal hepatitis is more common in infant boys who were small for gestational age and are failing to thrive. The most accurate diagnostic test for differentiating biliary atresia from other conditions is percutaneous liver biopsy. When obtained before laparotomy, liver biopsy has a diagnostic accuracy for biliary atresia when examined by experienced pathologists of 90% to 95% if an adequate biopsy size is obtained (92,93), and will prompt urgent surgical exploration if characteristic findings are present. Liver biopsy samples from patients with biliary atresia generally show bile ductular proliferation, canalicular and cellular bile stasis, portal or periportal inflammation, and fibrosis with the presence of bile plugs in portal tract bile ducts (Fig. 3) (9,92). Hepatocyte giant cell transformation is found in at least 25% of patients with biliary atresia, particularly if the biopsy is obtained during the first 6 weeks of life. It is important to note that liver histology in patients with α-1-antitrypsin deficiency, and occasionally in those with Alagille syndrome (94), cystic fibrosis, and total parenteral nutrition (TPN)-related cholestasis, can resemble all the features of biliary atresia, necessitating the use of other diagnostic studies to differentiate these disorders. Liver biopsy specimens from patients with idiopathic neonatal hepatitis show lobular disarray, a variable inflammatory infiltrate with marked giant cell transformation of individual hepatocytes, individual hepatocyte necrosis and apoptosis, increased extramedullary hematopoiesis, and cellular bile stasis. However, bile plugs in portal tract bile ducts are absent, and bile ductular proliferation is usually minimal or absent. Portal tract fibrosis is occasionally found but is not extensive (9). These histologic features, although characteristic, are not specific to idiopathic neonatal hepatitis and have been observed in patients with PFIC, bile acid synthesis disorders, α-1-antitrypsin deficiency, and other conditions.

Other diagnostic studies are less accurate in differentiating biliary atresia from intrahepatic causes of cholestasis. No serum or urine biochemical tests differentiate between these disorders. Hepatobiliary scintigraphy is of limited value. Imaging studies are inconclusive. Failure of isotope excretion into the small intestine during scintigraphy (iminodiacetic acid derivatives) has only 50% to 75% specificity for biliary atresia despite more than 95% sensitivity (95). Severe intrahepatic cholestasis and paucity of interlobular bile duct disorders may yield scinti-
graphic findings indistinguishable from biliary atresia. However, clear demonstration of secretion of the radioisotope into the small bowel essentially excludes biliary atresia. Likewise, ultrasonography of the liver and biliary tree is useful but not diagnostic for biliary atresia. For example, ultrasonography may identify a small, nondistended gallbladder (suggesting biliary atresia) if severe intrahepatic cholestasis is present and, conversely, may show a clear fluid-filled gallbladder remnant in a patient with biliary atresia that is indistinguishable from normal (96). This modality is also not sensitive enough to determine presence or absence of the common hepatic and common bile ducts in small infants. However, ultrasoundography is absolutely essential for the diagnostic evaluation of infants with cholestasis to exclude the presence of a choledochal cyst and to determine the presence of features of polysplenia syndrome, which is strongly associated with biliary atresia.

Newer ultrasonographic techniques have led to the description of two novel findings that may prove to be more diagnostic. Choi et al. (97) reported that a unique triangular or tubular echogenic density (the “triangular cord” sign), representing the fibrous cone of the bile duct remnant at the hepatic porta, is a specific ultrasonographic finding for biliary atresia. Another recent study suggests that using a higher frequency (13 MHz rather than 7 MHz) ultrasound transducer may identify abnormalities in gall bladder shape, wall thickness, and morphology that are characteristic of biliary atresia (96). The usefulness of these sonographic findings for evaluating infants with cholestasis will require further study at other centers. Early studies suggested that magnetic resonance cholangiopancreatography (MRCP) using T2-weighted turbo spin-echo sequences might hold promise as a noninvasive method for diagnosis of biliary atresia (98). However, Norton et al. (99) recently demonstrated limitations of MRCP for differentiation of severe intrahepatic cholestasis from biliary atresia because the ability to identify extrahepatic bile ducts depends on bile flow. The use of endoscopic retrograde cholangiopancreatography (ERCP) has been proposed for identification of the extrahepatic biliary tree, although it requires consider-
able technical expertise and general anesthesia, and the proper sized side-viewing endoscope is not widely available (100,101). Finally, several groups have reported the usefulness of analyzing a duodenal bile specimen for presence of bilirubin or bile acids (102,103) as a test to demonstrate patency of the extrahepatic biliary tree.

Delay in diagnosis of biliary atresia is a considerable problem in the United States because neonatal jaundice may be incorrectly ascribed to “breast milk jaundice;” infants with biliary atresia may have only mild jaundice and not be thoroughly investigated; infants are generally seen by health care providers only at age 2 and 8 weeks; and physician extenders unaware of these rare diseases may be providing the patient care. Thus, an infant aged 2 weeks with jaundice may not be seen again by a health care provider until after age 8 weeks, already the age limit for optimal results of portoenterostomy. To help reduce the average age for diagnosis of biliary atresia, and hence improve outcome from the portoenterostomy procedure, several groups in Japan and Taiwan have developed pilot programs in which stool color cards are given to mothers of newborns (104). These cards have seven or eight numbered color photographs of age-matched infant stools of varying colors, including three acholic stools. When infants are aged 1 month, the mothers are instructed to compare their infant’s stool color with those printed on the card, to fill in a corresponding number, and to take the card to their 1-month physician office visit. If the mother and the physician identified the child’s stool color as one of the acholic examples on the card, the physician calls or sends a fax to the screening center. The mother and an informed pediatric specialist are contacted, and the child is evaluated for cholestasis and biliary atresia. The results of large-scale screening using this method appear promising (104).

The diagnosis of biliary atresia is established after timely exclusion of intrahepatic (infectious, metabolic, genetic, and toxic) causes of cholestasis and choledochal cyst (by ultrasonography) (Table 1). In North America, biliary atresia is then generally diagnosed at minilaparotomy by intraoperative cholangiography that fails to demonstrate a lumen in some portion of the extrahepatic biliary tree, by surgical findings of a fibrotic, nonpatent extrahepatic bile duct, and by characteristic findings on liver and bile duct histology, in the absence of other known etiologies (17). In some countries, percutaneous transhepatic cholangiography (105) or ERCP (101) are alternative tests to define the anatomy of the extrahepatic biliary tree. Caution must be entertained in assigning the diagnosis of biliary atresia and performing the portoenterostomy if the only findings are failure to visualize the common hepatic duct and intrahepatic ducts on cholangiography because these findings may represent hypoplastic but patent proximal biliary structures in those with Alagille syndrome (106).

The diagnosis of idiopathic neonatal hepatitis is assigned only after infectious, metabolic, genetic, and structural causes of “giant cell hepatitis” are excluded (17). Therefore, as newer etiologies for intrahepatic cholestasis are discovered, infants thought previously to have “idiopathic” neonatal hepatitis may have their diagnosis reassigned.

### TREATMENT OF BILIARY ATRESIA AND NEONATAL HEPATITIS

Optimal therapy for patients with biliary atresia diagnosed before age 12 weeks is the surgical portoenterostomy, in which a Roux-en-Y loop of jejunum is connected by anastomosis to the porta of the liver to allow the liver to drain bile into the intestinal tract in at least 70% to 80% of patients, resulting in increased pigmentation of the stools and resolution of jaundice (6,92,107,108). If performed between 60 and 90 days of life, approximately 40% to 50% of patients show bile drainage; if performed after 90 days of life, up to 25% show bile drainage (108); and if performed after 120 days of life, only 10% to 20% of patients, at best, show evidence of bile drainage. Thus, many surgeons will not perform the portoenterostomy in infants with biliary atresia diagnosed after age 3 to 4 months (109). Consequently, it is absolutely essential that infants who remain jaundiced after age 2 to 3 weeks be evaluated expediently for cholestasis and, if present, for biliary atresia. Prompt surgical exploration and intraoperative cholangiogram must be performed if biliary atresia cannot be excluded using other diagnostic tests.

There is no standardized protocol for postoperative treatment of patients with biliary atresia in the United States. Antibiotic prophylaxis of cholangitis (trimethoprim–sulfamethoxazole or neomycin (110)), use of short courses of corticosteroid pulse therapy to manage refractory cholangitis (111), empiric use of oral ursodeoxycholic acid (UDCA) to stimulate bile flow and as a cytoprotective agent (112), optimization of nutrition (use of a medium chain triglyceride-containing infant formula), and prevention of fat-soluble vitamin deficiencies (113) are frequently used; however, there is no uniformity in practice (19,92). Supplementation with infant formula high in branched-chain amino acids, available in Europe and Australia, was well tolerated and associated with improved growth in one study (113). Patients with failure to thrive awaiting liver transplantation may require supplemental nasogastric tube feedings or parenteral alimentation.
Postoperatively, ascending cholangitis and sclerosis of patent intrahepatic bile ducts occur in 40% to 60% of patients and may lead to progressive biliary cirrhosis and liver failure (114). Because of the inflammatory component of cholangitis and the possible immune mechanisms involved in the pathogenesis of biliary atresia, anti-inflammatory therapy (e.g., corticosteroids) after portoenterostomy to prevent cholangitis and reduce intrahepatic bile duct injury and fibrosis could theoretically be of potential benefit. Unfortunately, there are no published randomized controlled trials of corticosteroid treatment for patients with biliary atresia. Short-term (1–2 weeks) postoperative therapy has been used in many centers (115), and an 8- to 10-week course of corticosteroids after portoenterostomy appeared to improve clinical outcome compared with historical control subjects in a prospective review (116). Many centers in Japan use intravenous and oral corticosteroid therapy along with antibiotic and bile acid (dehydrocholic acid intravenously and UDCA orally) therapy for up to several months after portoenterostomy (6). However, without a prospective randomized controlled trial of corticosteroid therapy after portoenterostomy, it is not possible to recommend routine treatment with this agent at this time. One additional therapy used in some Asian countries is the traditional Chinese medicine, kanzou (licorice root, glycyrrhizic acid), a hepatoprotective and cell-proliferative agent (117).

Liver transplantation can be life saving and is indicated for patients with biliary atresia who do not undergo an attempt at portoenterostomy because of delayed diagnosis, those in whom portoenterostomy has failed to reestablish hepatic-to-intestinal bile flow, and those with decompensated cirrhosis and end-stage liver disease despite initial “success” of portoenterostomy. Long-term survival after liver transplantation for biliary atresia approaches 80% to 90% (92).

The treatment for patients with idiopathic neonatal hepatitis is largely supportive, with optimization of nutrition, prevention of vitamin deficiencies, and use of choleretic agents and antipruritic agents (19) as needed. Therefore, infant formulas containing medium-chain triglyceride oil for improved fat absorption and adequate amounts of long-chain fat to provide for essential fatty acids are preferred; fat-soluble vitamin supplements are given; and oral UDCA or cholestyramine is used to induce choleresis. In up to 20% of those with idiopathic neonatal hepatitis, patients will show progression to cirrhosis and chronic liver failure and may require liver transplantation. In recent years, many of these patients have been found to harbor forms of PFIC (Byler disease and syndrome; PFIC types 1 and 2), MDR3 deficiency (PFIC type 3), bile acid synthesis defects, or neonatal iron storage disease (118). Patients with PFIC types 1 and 2 may benefit from partial external biliary diversion with improvement in pruritus, liver function, growth, and liver histology (119–121).

<table>
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<tr>
<th>TABLE 3. Transplant-free survival vs. age at portoenterostomy for biliary atresia at Tohoku University Hospital</th>
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<td><strong>Age at operation (days)</strong></td>
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OUTCOME AND COMPLICATIONS

If the portoenterostomy is not performed in patients with biliary atresia, 50% to 80% of children will die (without liver transplantation) from biliary cirrhosis by age 1 year, and 90% to 100% will die by age 3 years (109,122). Complications of portal hypertension, malnutrition, fat-soluble vitamin deficiencies, pruritus, and cholestasis add to the misery of these children before death. Successful portoenterostomy, when performed before 60 to 90 days of age, is associated with approximately a 30% to 40% 10-year survival at North American and European centers with the most experience (122–124), whereas 10-year survival after portoenterostomy performed before 60 days of age in Japan may approach 60% (Table 3) (6). Thus, the age at referral of the patient for evaluation is one of the most important factors determining surgical outcome. If the portoenterostomy is not successful in establishing bile flow, survival without transplantation is similar to or worse than that of patients not undergoing surgery. Postoperative care after portoenterostomy differs in Japan (6) from that used in the United States, as described previously. It is not clear if these differences in management or other factors (e.g., genetic or surgical technique) are responsible for the generally improved prognosis in Japan.

A large multicenter review of the outcome of all children diagnosed with biliary atresia in France between 1986 and 1996 was recently published (108). Combining the results of portoenterostomy with liver transplantation, 10-year overall survival for 472 patients with biliary atresia was 68%. The 10-year actuarial survival with the native liver after portoenterostomy was 29%, and 5-year survival after liver transplantation was 71%. Prognostic factors predictive of overall 10-year survival were the performance of the portoenterostomy, age at portoenterostomy (survival of 80.4% with surgery at age <45 days vs. 68.5% at >45 days), anatomic pattern of atresia (100% for atresia of the common bile duct only vs. 65.4% for complete extrahepatic atresia), presence of polysplenia syndrome (48.3% for yes vs. 69.9% for no),
and experience of the center performing the portoenterostomy (54% for ≤2 new patients per year, 59.8% for 3–5 per year, and 77.8% for >20 per year). The same factors predicted 5- and 10-year survival with the native liver after portoenterostomy. Improved survival with the native liver when portoenterostomy was performed at centers caring for more than five new patients per year was also reported from the United Kingdom and Ireland (125), resulting in a change in national policy that restricted performance of the portoenterostomy to the three most-experienced centers in the United Kingdom. Thus, the experience of the center performing the portoenterostomy is one of the most important factors determining surgical outcome.

The suggestion of delayed neurodevelopmental function in children with biliary atresia was put forth by Stewart et al. (126), who showed that children with end-stage liver disease with onset during the first year of life showed significant delays in verbal performance and full-scale IQs at a mean age of 8.4 years. They related these changes to nutritional status, severity of liver dysfunction, and duration of liver disease, and there was little improvement in these parameters 1 year after liver transplantation (127). However, because the patients reported included children with other liver diseases associated with delayed development (e.g., Alagille syndrome), its relevance to biliary atresia has been questioned. Wayman et al. (128) subsequently reported that children aged less than 1 year with biliary atresia who were evaluated for liver transplantation had a mean mental developmental score in the low average range and a mean psychomotor developmental score 1 SD below normal (Bayley Scales of Infant Development). Although many patients showed improvement 1 year after transplantation, 35% continued to be developmentally delayed. Developmental domains particularly affected included expressive language and independent walking. Factors associated with this persisting delay were decreased weight before transplantation, low serum albumin, length of hospitalization for transplantation, and younger age at transplantation. These data suggest that an improved neurodevelopmental outcome may be achieved using a more aggressive approach to nutritional rehabilitation before liver transplantation, attention to micronutrient supplementation, and transplantation before the onset of significant malnutrition. However, the degree to which the developmental delays may be attributed to other factors related to chronic liver failure has not been determined.

**Early complications**

The most important early complication after the portoenterostomy procedure is the development of ascending bacterial cholangitis. Breach of the barrier to penetration of intestinal bacteria into the biliary system is one of the unavoidable consequences of the portoenterostomy because the ampulla of Vater no longer provides this protection. Early diagnosis and management of ascending cholangitis is essential to prevent sclerosis and loss of remaining intrahepatic bile ducts, and to preserve function of the native liver. Cholangitis is suspected after a successful portoenterostomy if any of the following develop: fever, hypopigmented stools, elevated liver function tests (particularly the bilirubin concentration), generalized or right upper quadrant abdominal pain, right shoulder pain, or sudden increased pruritus. Ascending cholangitis can develop during or after viral infections because of poor oral intake causing decreased stimulation of bile flow and bile stasis, and because of the inhibitory effects of circulating cytokines on bile flow. Thus, it may be difficult to differentiate viral causes of fever and abnormal liver function tests versus ascending cholangitis. Particularly during the first 6 to 12 months after portoenterostomy, most fevers with any evidence of increased liver dysfunction or reduction in stool pigmentation are treated as if cholangitis is present. In our experience, imipenem/cilastin (or meropenem) has been the most effective broad-spectrum antibiotic for management of cholangitis in the absence of a positive blood culture. Others have used third-generation cephalosporins and aminoglycosides. If fever persists beyond 24 to 48 hours of therapy or if liver function tests do not show improvement, we initiate intravenous corticosteroid pulse therapy for 5 days in addition to the antibiotics. Intravenous methylprednisolone is administered in 1 hour as a single dose of 10 mg/kg on day 1, followed by 5 mg/kg, 2.5 mg/kg, 1 mg/kg, and 0.5 mg/kg on successive days. If cholangitis does not fully respond to this regimen, the patient may also be treated with 1 mg/kg oral prednisone for 2 to 4 weeks. Persistent or recurring cholangitis should also prompt evaluation with hepatic scintigraphy and ultrasonography of the Roux-en-Y loop for kinking or obstruction, which may require surgical revision. As a means of reducing ascending cholangitis, some have advocated the surgical construction of an intussuscepted valve in the Roux-n-Y loop, although its benefit is debatable.

**Late complications**

The majority of surviving patients with biliary atresia will develop portal hypertension. Portal hypertension may become clinically manifest simply by the finding of progressive hepatosplenomegaly with a very firm to hard liver texture, the development of gastrointestinal hemorrhage from esophageal or gastric varices or portal hypertensive gastropathy, hypersplenism, ascites, spontaneous bacterial peritonitis, portosystemic encephalopathy, or portopulmonary syndrome. Significant variceal hemorrhage has been reported in 20% to 60% of patients with biliary atresia. In a study from the University of Colo-
rado, 29% of 134 patients developed esophageal variceal hemorrhage (129). By age 5 years, 40% of the patients who were alive without a liver transplant had at least 1 episode of gastrointestinal hemorrhage, increasing to 60% by age 10 years. Recent interest has centered on attempts to prevent variceal hemorrhage by pharmacologic or endoscopic methods. Prophylactic sclerotherapy of esophageal varices before onset of hemorrhage has been evaluated in one randomized controlled trial (130). Although sclerotherapy significantly reduced the frequency of gastrointestinal hemorrhage from esophageal varices, there was a coincident increase in bleeding from gastric varices and portal hypertensive gastropathy, such that prophylactic sclerotherapy had no effect on the overall incidence of gastrointestinal hemorrhage or on survival. Another proposed approach to prevent variceal hemorrhage is the prophylactic use of pharmacologic agents (selective β-blockers and vasodilators) to reduce portal venous pressure. This therapy was effective in reducing the frequency of variceal hemorrhage in children with cirrhosis (131). However, this has not been studied in a randomized controlled fashion in children. There have been two open-label studies of propranolol for primary and secondary prophylaxis of variceal hemorrhage in children (132,133), showing reasonable safety, but bleeding rates on propranolol ranged from 15% to 53%. Thus, the efficacy and safety of β-blocker therapy in children remain to be determined.

Once gastrointestinal hemorrhage occurs, the initial management focuses on establishing hemodynamic stability. Subsequent treatment options consider on medical management with intravenous somatostatin (or its longer-acting analogs, such as octreotide), vasopressin, or terlipressin and endoscopic management with variceal sclerotherapy or variceal band ligation (134–137). If these methods are not successful in controlling hemorrhage, a surgical or radiologic portosystemic shunt (vs. liver transplantation) needs to be considered. Since the development of the technique of radiologic placement of a transjugular intrahepatic portosystemic shunt (TIPS), this has been the preferred procedure in some centers for patients weighing more than 12 kg to 15 kg (138,139). In most patients with biliary atresia, liver transplantation may be more appropriate than a surgical portosystemic shunt.

Ascites, portosystemic encephalopathy, portopulmonary syndrome, and severe hypersplenism are less common complications resulting from portal hypertension. Their incidence and prevalence have not been well studied in patients with biliary atresia. Management of the ascites is directed at salt restriction and diuretic treatment with large-volume paracentesis occasionally required. Encephalopathy is best managed with ammonia-binding agents such as lactulose, protein restriction, and oral antibiotics. Rarely, partial splenic embolization (140,141) or TIPS may be indicated for hypersplenism.

Pruritus caused by progressive cholestasis is managed with UDCA, antihistamines, rifampicin, and occasionally opiate antagonists or ultraviolet light exposure, and local measures to prevent scratching or skin dryness (19). We generally avoid bile acid-binding resins (e.g., cholestyramine) for pruritus after a portoenterostomy because of concern that the resin will enter and potentially obstruct the Roux-en-Y loop or intrahepatic bile ducts.

**Prognosis**

Factors shown to predict outcome after portoenterostomy include 1) age at operation, 2) experience of the center (surgeon), 3) site of atresia of the extrahepatic bile duct, and 4) number and severity of episodes of postoperative ascending cholangitis. Patients with a patent proximal common hepatic duct but distal atresia fare better than patients with atresia of the proximal biliary tree extending into the liver (6). Several groups have attempted to develop prognostic indices to predict survival and timing of liver transplantation for children with biliary atresia. The proposed indices include serum concentrations of hyaluronic acid (142) and procollagen III peptide and type IV collagen (143), the hepatic artery resistance index (144), urinary excretion of D-glucaric acid (145), postoperative bile bilirubin excretion (146), and a predictive model combining serum zinc, copper, zinc sulfate turbidity test, total bilirubin, GGT, and cholinesterase (147). These indices have not been validated prospectively in large numbers of patients, and many are not routinely available; thus, these indices have not proved valuable in clinical practice. Malatack et al. (148) developed a prognostic score for risk of death in pediatric patients at referral for liver transplantation that included serum concentrations of cholesterol, indirect bilirubin, partial thromboplastin time (PTT), and history of ascites. Although this prognostic index is simple and well characterized, it has not been validated earlier in the course of liver disease for patients with biliary atresia before referral for liver transplantation. Recently, the Pediatric End-Stage Liver Disease (PELD) scoring system has been developed using data accumulated by the Studies for Pediatric Liver Transplantation Research Group (149). Three-month survival of children listed for liver transplantation was predicted by five variables: serum total bilirubin, international normalized ratio (INR), albumin, growth failure, and age less than 1 year. Although the PELD score was derived in large part from infants and children with biliary atresia, it has not been validated for infants and children before reaching the stage of disease at which they would be evaluated for liver transplantation and, thus, may be of limited prognostic value earlier in the course of biliary atresia. In another study, serum total bilirubin concentration was predictive of survival after the first episode of esophageal variceal hemorrhage (128).
In the context of current therapeutic options, 70% to 80% of patients with biliary atresia in North America will require liver transplantation during the first two decades of life, despite initial success with portoenterostomy (92,122). Consequently, biliary atresia accounts for 40% to 50% of all liver transplants performed in children (152). It should be noted that there is no single liver disease in adults that accounts for as large a proportion of liver transplants. Factors that determine long-term survival without transplantation have not been carefully evaluated in North America since the advent of improved nutritional therapies, newer broad-spectrum antibiotics, and corticosteroid regimens. Moreover, quality of life outcome (QOL) measures for patients with biliary atresia and other cholestatic disorders have not been prospectively analyzed in a large enough cohort using age-specific tools that are now available (6,150).

**RECOMMENDATIONS**

Based on the data reviewed and the overall poor survival without liver transplantation of children with biliary atresia in North America, we propose the following steps that could be taken to improve the outcome of patients with biliary atresia.

1. A major public health campaign to educate primary care providers to follow all infants with jaundice at the 2-week well-baby examination (even if breast-fed) and to obtain a total and direct (conjugated) bilirubin if the jaundice persists when infant is aged more than 2 to 3 weeks or if the infant has pale stools, dark urine, or hepatomegaly (151).

2. Development of a national, Internet-based registry to track age at diagnosis of patients with biliary atresia and patient outcome, and to evaluate factors that influence outcome.

3. Explore the feasibility of piloting a stool color card program for infants at age 1 month (similar to the Japanese and Taiwanese programs) to determine if it will improve identification of patients with biliary atresia before age 6 to 8 weeks in a cost-effective manner.

4. Referral of patients with suspected biliary atresia, before surgical exploration, to centers with a defined care team and pediatric surgeons who perform at least four or five portoenterostomies per year to improve outcome after surgery.

5. Prospective controlled trial of corticosteroid therapy after portoenterostomy in newly diagnosed patients with biliary atresia to determine the efficacy and safety of such therapy.

6. Multicentered cooperative studies to investigate possible genetic, infectious, and immune/autoimmune mechanisms involved in the etiology and pathogenesis of biliary atresia.

7. Continued investigation of the pathogenesis of biliary atresia.

The new Biliary Atresia Clinical Research Consortium (BARC), funded for 5 years by the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health, is a network of 9 clinical centers and a data coordinating center in the United States, in cooperation with the Division of Digestive Diseases and Nutrition at NIDDK, that will embark on a number of these initiatives. The goals of BARC are 1) to develop and implement a clinical database and the collection of tissue, serum, and DNA specimens as a means of investigating the etiology and pathogenesis of biliary atresia; 2) to develop and initiate clinical trials and outcome studies; and 3) to stimulate others to investigate the etiology, pathogenesis, and management of biliary atresia. Through this and other multicentered collaborative groups, it is anticipated that major advances in our understanding of biliary atresia and related diseases will take place over the next 5 years, resulting in improved health and outcomes for children with biliary atresia.

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