Biliary Atresia
A Multidisciplinary Approach to Diagnosis and Management

Roger Klein Moreira, MD; Rodrigo Cabral, MD; Robert A. Cowles, MD; Steven J. Lobritto, MD

Context.—Biliary atresia is an inflammatory cholangiopathy of infancy that results in progressive fibrosis and obliteration of bile ducts and represents the main indication for liver transplant in young children. In spite of extensive investigation, its etiology has remained poorly understood. Timely surgical intervention (Kasai procedure) may result in significant benefit to these patients and represents the final goal of an accurate diagnostic evaluation.

Objective.—To present an overview of biliary atresia, including clinical and surgical approaches to this disease, with emphasis on the histopathologic evaluation.

Biliary atresia is an inflammatory cholangiopathy of infancy that results in progressive fibrosis and obliteration of extrahepatic and intrahepatic bile ducts. The etiology of biliary atresia has been subject of intense investigation and a number of possible pathogenic mechanisms have been proposed. The precise etiology of this disease, however, remains largely unknown. If untreated, affected children show progressive liver disease, with development of portal hypertension and liver failure, invariably resulting in death within the first 2 years of life.

Described in the 1950s by the Japanese surgeon Morio Kasai, portoenterostomy (Kasai) procedure remains the only form of therapy that can be offered to these patients besides liver transplant. Its effectiveness, however, is variable and probably dependent on early diagnosis with prompt surgical intervention. In spite of all efforts, biliary atresia remains the most common indication of liver transplant in young children.

Histopathologic examination of liver biopsy specimens represents a crucial element in the diagnostic evaluation of patients with suspected biliary atresia. Biliary obstructive features must be confirmed histologically and distinguished from various nonobstructive etiologies of neonatal cholestasis (ie, neonatal hepatitis syndrome). Given the potential complexities involved in the diagnosis of biliary atresia and the need for timely diagnosis, a well-coordinated multidisciplinary approach is essential for appropriate patient management. In this review, the authors present an overview of biliary atresia, including clinical and surgical perspectives on this disease, with emphasis on the histopathologic evaluation of biopsy and resection specimens.

Data Sources.—Review of relevant literature indexed in PubMed (US National Library of Medicine).

Conclusion.—A well-coordinated multidisciplinary approach is required in the assessment of suspected cases of biliary atresia. Pathologic examination of biopsy specimens is an integral part of the diagnostic algorithm and, therefore, plays a pivotal role in the diagnostic evaluation of this disease.


Epidemiology
The reported incidence of biliary atresia shows some regional variability, being higher in Asia and the Pacific region than in the rest of the world. The disease is diagnosed in approximately 5 to 6 per 100,000 live births in Europe and the United States, 10.6 per 100,000 in Japan, and up to 32 per 100,000 in French Polynesia. Small series have documented both seasonal variation in incidence as well as regional clustering of cases, but large studies have questioned these initial observations.

Familial clusterings are exceedingly rare and disease concordance in twins is unusual.

Classification
Biliary atresia is broadly classified into 2 main forms. The first is the embryonic/fetal, “early,” or syndromic form, accounting for 10% to 20% of cases, which is associated with a high frequency of additional congenital malformations (including asplenia, polysplenia, cardiovascular defects, situs inversus, intestinal malrotation, small-intestinal atresia, anomalous choledochopancreatic ductal junction, and various positional abnormalities of the portal vein and hepatic artery), and is referred to as biliary atresia-splenic malformation (BASM) syndrome. The second is the perinatal/postnatal, “late,” or nonsyndromic form, representing 80% to 90% of cases, generally...
occurring as an isolated abnormality. These 2 forms of biliary atresia have been proposed to represent different etiologic subgroups. In addition, cystic dilatation of biliary remnants may be seen in a small minority of cases of fetal-type biliary atresia (approximately 5%-10% of cases). These cases are referred to as cystic biliary atresia and, likewise, have been postulated to form a distinct subgroup of patients whose prognoses were found to be more favorable in 1 study but, as seen in BASM, may be more dependent on age at Kasai procedure than perinatal biliary atresia.

**ETIOLOGY AND PATHOGENESIS**

Biliary atresia is thought to represent the end result of intra-uterine or perinatal injury to bile ducts, leading to fibrous obliteration of these structures and severe cholestatic liver disease in the neonatal period. Various parts of the extrahepatic biliary system are initially affected, but intrahepatic bile ducts are subsequently involved in a significant proportion of patients, even in those who undergo an initially successful Kasai procedure. Histologic examination of surgically excised bile duct remnants and postmortem specimens supports the contention that, in most cases, the observed fibro-obliterative cholangiopathy in biliary atresia results from destruction of a presumably well-formed biliary system rather than from primary failure of normal embryologic development of these structures.

In the embryonal form/BASM syndrome, the coexistence of malformations in multiple organs (many of which are embryologically related) suggests an underlying abnormality originating during early phases of fetal development. To date, however, no single agent or abnormality has consistently been implicated as a cause of biliary atresia in humans. Instead, multiple etiologic factors have been postulated to be part of the pathogenesis of this complex disease (Table 1).

**IMMUNOLOGIC INJURY**

The presence of mononuclear inflammatory infiltrates in the vicinity of damaged intrahepatic bile ducts and within the biliary epithelium has been regarded as evidence of immune-mediated injury since early studies of biliary atresia. Numerous subsequent studies have confirmed these findings. Although the mechanism and precise triggers for the immune response seen in cases of biliary atresia have not been elucidated, it is currently hypothesized that an initial insult to the biliary tree leads to expression of new or altered antigens, which in turn are presented to T lymphocytes by antigen-presenting cells. Primed Th1 lymphocytes would then orchestrate an immune response by releasing proinflammatory cytokines and recruiting cytotoxic T cells, ultimately resulting in damage to bile ducts and liver parenchyma. In this regard, Mack et al identified increased numbers of lymphocytes (both CD4+ and CD8+) and a Th1-type cytokine profile (expression of interleukin [IL] 2, interferon γ, tumor necrosis factor α, and IL-12) in cases of biliary atresia in contrast to normal liver controls and other neonatal cholestatic liver diseases, such as idiopathic neonatal hepatitis, total parenteral nutrition-associated liver disease, and choleodochal cysts; these findings suggest that the inflammatory infiltrate seen in biliary atresia is a specific immune response rather than a secondary phenomenon of cholestatic diseases. Other investigators have suggested an activation of Th2 response in cases of biliary atresia but not in other neonatal cholestatic diseases. Interestingly, Narayanaswamy et al performed serial plasma measurements for a panel of inflammatory mediators (IL-2, IL-4, IL-10, IL-18, tumor necrosis factor α, and interferon γ) and cellular adhesion molecules (soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1) in 21 cases of biliary atresia. The authors concluded that the inflammatory process in biliary atresia is nonpolarized (involving Th1, Th2, and macrophage-associated cytokines) and shows consistent overexpression of cellular adhesion molecules in the biliary epithelium and vessels. In this study, the inflammatory response (Th1 in particular) and adhesion-molecule expression continued to increase after Kasai procedure in spite of amelioration of bilirubin and transaminase levels.

In addition, macrophages/Kupffer cells, natural killer cells, and mast cells have also been identified within the inflammatory infiltrate in biliary atresia. Described the presence of natural killer cells in the vicinity of intrahepatic bile ducts with accompanying overexpression of genes involved in cytotoxicity, while Harada et al suggested a role for Toll-like receptor (TLR)–related receptor in the presence of fetal-type biliary atresia than patients with neonatal hepatitis. These findings suggest that innate immune responses may play a role in the pathogenesis of biliary atresia.

A number of other abnormalities have also been reported in biliary atresia that may have important pathogenetic implications. For instance, biliary epithelial cells in biliary atresia has been shown to aberrantly express human leukocyte antigen (HLA) class I and II molecules—a phenomenon also documented in cases of liver allograft rejection, graft-versus-host disease, and primary biliary cirrhosis and that may have a role in presentation of neoantigens to naive T lymphocytes. Aberrant expression of Fas ligand and increased numbers of apoptotic cells in biliary epithelium have also been reported.

In 2004, Suskind et al, using X and Y chromosome fluorescence in situ hybridization and maternal HLA genetic polymerase chain reaction, identified significantly higher numbers of maternal cells in livers of patients with biliary atresia than patients with neonatal hepatitis. These findings were subsequently confirmed by other authors.

In Japan, Muraji and colleagues characterized these maternal cells in biliary atresia as mainly CD8+ lymphocytes (but also CD4+ lymphocytes) as well as cytokeratin-positive biliary epithelial cells. Therefore, a maternal microchimerism hypothesis was proposed, whereby both immune cells and biliary epithelial cells of maternal origin are found in increased numbers in patients with biliary atresia, raising the possibility that an alloimmune or a graft-versus-host–like phenomenon may be part of the pathogenesis of this disease (Figure 1).

Finally, while the precise timing and extent of the prenatal damage to the biliary tree due to the factors above are unclear, it is likely that in the perinatal period, when bile flow increases, tissue damage is amplified. It is postulated that bile leakage from an abnormal biliary system, and impaired bile flow, may trigger further inflammatory response and tissue damage. This contention
Jagged1 gene mutation was identified in 9 of CFC1 human papillomavirus, Innate immune response (natural killer cells and Toll-like receptor signaling). Medial hypertrophy of hepatic artery branches by reovirus type A, likewise, leads to inflammation and injury of the extrahepatic biliary tree with subsequent development of fibrous obliteration of extrahepatic bile ducts as well as intrahepatic histologic changes, including fibrosis and ductular reaction, resembling human biliary atresia. In fact, reovirus has been detected in bile duct remnant tissue by immunohistochemistry as well as in frozen liver tissue by real-time polymerase chain reaction in humans. These results, however, have not been confirmed by other investigators. Similarly, a murine model of biliary atresia induced by group A rotavirus is well described and closely mimics human disease. Interestingly, transfer of T cells from rotavirus-induced biliary atresia to severe combined immunodeficiency mice has been shown to be sufficient to cause bile duct–specific inflammation. As with reovirus, however, the detection of rotavirus in human biliary atresia has been inconsistent. Therefore, in spite of all the evidence from well-characterized experimental models, the role of viral infection in human biliary atresia remains unresolved.

**VIRAL INFECTION**

The reported seasonal variation of cases reported in some studies and the experimental evidence of virus-induced biliary atresia in animal models have suggested a role of virus infection in human cases of biliary atresia. The possible role of numerous viruses has been investigated in this setting, including hepatotropic viruses (especially hepatitis B virus), cytomegalovirus, human herpesvirus, human papillomavirus, group C rotavirus, and reovirus. The data regarding a possible role of each of the above viral agents in biliary atresia are inconsistent and often conflicting. However, there is particular interest in reovirus and rotavirus, given their role in experimental animal models of biliary atresia. Infection of weanling mice by reovirus type III causes biliary and liver damage that is, in many ways, similar to that seen in human biliary atresia. In this animal model, reovirus infection in the neonatal period induces hepatitis and biliary epithelial necrosis of intrahepatic and extrahepatic bile ducts, with associated bile duct edema and inflammation. Upon repeated intraperitoneal viral injections, mice develop fibrosis of the extrahepatic biliary tree but without progressing to irreversible luminal obstruction. Intraperitoneal infection of newborn mice by rotavirus type A, likewise, leads to inflammation and injury of the extrahepatic biliary tree with subsequent development of fibrous obliteration of extrahepatic bile ducts as well as intrahepatic histologic changes, including fibrosis and ductular reaction, resembling human biliary atresia.

**GENETIC FACTORS**

The role of several different candidate genes has recently been investigated in patients with biliary atresia, in most cases owing to identification of genetic abnormalities associated with laterality defects and biliary atresia–like diseases in murine models. The CFC1 gene, which codes for the CRYPTIC protein, thought to act as a cofactor in pathways determining left-right axis, has been found to be mutated in a minority of patients with sporadic and familial laterality defects (including patients with BASM). Likewise, mutations in the human Jagged1 gene (JAG1), which encodes a ligand for the Notch signaling pathway and is found in most patients with Alagille syndrome, has also been investigated in biliary atresia. In one study, Jagged1 gene mutation was identified in 9 of...
102 patients with biliary atresia and in none of 100 healthy controls. The same investigators\textsuperscript{55} report the presence of Jagged1 mutation in 6 of 28 patients with biliary atresia (21.4\%) who required early liver transplant after Kasai procedure (before the age of 5 years), compared to 3 of 72 patients (4.1\%) who had not undergone liver transplant. In addition, they describe a immunoregulatory role of the Jagged1 gene, which suppresses IL-8 production in cell culture, and postulate that abnormal regulation of inflammatory cytokines by a dysfunctional Jagged1 gene may explain its putative role as an aggravating factor in biliary atresia. Similarly, Campbell et al\textsuperscript{66} reported an increased frequency of non–M α1-antitrypsin heterozygosity in patients with biliary atresia, compared to the general population, as well as a more rapid progression of disease and earlier need for liver transplant in these patients.

The presence of mutations involving the inversin gene (inv) in chromosome 4, known to cause anomalous development of the hepatobiliary system in mice,\textsuperscript{37,38} has also been investigated in human biliary atresia but no consistent abnormalities have been identified.\textsuperscript{59} Likewise, some studies have shown an association between certain HLA alleles and biliary atresia,\textsuperscript{60–62} while others did not confirm these findings.\textsuperscript{63} Finally, vascular endothelial growth factor gene (VEGF) polymorphism (particularly the +936 C allele) has been associated with biliary atresia, possibly by conferring increased susceptibility to the disease rather than by direct mechanisms.

Therefore, although there is evidence for the contribution of several genetic abnormalities in the pathogenesis of biliary atresia, their precise role in this disease remains unclear and is still under investigation. In addition, some authors\textsuperscript{6} have suggested that the fetal/embryonic form of biliary atresia represents a different entity than the more common perinatal form, and different developmental and genetic abnormalities may be involved in these 2 forms of biliary atresia.

**VASCULAR ABNORMALITIES**

Because the biliary tree receives its blood supply exclusively from the arterial system, and impaired arterial flow may lead to necrosis and fibrous obliteration of extrahepatic bile ducts in other clinical settings (eg, hepatic artery thrombosis in liver transplant recipients), it has been hypothesized that primary vascular abnormalities participate in the pathogenesis of biliary atresia. This hypothesis stems from early studies\textsuperscript{64} during which Pickett and Briggs\textsuperscript{65} successfully created an animal model of biliary atresia. Subsequently, Ho and colleagues\textsuperscript{66} reported the presence of tortuous hepatic artery branches showing thickened walls with medial hypertrophy in both the extrahepatic and intrahepatic locations in all 11 analyzed cases of biliary atresia. This is in agreement with the hepatic artery dilatation seen in biliary atresia by ultrasonography, as compared to controls observed by the same authors. Similarly, dos Santos et al\textsuperscript{67} found significant medial hypertrophy of hepatic arteries at the portoenterostomy site in infants with biliary atresia as compared to healthy infants and others with cholestatic diseases. They also documented progressive arterial thickening when the Kasai specimen was compared to the explanted liver in several cases, which was associated with disappearance of interlobular bile ducts. Arterial thickening has also been confirmed by comprehensive studies using imaging techniques.\textsuperscript{68} In the context of a possible role of vascular/hypoxic injury in biliary atresia, the contribution of VEGF polymorphism, as described above, becomes particularly worthy of further investigation.

**ENVIRONMENTAL FACTORS**

A number of exogenous/environmental insults have been postulated to have a role in the pathogenesis of biliary atresia, including drugs used during gestation (amphetamine and alcohol), phytotoxins, mycotoxins, toxic agricultural products, and industrial toxins.\textsuperscript{69} A definite contribution of environmental factors to the pathogenesis of human biliary atresia, however, remains unproven.

**CLINICAL EVALUATION**

Jaundice and hyperbilirubinemia extending beyond the immediate neonatal period is abnormal and always merits investigation. Laboratory and radiologic testing focus specifically on establishing the cause of the disorder as early as possible, permitting judicious intervention. The disorders leading to direct hyperbilirubinemia in this period include infectious diseases, endocrine disorders, metabolic disorders, inflammatory disorders, drug reactions, immunologic disorders, and anatomic disorders of the biliary tree. Clinically, these disorders may appear quite similar, but the prognosis and treatment vary widely. Biliary atresia represents one such rare disorder presenting in the first few weeks of life that requires early recognition and prompt intervention to prevent or delay progressive liver dysfunction.

**CLINICAL PRESENTATION**

**Diagnosis**

Recognition of the Disorder.—The first impedance to the diagnosis of biliary atresia is the recognition that the first signs and symptoms of poor bile flow are abnormal, as jaundice that develops outside of the immediate neonatal period is often confused with physiologic (breast milk) jaundice, causing delay in the diagnostic workup. Associated clinical observations, such as poorly pigmented stool, dark urine, and hepatomegaly, serve as additional clues that further workup is warranted. In cases of BASM, presence of associated malformations, especially congenital heart disease, may serve as a clinical clue to the diagnosis of biliary atresia.

**Laboratory Evaluation.**—Routine laboratory analysis will demonstrate a direct hyperbilirubinemia and variable levels of transaminases, γ-glutamyltranspeptidase, and alkaline phosphatase, which overlap significantly with other causes of neonatal cholestasis. γ-Glutamyltranspeptidase levels, however, are usually elevated in biliary atresia,\textsuperscript{62,71} and normal or low values should prompt evaluation of progressive familial intrahepatic cholestasis types I and II, as well as bile acid synthesis disorders.

Early analysis should focus on excluding diagnoses of galactosemia, viral hepatitis, hypothyroidism, and choledochal cyst. Studies of synthetic function, such as albumin and coagulation profiles, provide clues to the degree and duration of hepatic insult. Maternal history, neonatal history, and physical examination will guide further laboratory testing for metabolic disorders, α1-antitrypsin
deficiency, progressive familial intrahepatic cholestasis, Alagille syndrome, and cystic fibrosis.

**Imaging Studies.**—The imaging study most often used is abdominal sonography. This study will assess for liver masses, choledochal cysts, biliary ductal dilatation, vascular anomalies, polysplenia, and signs of portal hypertension. The infant gallbladder is commonly not observed and this finding does not suffice for diagnosis of biliary atresia. The sonographic demonstration of a triangular cord in the vicinity of the portal vein has been suggested to be a specific finding in patients with biliary atresia, but may have low sensitivity. In cases of “cystic” biliary atresia, prenatal diagnosis is possible by routine fetal ultrasonography.  

If no diagnosis emerges from the initial laboratory or imaging studies, patients are routinely given phenobarbital for 5 to 7 days to prepare for hepatobiliary iminodiacetic acid scan. Barbiturates are thought to enhance bile acid—dependent biliary flow, decreasing false-positive scans (demonstrating failure to excrete marker into the intestine). Such results are seen with biliary atresia, as well as any form of severe cholestasis, and are therefore not diagnostic of mechanical obstruction. Unequivocal intestinal excretion of marker, however, effectively excludes the diagnosis of biliary atresia.

Magnetic resonance imaging of the biliary tree has also been used in this setting with reported diagnostic accuracy of 71% to 82%. Direct imaging of the biliary tree by cholangiography is performed either percutaneously or via endoscopy. Demonstration of a patent extrahepatic biliary tree effectively excludes biliary atresia. These procedures are invasive, however, and results are operator-dependent and may be technically challenging. Finally, intraoperative cholangiogram is still considered the gold standard for the diagnosis of biliary atresia and is performed routinely in many institutions.

**PATHOLOGIC EVALUATION**

**Liver Biopsy Findings**

Needle core biopsy sample is currently the most common specimen submitted for pathologic evaluation in cases of neonatal cholestasis and is generally considered the most reliable tool for the prelaparotomy diagnosis of biliary obstruction, with reported diagnostic accuracy of up to 93% to 94% in large series. However, several of the histopathologic features of biliary atresia may overlap significantly with those of nonobstructive (ie, “nonsurgical”) etiologies of neonatal cholestasis; hence, the need for a careful and systematic approach to these cases.

The histopathologic changes of the portal tracts are the key to a correct diagnosis of biliary atresia. Portal findings in biliary atresia are broadly similar to what is seen in large-duct obstruction due to other etiologies (Figure 2, A through E). In typical cases, ductular reaction is prominent and consists of a proliferation of small, interanastomosing ductules located at the periphery of the portal tracts. This finding represents the most consistent indicator of the presence of a biliary obstructive process and has repeatedly been shown to be a key feature of biliary atresia. Bile plugs are frequently seen within dilated lumens of ductules and represent a useful diagnostic feature (Figure 2, C). As in other settings, the term ductular reaction encompasses the presence of a mesenchymal proliferation, including extracellular matrix–producing myofibroblasts, as well as a variable amount of inflammatory cells, especially neutrophils, in addition to proliferation of ductular structures. It must be emphasized, however, that variable degrees of ductular reaction (albeit generally of milder degree than in biliary atresia) may also be seen in a variety of nonobstructive liver diseases, including neonatal hepatitis of various etiologies; therefore, this finding must be interpreted with caution (see “Differential Diagnosis and Diagnostic Pitfalls” below).

Portal-based fibrosis, likewise, represents a typical diagnostic finding in this context. Although early findings (within the first month of life) may be variable, fibrous expansion of portal tracts, and often more advanced stages of fibrosis, are expected to be present in most cases of biliary atresia, particularly those with biopsy in the second month of life or later (Figure 2, D). The portal fibrosis is typically accompanied by ductular reaction and mild inflammation, as described above, as well as by some degree of portal and periductal edema, which imparts a distinctive “obstructive” appearance to the expanded portal tracts (Figure 2, A and B).

At presentation, extrahepatic bile injury and obliteration represent the main finding in biliary atresia and the underlying cause of most, or all, of the initial clinical manifestations. Injury and destruction of smaller intrahepatic bile ducts are thought to occur later in the course of the disease and may represent an important factor leading to the progressive liver dysfunction that occurs in a significant proportion of cases, which eventually results in liver failure and need for liver transplant. However, a few studies have shown a greater degree of portal and periductal edema, which imparts a distinctive “obstructive” appearance to the expanded portal tracts (Figure 2, A and B).

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Figure 2. Typical histopathologic features of biliary atresia. A, Low-power image showing multiple markedly expanded portal tracts. B, Expanded portal tract with mild inflammation, edema, prominent ductular reaction, and focal ductular bile plugs. C, Ductular reaction with prominent ductular bile plugs. D, Portal-based fibrosis is seen in most cases of biliary atresia. This case shows fibrous expansion as well as presence of fibrous septa. E, Prominent cholestasis is typical of biliary atresia, while giant cell transformation is highly variable (conspicuous in this example) (hematoxylin-eosin, original magnifications ×40 [A], ×100 [B], ×200 [C and E]; Masson trichrome, original magnification ×100 [D]).
represents a common finding in neonatal livers and should not be misinterpreted as inflammation.

Lobular findings in biliary atresia are variable and generally less useful than portal findings in the differential diagnosis with nonobstructive causes of neonatal cholestasis (discussed below). Significant cholestasis, in the form of canalicular and intracellular bile, is present in nearly all cases. Cholate stasis (encompassing periportal hepatocyte swelling due to bile salt retention, Mallory hyaline, and copper/copper-binding protein accumulation in hepatocytes) and bile infarcts may be seen on initial biopsies but are more common later in the course of the disease, especially on explant specimens. Lobular inflammation is typically mild and often difficult to differentiate from EMH. Giant cell transformation, likewise, is a common but nonspecific finding (Figure 2, E). While lobular disarray may be prominent, particularly in cases with significant giant cell transformation, confluent necrosis is not a characteristic finding.

**Differential Diagnosis and Diagnostic Pitfalls**

The most crucial distinction in cases of neonatal cholestasis from a histopathologic standpoint is between obstructive (mainly represented by biliary atresia) and nonobstructive etiologies. While the histopathologic features of biliary atresia are well described, there is significant overlap with changes seen in nonobstructive neonatal diseases, including idiopathic neonatal hepatitis and various genetic/metabolic diseases and infectious conditions, often referred to as *neonatal hepatitis* (reviewed by Torbenson et al). In a recent study by the Biliary Atresia Research Consortium (BARC), in which 16 different histopathologic parameters were evaluated by 10 pathologists, features shown to be useful in distinguishing biliary atresia (obstructive pattern) from neonatal hepatitis (nonobstructive pattern) included ductular reaction, presence of bile plugs within bile ducts, significant portal-based fibrosis, portal edema, and lack of sinusoidal fibrosis (Figure 2, A through D). Among these, the most predictive features of biliary atresia by logistic regression analysis (also taking into account interobserver variability) were ductular reaction, portal fibrosis, and absence of sinusoidal fibrosis. In contrast, features such as amount of EMH, lobular inflammation, and giant cell transformation were seen with similar frequency in biliary obstruction and other forms of
Table 2. Reported Frequency of Selected Histopathologic Features Distinguishing Biliary Atresia From Nonobstructive Etiologies of Neonatal Cholestasis

<table>
<thead>
<tr>
<th>Biliary Atresia, %</th>
<th>Nonobstructive Diseases, %</th>
<th>Source, y</th>
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<tbody>
<tr>
<td>Ductular reaction (moderate to severe)</td>
<td>76–100</td>
<td>13.4–22</td>
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<tr>
<td>Fibrosis (at least fibrous expansion of portal tracts)</td>
<td>53.6–100</td>
<td>6.7–87.8</td>
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<tr>
<td>Advanced fibrosis (bridging fibrosis or cirrhosis)</td>
<td>42.9–70</td>
<td>0–14</td>
</tr>
<tr>
<td>Ductal/ductular bile plugs</td>
<td>42.9–69</td>
<td>0–23</td>
</tr>
<tr>
<td>Sinusoidal fibrosis (zone 3)</td>
<td>20</td>
<td>32</td>
</tr>
<tr>
<td>Hepatocyte swelling</td>
<td>30</td>
<td>23</td>
</tr>
<tr>
<td>Giant cell transformation of hepatocytes (&gt;mild/focal)</td>
<td>14–43</td>
<td>23–80</td>
</tr>
<tr>
<td>Extramedullary hematopoiesis</td>
<td>47–56</td>
<td>36–68.2</td>
</tr>
<tr>
<td>Acute cholangitis</td>
<td>17.9–37</td>
<td>14–20</td>
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<tr>
<td>Portal inflammation (at least moderate)</td>
<td>28</td>
<td>20</td>
</tr>
<tr>
<td>Hepatocellular necrosis</td>
<td>35</td>
<td>37</td>
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<tr>
<td>Duct plate malformation</td>
<td>10–48.8</td>
<td>0–0.5</td>
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<tr>
<td>Bile duct inflammation</td>
<td>31</td>
<td>18.5</td>
</tr>
<tr>
<td>Bile duct loss</td>
<td>7.3–8.5</td>
<td>0–95*</td>
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*Variation in the reported frequency of bile duct loss may be related to the overall prevalence of Alagille syndrome and other bile duct paucity syndromes in different studies.

neonatal cholestasis. Similarly, previous studies78,96,97 evaluating distinguishing histopathologic features between biliary atresia and neonatal hepatitis have consistently identified ductular reaction, ductal/ductular bile plugs, and portal-based fibrosis as key distinguishing findings in this scenario (Table 2).

Features of biliary obstruction may be poorly developed in cases in which the biopsy is performed in the first few weeks after birth (usually before 4–6 weeks).27,82,83 In this stage, although cholestasis may be readily identified, portal fibrosis and ductular reaction may be minimal and the changes are difficult to differentiate from nonobstructive causes of neonatal cholestasis (Figure 3, C and D). Therefore, repeated biopsies are advisable in this situation if biliary atresia remains in the clinical differential diagnosis. Inadequate specimens (containing <5–6 portal tracts), likewise, may be an important reason for underrecognition of obstructive features, as acknowledged by Russo et al.27 Conversely, nonobstructive processes may at times show features that closely mimic an obstructive pattern, most notably α1-antitrypsin deficiency and total parenteral nutrition–associated liver disease (Figure 4, A through D).74,75 In these 2 conditions, portal fibrosis and significant ductular reaction are often present and may lead to an erroneous diagnosis of an obstructive process (Table 3). Therefore, clinical history of total parenteral nutrition should always be excluded and α1-antitrypsin deficiency evaluated by immunohistochemistry (or clinically by determining serum α1-antitrypsin activity or phenotype) before a diagnosis of biliary atresia is rendered. Finally, when DPM-like structures are present on biopsy samples, fibropolycystic diseases, such as congenital hepatic fibrosis and Caroli syndrome, may enter the histologic differential diagnosis (Figure 3, A and B). Age of presentation, clinical manifestations, and imaging features of this group of diseases, however, differ significantly from biliary atresia.

**Kasai Specimen**

The Kasai procedure typically yields a fibrotic segment of extrahepatic bile duct. On its proximal end lies the so-called portal plate—the resected end of the extrahepatic biliary tree at the level of the porta hepatis, sometimes including a small amount of surrounding liver parenchyma. Distally to this area, a segment of common hepatic duct, cystic duct, gallbladder, and segment of common bile duct are found, and each of these areas should be sampled on gross examination (Figure 5, A through C). Abnormalities in each segment will depend on the type of biliary atresia and extent of extrahepatic obliteration, which can be included in the surgical pathology report. Early studies by Chandra and Altman,46 analyzing bile duct remnants at the level of the portal plate, have claimed significant correlation between residual bile duct size greater than 150 μm and successful post-Kasai bile drainage and overall patient outcome. While some subsequent studies55,94,99 have confirmed a more favorable outcome in cases showing bile duct sizes greater than 150 to 200 μm at the portal plate, others50,102 have found no significant correlation. In a large study including 205
cases, Tan et al\textsuperscript{102} reported significantly lower 5-year survival in patients with no or fewer than 5 small bile ducts (<100 µm) at the portal plate, compared to other groups; however, the presence of medium-sized (100–300 µm) or large bile ducts (>300 µm) did not confer additional survival benefit. Irrespective of its prognostic value, documentation of fibrous obliteration of the extrahepatic biliary tree in Kasai procedure specimens represents the final histopathologic confirmation of the diagnosis of biliary atresia, and systematic histopathologic examination of these specimens remains important.

<table>
<thead>
<tr>
<th>Table 3. Histopathologic Diagnostic Pitfalls in Biliary Atresia</th>
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<tbody>
<tr>
<td>False-positive interpretation</td>
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<tr>
<td>• Total parenteral nutrition–associated liver disease</td>
</tr>
<tr>
<td>• α1-Antitrypsin deficiency</td>
</tr>
<tr>
<td>False-negative interpretation</td>
</tr>
<tr>
<td>• Early age at diagnosis (usually &lt;4–6 weeks)</td>
</tr>
<tr>
<td>• Small/inadequate sample (&lt;5–6 portal tracts)</td>
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</table>

Explant Specimen

Examination of the explanted specimen usually shows typical features of biliary cirrhosis, with broad fibrous septa and irregularly shaped regenerative nodules with a “geographic” or “jigsaw” appearance. Large–bile duct disease is readily apparent, with inflammation and injury of hilar bile ducts, sometimes associated with periportal fibrosis and variable luminal obliteration. Bile duct inflammation/injury and bile duct loss involving small, peripheral bile ducts are also evident in many cases. Parenchymal changes in these specimens typically reflect end-stage biliary disease and include cholestasis, periporal hepatocyte swelling/feathery degeneration, bile infarcts, as well as periportal Mallory-Denk bodies and accumulation of copper and copper-binding protein. Extramedullary hematopoiesis and giant cell transformation may be present in patients undergoing transplant at a very early age but, otherwise, are generally absent. Histopathologic features of explanted specimens of biliary atresia cases may differ depending on whether individual patients underwent a Kasai procedure. In one study, prominent perihilar regenerative nodules measuring up to 14 cm (some mimicking neoplasms) were commonly present in patients with history of a prior Kasai procedure.
but were not seen in patients undergoing primary liver transplant. These nodular areas were more prominent in the group of patients whose native livers survived several years before liver transplant as compared to patients receiving an allograft within months of the Kasai procedure. Histopathologic examination of these large regenerative areas showed relatively preserved liver tissue, with little to no fibrosis and spared bile ducts, in contrast to the surrounding liver, which showed well-developed biliary cirrhosis and, in many cases, bile duct loss. These nodular areas of noncirrhotic liver with preserved bile ducts (and presumably preserved bile drainage) have been postulated to have a role in long-term transplant-free survival after Kasai procedure (Figure 6).

**Figure 6.** Gross appearance of native liver resected several years post Kasai procedure with corresponding histologic sections. The liver explant shows large nodular areas of regeneration near the hepatic hilum (asterisk) (gross specimen). Section from the edge of one of the nodular areas shows preserved liver architecture within the nodule and cirrhosis of the adjacent liver (right inset). In contrast to the cirrhotic liver, there was no bile duct loss within nodular areas (left inset) (Masson trichrome, original magnification ×20 [right inset]; cytokeratin 7, original magnification ×100 [left inset]).
Hepatocellular carcinomas develop in less than 1% of patients with biliary atresia, as described in a recent series.105

**SURGICAL ASSESSMENT AND MANAGEMENT**

Several systems have been proposed to classify the surgical anatomy in cases of biliary atresia. The Japanese Association of Pediatric Surgeons proposed that cases should be classified according to the location of atresia. In this system, type I anatomy is associated with atresia at the level of the common bile duct (approximately 12% of cases); in type II, atresia is at the level of the hepatic duct (2.5% of cases); and in type III, the most frequent type, atresia occurs at the porta hepatis (approximately 85% of cases) (Figure 7). These main types are subdivided into subtypes, according to the morphology of the distal bile duct (α-patent, β-fibrous, c-aplasia, or d-miscellaneous), and subgroups, according to the pattern of hepatic radicles at the porta hepatis (α-dilated ducts, β-hypoplastic ducts, γ-bile lake, μ-fibrous ducts, ζ-fibrous mass, and o-aplasia of ducts).106,107

**Surgical Technique**

The Kasai procedure (Roux-en-Y hepatic portoenterostomy) is the standard initial operation for treatment of infants with biliary atresia. The operation involves excision of the entire extrahepatic biliary tree with transection of the fibrous portal plate near the hilum of the liver. Bilioenteric continuity is then reestablished with a Roux-en-Y jejunal limb.108 The ultimate goal of the procedure is to allow drainage of bile from the liver into the Roux limb via microscopic ductules in the portal plate. The general steps taken during surgery are described below.

The exploration begins via a right upper abdominal incision. The left upper quadrant is examined first, searching for the spleen. Absence of the spleen or the finding of polysplenia can alert the surgeon to the presence of important associated anomalies such as malrotation, preduodenal portal vein, and interrupted inferior vena cava withazygous continuation. Next, the liver, biliary structures, and porta hepatitis are inspected. The liver in biliary atresia can appear nodular and fibrotic with a greenish color. This finding is not common in neonatal hepatitis or bile duct paucity syndromes, where the liver is smooth and dark brown in color. Many infants with biliary atresia have a contracted, fibrotic gallbladder (Figure 8). If a rudimentary fibrous gallbladder is noted at initial exploration, and if it clearly has no lumen, then the diagnosis of biliary atresia has been confirmed and the operation can proceed with dissection of the portal plate. If the gallbladder is normal appearing or if it is felt to have a lumen, then additional intraoperative diagnostic maneuvers are warranted before dissecting the portal plate. In this situation, a purse-string suture can be placed in the fundus of the gallbladder and the fluid within the lumen of the gallbladder is aspirated. If clear fluid (white bile) returns, then our approach has been to proceed with portal plate dissection without a cholangiogram. If, however, the aspirated fluid is darker in color or if there is any ongoing question regarding the diagnosis, then an intraoperative cholangiogram should follow.

While simple in concept, the cholangiogram can be difficult to perform and interpret successfully during surgical exploration. Diatrizoic acid (Hypaque [GE Healthcare, Inc, Princeton, New Jersey] or Gastrografin [Schering, Berlin, Germany]) is diluted 1:1 with normal saline and injected as the contrast agent via the cholangiogram catheter to assess for patency or obstruction of the biliary tree. Real-time fluoroscopy facilitates rapid intraoperative interpretation of the study. If contrast flows freely into the duodenum and into intrahepatic bile ducts, then patency of the biliary tree has been established and the diagnosis of biliary atresia excluded. In this scenario, a
wedge liver biopsy should be performed as an aid to diagnosis, the cholangiogram catheter removed, the cholecystostomy closed, and the operation concluded. Conversely, failure to reliably delineate patent intrahepatic and extrahepatic biliary structures mandates that the surgeon proceed with portal dissection.

Regardless of the presence of a patent gallbladder or distal common bile duct, a direct Roux-en-Y hepatic portoenterostomy affords outcomes that are superior to other forms of reconstruction such as the portocholecystostomy.\textsuperscript{109} The peritoneum overlying the hepatoduodenal ligament is opened to allow identification of the structures in this area. The fibrous remnant of the distal common bile duct is often present here. It can be identified in the anterolateral aspect of the hepatoduodenal ligament, isolated and divided. With traction on the cut end of the obliterated distal common bile duct, the fibrous biliary remnant can be dissected toward the porta hepatis. During this dissection, the gallbladder remnant is also dissected away from the liver in continuity with the rest of the extrahepatic biliary remnant. As dissection continues proximally, the biliary remnant develops into a cone of fibrotic tissue (in most cases, type III biliary atresia) that is located at the bifurcation of the main portal vein into its left and right branches. This constitutes the most important landmark during the dissection of the portal plate and should be the goal of every dissection.\textsuperscript{109,110} Once the fibrous cone and portal plate region have been identified, the fibrous cone is placed on gentle traction and transected with a sharp scissors or knife. It is not beneficial to cut deeply toward the liver parenchyma as this may result in more scar formation and inhibition of bile drainage. While advocated by some,\textsuperscript{112} we have not found frozen section for histopathologic measurement of the diameter of biliary ducts at the portal plate to be helpful in guiding the level of transection.

With a completed portal plate dissection, the operation shifts to the construction of the Roux limb. The proximal jejunum is identified and transected about 10 cm distal to the ligament of Treitz. The distal end, destined for the right upper quadrant, is oversewn and the Roux limb is measured to from 40 to 50 cm. At this location, an end-to-side jejunojunostomy is performed. Finally, the side of the Roux limb is opened and the portoenterostomy is created. When complete, the entire surface of the portal plate must be contained within the jejunal lumen of the Roux limb, so that any bile drainage via biliary ductules at the portal plate will be collected by the Roux limb and allowed to proceed distally. A diagram of the completed operation is shown in Figure 9.

Surgical Controversies

Much of the controversy surrounding surgery for biliary atresia has subsided. Previously reported techniques using stomas to exteriorize the Roux limb or antireflux valves\textsuperscript{113–115} have been subsequently abandoned either owing to associated complications or lack of effectiveness.\textsuperscript{116–118} Similarly, use of the appendix as a conduit between the liver and the small intestine has been proposed but its use has been limited, with some reports suggesting an inferior surgical outcome.\textsuperscript{119}

With the widespread application of minimally invasive techniques even to the most complex operations, the laparoscopic Kasai procedure has been described and used at several centers worldwide.\textsuperscript{120–122} Most reports, however, involve single cases or small series of carefully selected patients. A recent prospective trial confirmed the feasibility of the operation but revealed a significantly poorer outcome at 6 and 24 months, causing the trial to be stopped.\textsuperscript{122} Outcomes of other series have been variable\textsuperscript{123} and, therefore, the laparoscopic Kasai procedure currently lacks support among general pediatric surgeons including those who specialize in minimally invasive pediatric surgery.

Some investigators have proposed that primary liver transplant be considered the initial treatment for infants

Figure 8. Intraoperative image of a patient with biliary atresia, showing a slightly nodular liver and fibrous obliteration of the gallbladder (arrows).

Figure 9. Illustration of a portoenterostomy (Kasai) procedure with a completed anastomosis between the Roux limb and the portal plate.
with biliary atresia, citing deleterious effects of the Kasai procedure on possible subsequent liver transplant. This approach, however, would subject a percentage of children who may have been managed successfully by the Kasai procedure to the dangers of transplant and its associated short- and long-term complications. For this reason, primary liver transplant has been reserved for selected cases, such as delayed diagnoses presenting with severe liver failure, in which a Kasai procedure would be risky and have a high failure rate. For all other children with compensated liver function and a timely diagnosis, the Kasai procedure and liver transplant are considered by most to be sequential, complementary procedures.

**NATURAL HISTORY AND PROGNOSIS**

If left untreated, biliary atresia is universally fatal within the first 2 years of life. Patients develop progressive biliary cirrhosis and succumb to either hepatic synthetic failure or complications of portal hypertension. After the Kasai procedure, resolution of jaundice is achieved in 40% to 60% of patients, but one-third to half of all patients require liver transplant within the first year of life. Risk factors for Kasai failure include anatomic (type III) and histologic (small or absent bile ducts at the portal plate) features of extrahepatic bile duct remnants, age at Kasai procedure, postoperative cholangitis, presence of cirrhosis, and failure to establish bile flow. Long-term survival without transplant is approximately 30% at 10 years and 14% to 23% at 20 years. Liver transplant is the preferred treatment for patients experiencing Kasai failure, with reported 10-year patient survival of approximately 85%. The disease does not recur in the liver allograft.

**CONCLUSION**

Biliary atresia, although uncommon, represents one of the most important forms of liver diseases in neonates and the main indication for liver transplant in this age group. The precise etiology and pathogenic mechanisms involved in biliary atresia, in spite of decades of investigation, have thus far remained largely elusive. From a pathologic perspective, recognition of unequivocal biliary obstructive features is essential for an accurate assessment, with awareness of the numerous well-described diagnostic pitfalls, including early age at biopsy, which may result in false-“negative” interpretation, and conditions such as total parenteral nutrition–related liver disease and α1-antitrypsin deficiency, which may result in false-“positive” interpretation. Finally, a well-coordinated multidisciplinary approach to suspected cases of biliary atresia is key to timely diagnosis and optimal outcomes.

**References**

117. and extrahepatic biliary atresia.


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