Neonatal Cholestasis

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KEYWORDS

• Neonatal cholestasis • Neonatal liver disease • Biliary atresia • Jaundice
• Cholestasis

KEY POINTS

- The initial evaluation of a jaundiced infant should always include measuring serum conjugated (or direct) and unconjugated (or indirect) bilirubin levels.
- Jaundice in an infant that is of very early onset (less than 24 hours of age), persistent beyond 14 days of life, or of new-onset is abnormal and should be investigated.
- Conjugated hyperbilirubinemia in an infant (direct bilirubin levels >1.0 mg/dL or >17 μmol/L, or >15% of total bilirubin) is never normal and indicates hepatobiliary abnormality.
- Infants with cholestasis should be evaluated promptly for potentially life-threatening and treatable causes whereby timing of intervention directly impacts clinical outcomes.

INTRODUCTION

Jaundice in the neonate is common, usually secondary to unconjugated or indirect hyperbilirubinemia, and is most typically not dangerous to the infant. However, even in the setting of the well-appearing neonate, jaundice should be investigated if it is of very early onset (less than 24 hours of life), prolonged beyond 14 days of life, of new-onset, or at high levels. In these settings, it is critical to evaluate for potentially life-threatening causes, such as infection or evolving hepatobiliary dysfunction, and determine if urgent therapeutic intervention is required. Conjugated hyperbilirubinemia warrants expedient evaluation as timing of invention in some cases directly impacts clinical outcomes.

Bile is primarily composed of bile acids, bilirubin, and fats, is formed in the liver, and is secreted into the canaliculus. From the canaliculus, bile flows into biliary ducts from where it is ultimately secreted into the intestine after transient storage within the gallbladder. Disruption of this process at any level results in cholestasis. Cholestasis is the end result of obstruction of the normal excretion of bile from the liver, resulting in the abnormal accumulation of bile salts, bilirubin, and lipids in liver and the blood. Although cholestasis is not synonymous with conjugated hyperbilirubinemia, the abnormal retention of bilirubin, elevated serum levels in cholestasis, low cost, and

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wide availability of testing make serum-conjugated bilirubin the most clinically useful marker of cholestasis.

Clinically, cholestasis in the infant may present as jaundice, pruritus, fat-soluble vitamin deficiency, or may evolve during or following acute liver failure. Functional or anatomic biliary obstruction is often heralded by the presence of acholic stools. Although cholestasis is frequently the primary presenting symptom of neonatal hepatobiliary disease, it also commonly represents the final common pathway of any disease that affects the neonatal liver. As such, cholestasis is often classified by origin and is designated as either (1) biliary, referring to structural abnormalities and obstruction of extrahepatic or intrahepatic bile ducts; or (2) hepatocellular, resulting from impairment in bile transport, genetic or metabolic abnormalities, and infection.

This review presents an approach to the evaluation of the jaundiced infant. The authors discuss the most common causes, disease-specific evaluation, and clinical management of neonatal cholestasis. In addition, general concepts of supportive care for infants with cholestasis are reviewed.

EVALUATION OF THE JAUNDICED INFANT

Jaundice in the infant is usually clinically evident when the total serum bilirubin level exceeds 2.5 to 3.0 mg/dL (42–51 μmol/L) and is seen as scleral icterus or yellowing of the oral mucosa. However, visual estimates of serum bilirubin levels are inadequate and not precise,[1] and hence, levels should be determined when concern for elevation is raised. Although jaundice in neonates is common and can be physiologic, the continued presence of jaundice at 2 weeks of age should alert providers to the possibility of a pathologic process. A thorough examination and history evaluating for the possibility acute life-threatening conditions such as sepsis are paramount. In addition, clinical evaluation should survey for stigmata of hepatobiliary disease that may be heralded by the presence of dark urine or acholic stools or examination findings of hepatosplenomegaly and ascites. If the infant is exclusively breastfed and is well, the evaluation of serum bilirubin levels may be delayed up to 1 week (until 3 weeks of age) after repeat clinical evaluation.[2] However, if the infant is ill appearing, is formula fed, or carries any additional “red flags” such as poor growth or dysmorphic features, the provider should obtain total and fractionated (direct and indirect) serum bilirubin levels.[2] Conjugated hyperbilirubinemia in an infant (direct bilirubin levels >1.0 mg/dL or >17 μmol/L, or >15% of total bilirubin) is never normal and indicates hepatobiliary abnormality. The identification of elevated unconjugated hyperbilirubinemia warrants a different approach to management and is beyond the scope of this review.

If conjugated hyperbilirubinemia is identified, referral to a pediatric hepatologist is mandatory because timely identification of treatable causes of cholestasis can improve clinical outcomes. Secondary laboratory evaluations after cholestasis is identified may include serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase, prothrombin time and international normalized ratio (INR), and albumin levels. The initial diagnostic imagining should include an abdominal ultrasound (US), which can identify congenital anatomic or obstructive causes of cholestasis, including choledochal cysts and gallstones, and screen for vascular anomalies and evidence of portal hypertension such as splenomegaly. Liver biopsy often provides critical information to the diagnostic evaluation of neonates with cholestasis.

An algorithmic approach to the evaluation of the cholestatic infant is summarized in Fig. 1. Specific causes of neonatal cholestasis are reviewed in the text and tabulated in Table 1.

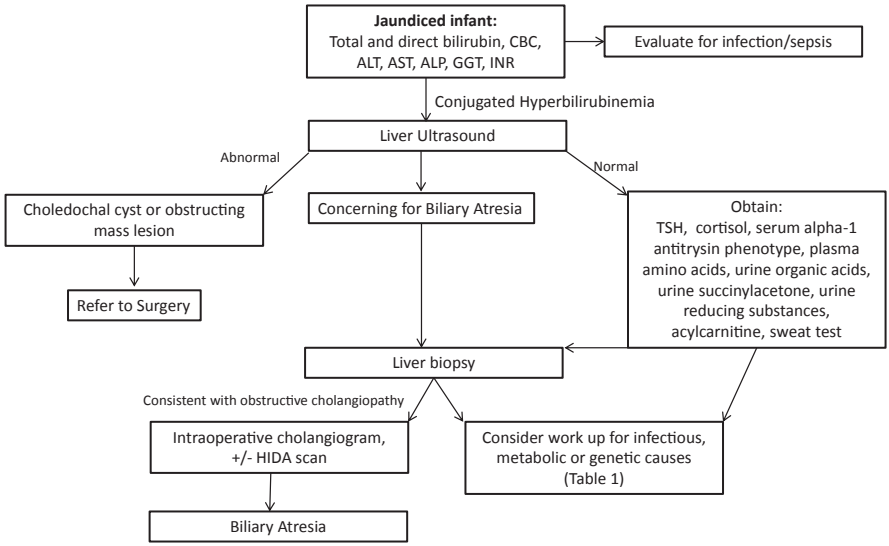

Fig. 1. Algorithmic approach to evaluation of neonatal cholestasis. ALP, alkaline phosphatase; CBC, complete blood count; TSH, thyroid stimulating hormone.

Structural (Biliary) Causes of Neonatal Cholestasis

Biliary atresia

Biliary atresia (BA) is the most common cause of infantile obstructive cholangiopathy and most frequent indication for liver transplantation in the pediatric population. The reported incidence of BA is 0.5 to 3.2 per 10,000 live births, but varies based on geography and ethnicity.[2–5] BA is characterized by progressive inflammation and fibrosis of the bile ducts, resulting in progressive obliteration of the extrahepatic and variably intrahepatic bile ducts.[6,7] The cause of BA is currently unknown. Hypotheses regarding pathogenesis range from abnormal genetic programming of bile duct formation, to viral infections, toxins, or autoimmune-mediated chronic biliary inflammation.[8–11]

BA is characterized anatomically, by the level of extrahepatic biliary obstruction.[12] Two clinical phenotypes exist: "classical" BA, which is not associated with extrahepatic congenital anomalies, and "biliary atresia with splenic malformation" that presents with other congenital anomalies, most frequently situs inversus, asplenia, or polysplenia, cardiac malformations, and intestinal malrotation.

BA presents most commonly with cholestasis between 2 and 5 weeks of life. Acholic stools may be present and indicate biliary obstruction; however, onset commonly follows the onset of jaundice. Unfortunately, if an affected infant has a preceding history of physiologic jaundice, the development of cholestasis may go unrecognized and delay appropriate evaluation and management. This clinical scenario highlights the importance of evaluating any prolonged or new jaundice in infants. Infants with delayed evaluation or presentation may demonstrate signs of chronic liver disease with portal hypertension such as hepatosplenomegaly or ascites. As chronic inflammation and cholestasis lead to malabsorption, many infants with BA present with inadequate weight gain and are characterized as failure to thrive.

Expedient differentiation of BA from other causes of neonatal cholestasis is critical, because surgical intervention before 2 months of age has been shown to improve surgical success and clinical outcome.[13–15] Without rapid intervention, the natural history

Table 1
Causes of neonatal cholestasis

Metabolic/genetic	Galactosemia Tyrosinemia type 1 Dubin-Johnson syndrome Rotor syndrome Disorders of BAD A1AT deficiency CF Defects of bile transport (PFIC) Peroxisomal disorders
Syndromic	Trisomy 21 Trisomy 13 Trisomy 18 Joubert syndrome Ivemark syndrome Beckwith-Weidemann syndrome Bardet-Biedl syndrome
Biliary	BA Choledochal cyst ALGS Choledocholithiasis Neonatal sclerosing cholangitis Caroli disease Obstruction from mass or stricture
Nutritional	Total parenteral nutrition
Cardiovascular	Heart failure Shock Hepatic ischemia
Infection	Herpes simplex virus Cytomegalovirus Adenovirus Hepatitis B Sepsis Urinary tract infection Cholecystitis Cholangitis
Endocrine	Hypothyroidism Panhypopituitarism Adrenal insufficiency

of BA is uniform fatality secondary to progressive end-stage liver disease by 2 years of age. Early in the course of disease, infants with BA typically demonstrate conjugated hyperbilirubinemia (direct bilirubin 2–7 mg/dL with total bilirubin levels between 5 and 12 mg/dL), with elevations in transaminases (ALT, AST) and GGT; the GGT elevation is usually more significant than that of ALT because the focus of the hepatocellular injury is in the bile ducts.[16]

Abdominal US is recommended early in the evaluation of a cholestatic infant. In the setting of BA, the US typically demonstrates absence, or nonfilling, of the gallbladder after adequate fasting, and an atretic extrahepatic bile duct; a normal gallbladder appearance, however, does not eliminate BA as the cause. The presence of an echogenic or fibrotic triangular cord at the porta hepatis representing the biliary remnant may be described as the "triangular cord sign" (**Fig. 2**) and has a diagnostic sensitivity

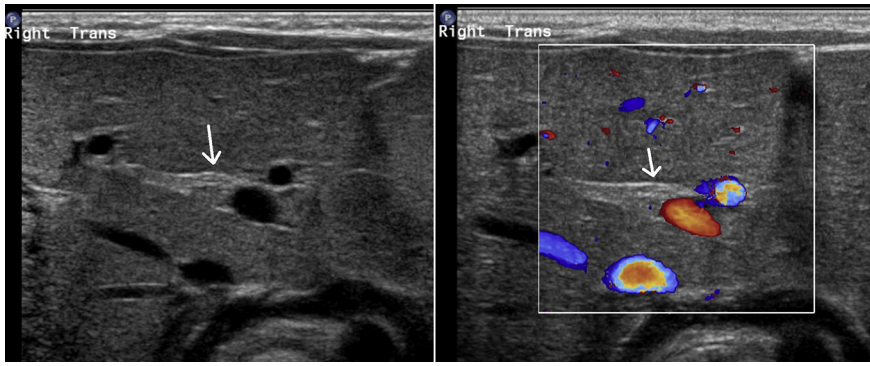

Fig. 2. Abdominal US in BA. Triangular-shaped homogenous echogenicity near the bifurcation of the portal vein consistent with triangular cord sign. *White arrows* indicate triangular cord of hyperechoic fibrous tissue seen at the porta hepatis. *Square* on figure at right indicates application of Doppler, highlighting vascular structures.

of 73%.[17] Functional abdominal imaging, including hepatobiliary scintigraphy with technetium-labeled iminodiacetic acid derivatives (HIDA scan), can assist in the differentiation between obstructive and nonobstructive causes of neonatal cholestasis. Pretreatment with phenobarbital (5 mg/kg/d) for 5 days before HIDA scan may increase the sensitivity of this test, but specificity is limited. On HIDA, the demonstration of rapid update of tracer but absence of excretion into the bowel at 24 hours is suggestive of BA (**Fig. 3**) or other obstructive process (eg, plugging in cystic fibrosis, CF); however, the low specificity (45%–72%) of the examination makes it better suited for exclusion rather than diagnosis of BA.[18,19] A normal HIDA does eliminate BA from the differential of possible diagnoses. A false "positive" nonexcreting HIDA scan finding may result from functional causes of cholestasis such as hypothyroidism.

In many cases, percutaneous liver biopsy is helpful in excluding alternate causes of neonatal cholestasis. Histopathological findings supportive of a diagnosis of BA include demonstration of bile ductular proliferation and bile duct plugging with relative preservation of normal hepatic lobular architecture (**Fig. 4**). Given the progressive nature of BA, however, the extent of liver fibrosis at the time of biopsy may vary, as can the extent of bile duct proliferation and destruction.

Failure to exclude BA, or a high suspicion for BA, necessitates surgical exploration with intraoperative cholangiogram. The diagnosis of BA is confirmed or excluded at the time of laparotomy, and intraoperative cholangiogram remains the gold standard for verifying a diagnosis of BA[16,20]; the identification of an atretic extrahepatic biliary tree confirms the diagnosis (**Fig. 5**). If BA is confirmed, surgical intervention at the time of initial laparotomy and intraoperative cholangiogram, with a Kasai hepatic portoenterostomy, is recommended. The Kasai aims to restore bile flow from the liver to bowel by excising the biliary obstruction and establishing biliary drainage through an anastomosis of the jejunal limb of a Roux-en-Y with the liver at the porta hepatis. The younger the age of diagnosis of BA and Kasai, the more likely the Kasai will be successful.[2,16] Although restoration of bile flow can significantly slow the progression of disease, most children progress to develop cirrhosis and portal hypertension despite effective bile drainage and ultimately require liver transplantation.

The importance of early diagnosis and surgical intervention implies a role for screening in the identification of BA. Screening for BA using stool color cards is currently used in Japan and Taiwan. Implementation of these programs, which use

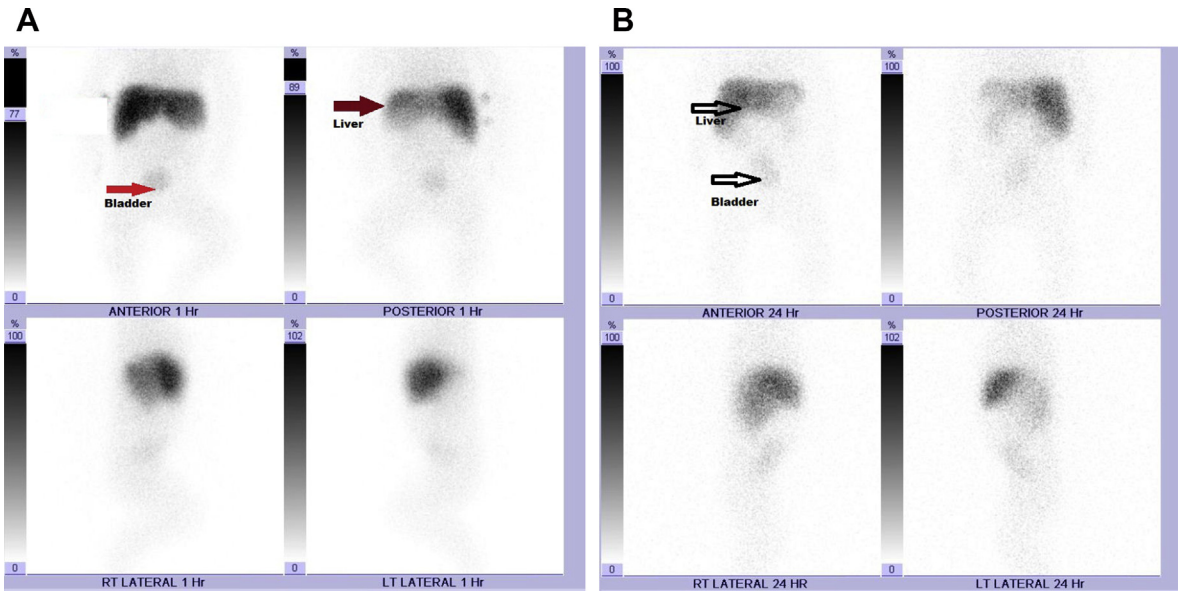

Fig. 3. HIDA scan in BA. Hepatobiliary scan at 1 hour (*A*) demonstrates rapid hepatic uptake (*red arrow*). Hepatobiliary scan at 24 hours (*B*) demonstrates lack of visualization of the biliary tree, gallbladder, and small bowel. Radiotracer is visualized in the kidneys and urinary bladder (*black arrow*). These findings are suggestive of, but not diagnostic for, BA.

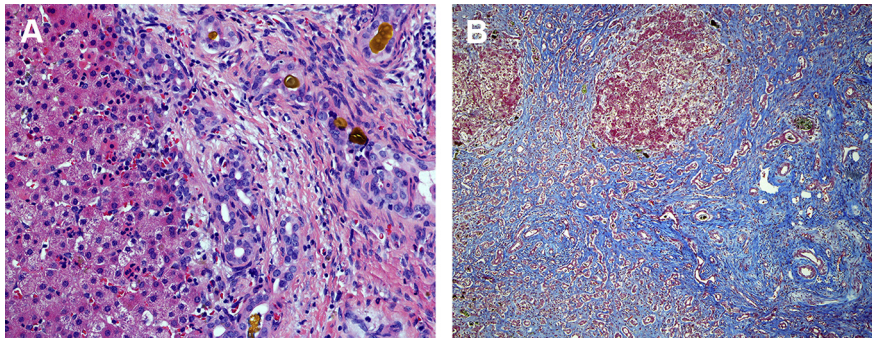

Fig. 4. BA histology. (*A*) Hematoxylin and eosin stain of a liver biopsy from a 3-month-old girl demonstrating a proliferation of bile ductules. Bile plugs are present (original magnification ×200). (*B*) Masson trichrome stain from the liver transplant specimen from the same girl at 8 months of age. Diffuse cirrhosis is identified with marked fibrous expansion of portal tracts. The portal triads lack bile ducts, but there is a marked bile ductule reaction, many containing bile plugs (original magnification ×40). (*Courtesy of* Dr Karen Chisholm, Seattle Children's Hospital, Seattle, WA.)

parents and caregivers to observe and report the infant's stool color at 1 month of age, has improved the timeliness of diagnosis and resulted in a significantly higher proportion of infants undergoing portoenterostomy before 60 days of age.[21–23] Stool color screening cards have not been widely adopted in North America or Europe, placing responsibility on primary care practitioners to have a high level of suspicion at the earliest routine well-child clinic visits.

Alagille syndrome

Alagille syndrome (ALGS) is a genetic disorder characterized by chronic, progressive cholestasis secondary to a paucity of intralobular bile ducts. The estimated prevalence

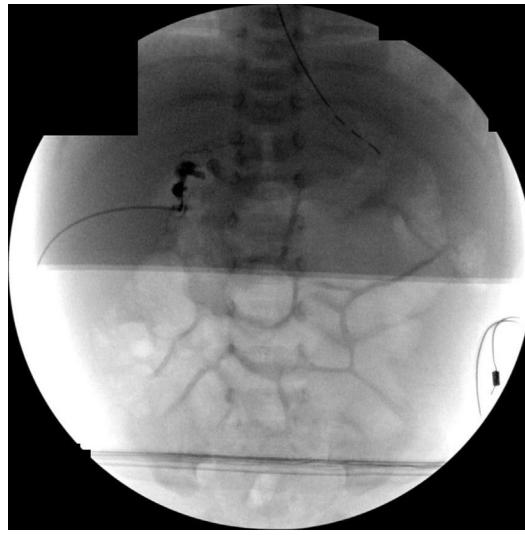

Fig. 5. Intraoperative cholangiogram. Catheter is demonstrated within a rudimentary gallbladder. Contrast injection does not show normal branching of extrahepatic or intrahepatic bile ducts concerning for BA.

is 1:30,000.[24] ALGS is inherited in an autosomal dominant fashion, but may occur sporadically due to de novo mutation. Most individuals with ALGS carry a mutation in *JAG1*, a gene located on chromosome 20, but a small number have mutations in *NOTCH2*.[25–29] The product of *JAG1* and *NOTCH2* is a ligand in the Notch signaling pathway, which plays a key role in embryogenesis.

Multiple organ systems are affected in infants with ALGS. Typically, ALGS is characterized by progressive cholestatic liver disease, stereotypical facial features, congenital heart disease, posterior embryotoxon, butterfly vertebrae, and renal disease. Most infants with ALGS present with cholestasis within the first 3 months of life. Those with severe congenital heart disease may present at birth or may initially come to attention after a cardiology evaluation. Although many forms of congenital heart disease have been associated with ALGS (eg, tetralogy of Fallot and transposition of the great arteries), the most common is peripheral pulmonary stenosis. The characteristic facial features are frequently difficult to appreciate in the neonatal period but include a prominent forehead and pointed chin, giving the face a triangular appearance, deep-set eyes with hypertelorism, and a saddle nose.

Care must be taken to discriminate ALGS from alternate causes of neonatal cholestasis, particularly BA. As in BA, standard neonatal cholestasis evaluation typically demonstrates conjugated hyperbilirubinemia associated with elevated serum aminotransferases and especially GGT, reflective of the biliary involvement. Recommended assessments for the extrahepatic manifestations include abdominal US, radiographs of the spine to identify hemivertebra or butterfly vertebra, echocardiogram, and ophthalmologic evaluation to identify the presence of posterior embryotoxon. Children with ALGS may also benefit from routine neuroimaging, because cerebrovascular anomalies, such as Moyamoya, resulting in increased risk of intracranial bleeding or stroke, have been described.[30]

Although a liver biopsy is not required for diagnosis when other stereotypical syndromic features are present, histologic evaluation may be needed when the diagnosis is in question or hepatic disease advancement is suspected of being advanced. The histopathology in ALGS is characterized by bile ductular paucity. The number of bile ducts is normally diminished in preterm infants, however, so care must be taken to not to make the diagnosis of pathologic paucity incorrectly[24] (**Fig. 6**). In term infants

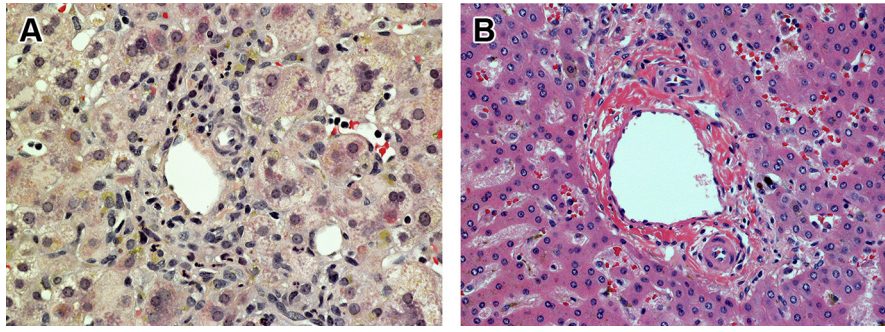

Fig. 6. ALGS histology. (*A*) Hematoxylin and eosin stain of a liver biopsy from a 3-month-old boy demonstrating loss of bile ducts in a portal triad. The adjacent hepatocytes are swollen and show hepatocanalicular cholestasis (original magnification ×400). (*B*) Hematoxylin and eosin stain of a different boy at 10 months of age who was transplanted for ALGS. His liver demonstrated paucity of bile ducts in the portal triads and mild hepatocanalicular cholestasis (original magnification ×200). (*Courtesy of* Dr Karen Chisholm, Seattle Children's Hospital, Seattle, WA.)

and older children, the normal bile duct to portal tract ratio ranges from 0.9 to 1.8, with ratios less than 0.9 suggestive of paucity. Without the other features of ALGS, infants with cholestatic jaundice and elevated GGT usually require a liver biopsy, hepatobiliary scintigraphy, and possibly an intraoperative cholangiogram to verify patency of the extrahepatic biliary system; care must be taken to interpret the intraoperative cholangiogram correctly, because the extrahepatic bile ducts in ALGS are typically very small due to few feeding intrahepatic ducts, but they are patent.

In addition to the syndromic features characteristic of ALGS, children with ALGS commonly suffer from severe metabolic bone disease, dyslipidemia, and refractory pruritis. Significantly elevated serum alkaline phosphatase typically reflects abnormal bone metabolism in addition to the biliary disease. Hypercholesterolemia and hypertriglyceridemia can lead to the development of xanthomas, which may appear most prominently on extensor surfaces and areas of minor trauma, such as the diaper area, plantar surfaces of the feet, abdomen, and neck. In addition, serum bile salt levels can be extremely elevated, even in the absence of jaundice, leading to intractable and refractory pruritus.

Treatment of ALGS is directed at maintaining adequate nutrition, treating the complications of cholestasis, and supporting cardiovascular health. Twenty-five percent to 50% of children with ALGS have debilitating and disfiguring pruritus despite medical therapy, or develop progressive liver disease, and ultimately require liver transplantation.[24]

Gallstones

Gallstones are uncommon in infants; however, sepsis, prematurity, and prolonged exposure to total parenteral nutrition may increase the risk of their development. Most infants identified to have gallstones have congenital biliary abnormalities or hemolytic disease (leading to development of black pigment stones).[31] For most infants, gallstones are incidental and asymptomatic. Screening studies have identified a prevalence of gallstones in approximately 2% of well children. In a cohort of children with incidentally identified gallstones followed over 15 years, there was only a 2% annual risk of biliary pain, and that risk decreased after 5 years.[31] Therefore, unless there is the development of obstruction (choledocholithiasis) or infection (cholecystitis or cholangitis), treatment is generally unnecessary. In select cases, ursodeoxycholic acid (ursodiol) may be considered an oral therapy for gallstone dissolution.[31]

Choledochal cysts

Choledochal cysts are congenital dilations or aneurysms of the biliary system. They may be single or multiple and may involve any part of the biliary system. The highest incidence is in Asia, occurring in approximately 1 in 1000 live births.[32] There are 5 types, classified by location of biliary dilation, with the most commonly seen (accounting for >85% of all choledochal cysts) variant being cystic or fusiform dilations of the common bile duct.[32] Most infants with choledochal cysts present with cholestasis; however, they may initially present with cholangitis or pancreatitis.

Diagnosis of choledochal cysts relies on imaging. Abdominal US is the diagnostic imaging modality of choice in evaluating intrahepatic and extrahepatic biliary anatomy. Secondary imaging may be required to delineate complicated biliary anatomy, including HIDA scans and magnetic resonance cholangiopancreatography. Serum laboratory testing may reveal elevated conjugated hyperbilirubinemia and GGT (reflecting biliary obstruction), and usually less dramatically elevated serum aminotransferases.

Definitive treatment is surgical resection, although treatment of pre-existing cholangitis or pancreatitis is necessary before surgical intervention. Surgical treatment is aimed at resolving biliary obstruction, restoring normal biliary drainage, and eliminating the long-term risk of cholangiocarcinoma or squamous cell carcinoma in any residual cyst.[32–35]

Genetic and Metabolic Causes of Neonatal Cholestasis

Alpha-1 antitrypsin deficiency

Alpha-1 antitrypsin (A1AT) deficiency is the most common genetic cause of liver disease and affects approximately 1 in 2000 live births.[36–38] The gene mutation leading to A1AT deficiency is inherited as an autosomal dominant disorder and results in a single amino acid substitution within the A1AT protein. This amino acid change causes abnormal molecular folding of the A1AT protein, and inability of the protein to be processed beyond and excreted from the endoplasmic reticulum. Inability of the abnormal protein to be excreted from hepatocytes leads to both low plasma levels of circulating A1AT and to hepatocellular injury from excessive accumulation. A1AT functions as a serine protease, which primarily acts to inhibit other proteases and elastases; without appropriate inhibition, the activities of proteases and elastases lead to cellular destruction.

The clinical phenotypes of A1AT deficiency include both liver and pulmonary manifestations, but penetrance is highly variable. Liver disease commonly presents in the neonatal period and is frequently characterized by transient cholestatic jaundice. Despite similar levels of circulating protein levels, advancement of the liver disease with stigmata of portal hypertension or the development of liver failure is uncommon, occurring in roughly 20% of homozygotically affected individuals. Pulmonary disease is a later development, manifesting in adulthood.

A1AT deficiency is diagnosed by protein phenotyping. Although widely available, the serum level of A1AT is less reliable for diagnosis because it can be misleadingly elevated into the normal range in times of systemic inflammation or infection (as an acute-phase reactant). A1AT phenotyping (Pi type) is the most specific and preferred diagnostic serum test. A1AT variants are named according to their electrophoretic migration pattern,[39] with normal A1AT protein designated M. The S and Z variants are the most common mutations leading to a reduction in serum A1AT, and disease when inherited homozygotically. The PiZZ variant, named for its slowest gel migration, causes the most severe clinical disease phenotype. Generally, liver disease manifests only in PiZZ, PiSZ, or rarely, PiSS variants.[40]

The classic, although not pathognomonic, histologic finding in A1AT deficiency is periodic acid Schiff-positive, diastase-resistant, eosinophilic globules within the hepatocytes. This finding represents the accumulated abnormal protein trapped within the endoplasmic reticulum. Liver histology may also demonstrate bile duct destruction, proliferation, and potentially bile duct paucity, making it important to distinguish from BA and ALGS.

Management of liver disease in A1AT is primarily supportive, because there are no specific or targeted therapies currently available. As cholestasis tends to be the primary clinical phenotype in neonates, fat malabsorption and fat-soluble vitamin deficiency are possible. Most infants will benefit from supplementation with medium-chain triglyceride (MCT) and fat-soluble vitamins as needed. Ursodeoxycholic acid may be used, but no study to date has demonstrated clear benefit. Although breastfeeding may be supported, there is no evidence that demonstrates clear benefit of breastfeeding over formula.[41,42]

Liver transplantation is indicated for infants and children with end-stage liver disease secondary to A1AT. Importantly, because A1AT is primarily manufactured in

the liver, the recipient assumes the donor's Pi phenotype. Thus, after transplant, the recipient experiences normal serum levels of functional A1AT, a decreased risk of pulmonary disease, and no chance of recurrent disease in the transplanted organ.

To prevent the development of pulmonary manifestations, including early emphysema, avoidance of smoking and environmental pollution is critical. It should be noted that recombinant A1AT is available and approved for the treatment of pulmonary manifestations. However, recombinant A1AT has no role in the treatment or prevention of hepatic injury, because it has no effect on the direct hepatocellular injury caused by the presence of misfolded A1AT.

Cystic fibrosis liver disease

Although CF is common, affecting approximately 1:2500 live births in North America, CF-related liver disease affects less than 2% of infants.[43] Given the low incidence of CF-related liver disease in neonates, testing for CF in jaundiced infants should be reserved for infants affected with meconium ileus, inadequate weight gain despite theoretically adequate caloric intake, those with an obstructive cholangiopathy without other explanation, or those infants in whom alternate causes of cholestasis have been excluded. Diagnosis of CF-related liver disease relies on diagnosis of CF, commonly supported by newborn screening for immunoreactive trypsinogen. The gold standard remains sequencing of the CFTR gene or a positive sweat chloride test.

Disorders of bile acid synthesis

Cholic acid and chenodeoxycholic acid are the primary bile acids manufactured in humans; disruption in the normal synthetic pathways results in the accumulation of toxic intermediate metabolites. Liver injury is also mediated by abnormal accumulation of cholesterol, drugs, and other toxins within the liver from abnormal bile excretion. Although rare, disorders of bile acid synthesis (BAD) should be included in the differential for a neonate presenting with progressive cholestasis when alternate causes have been ruled out.

In the neonatal period, infants with disorders of BAD may present with persistent cholestasis, whereas others may present with acute hepatitis or liver failure. The most common clinical presentation of disorders of BAD includes neonatal jaundice, failure to thrive, hepatosplenomegaly, rickets, and bleeding. Some disorders of BAD are associated with neurologic disease, including seizures, developmental delay, deafness, blindness, and neuromuscular weakness.

Diagnosis of bile acid synthetic disorders should include serum and urine analyses of the bile acids. Serum tests may demonstrate low bile acid levels, elevated serum aminotransferases, normal GGT, and evidence of fat malabsorption. If serum bile acids are low, urinary bile acids should be measured to identify the particular synthetic defect; the subject must not be on ursodeoxycholic acid therapy at the time of the analysis. Hepatic histology is generally nondiagnostic and may demonstrate nonspecific canalicular bile plugging, inflammation without bile duct proliferation, or giant cell transformation.[44]

Treatment of inborn errors of BAD, when possible, focuses on suppressing production of toxic metabolites and supporting normal growth. For the most treatable forms of BAD, these objectives are best achieved by treatment with cholic acid. Ursodiol is not indicated because it does not suppress production of abnormal bile acid intermediates.

Progressive familial intrahepatic cholestasis

Progressive familial intrahepatic cholestasis (PFIC) is a group of disorders characterized by defective bile export and subsequent cholestasis. This group of autosomal

recessive disorders includes PFIC 1, PFIC 2, and PFIC 3 and is named based on the specific genetic mutation. In PFIC, liver disease results from the accumulation of bile salts within the hepatocytes, leading to profound cholestasis. Infants commonly present with profound pruritus, but may also present with jaundice or occasionally life-threatening hemorrhage secondary to vitamin K deficiency.

PFIC 1, also known as Byler disease, is caused by a mutation in the gene ATP8B1 on chromosome 18q21-22.[45] This gene codes for a protein flippase (FIC 1), which facilitates the flipping of aminophospholipids from the outer to inner canalicular membrane. Affected individuals typically present in infancy with recurrent episodes of jaundice within the first few months of life. Later, affected children may develop short stature, deafness, pancreatitis, and persistent diarrhea.

PFIC 2 results from a defect in the bile canalicular bile salt export pump (BSEP) caused by a mutation in the gene ABCB11 on chromosome 2q24.[45] BSEP is responsible for transporting bile acids from inside the hepatocyte to the canaliculus. Disruption of BSEP results in accumulation of bile acids within hepatocytes, resulting in severe cholestasis. PFIC 2 presents in infancy with rapidly progressive cholestasis that often progresses to liver failure within the first few years of life. Children with PFIC 2 have an increased risk of developing hepatocellular carcinoma and cholangiocarcinoma.[45]

PFIC 3 is caused by a mutation in the gene ABCB4 on chromosome 7q21, which encodes for multidrug resistance–associated protein 3 (MDR3). MDR3 is a "floppase," which mediates flopping of aminophospholipids from the inner to outer canalicular lipid bilayer, resulting in a deficiency in export of phospholipids. Bile in infants with PFIC3 has insufficient phospholipid concentration, making the micelles unstable and toxic to bile ducts, which ultimately leads to the development of progressive intrahepatic cholangiopathy. In contrast to PFIC 1 and 2, only a third of children with PFIC 3 present with cholestasis during infancy. When infants with PFIC 3 do present with liver disease, they commonly have concurrent cholesterol gallstones complicating their intrahepatic cholestasis.

Definitive diagnosis of PFIC is dependent on specific genetic testing. However, routine serum laboratory testing can suggest PFIC as a cause of neonatal cholestasis. Infants with PFIC generally have markedly elevated serum bile acid levels with only mildly elevated serum bilirubin. The characteristic biochemical marker of PFIC 1 and 2 is a normal or low GGT, normal serum cholesterol, and only mild transaminitis. PFIC 3 presents with an elevated GGT in the absence of extrahepatic biliary obstruction.

Treatment of PFIC initially focuses on nutritional support to optimize absorption of fat and fat-soluble vitamins and achieve weight gain, in the presence of profound cholestasis. Aggressive treatment of pruritus often requires multiple concurrent therapies, including ursodiol, cholestyramine, rifampin, and opioid antagonists. In medically refractory cases or in the presence of advanced liver disease, treatment may include partial biliary diversion, interruption of the enterohepatic circulation by surgical ileal exclusion, and liver transplantation.[16,45,46]

Disorder of amino acid metabolism: type 1 tyrosinemia

Type 1 tyrosinemia is a metabolic disorder of amino acid metabolism that results from deficiency of fumarylacetoacetate hydrolase, the enzyme responsible for the final step of tyrosine degradation.[47] Type 1 tyrosinemia is an autosomal recessive disorder with an incidence of 1:100,000. Tyrosinemia generally presents acutely in the neonatal period and should be included in the differential of acute neonatal liver failure. In addition to acute liver failure, neonates with tyrosinemia may present with failure to thrive, vomiting, ascites, coagulopathy, hypoglycemia, and hyperbilirubinemia. In older

infants, a more chronic presentation characterized by growth failure, Fanconi syndrome, and neurologic manifestations may develop. Diagnosis of type 1 tyrosinemia is made by identifying elevated urinary succinylacetone.

Support of the infant diagnosed with tyrosinemia in the neonatal period consists of correcting any metabolic derangements, treating sepsis when present, and correcting coagulopathy as needed, followed by the restriction of dietary tyrosine. Usage of low-tyrosine formulas alone, however, results in less than 40% survival at 1 year of age.[48–50] More definitive treatment with NTBC (2-(2-nitro-4-trifluromethylbenzoyl)-1,3-cyclohexanedione, nitisinone) improves survival to greater than 85% at 1 year of age and is the standard of care.[51] NTBC works by inhibiting the formation of maleyl acetoacetic acid and fumaryl acetoacetic acid, the precursors to the hepatotoxic compound succinylacetone. Despite adequate treatment, children with tyrosinemia type 1 carry a long-term risk of developing hepatocellular carcinoma and therefore require close follow-up.

Galactosemia

Galactosemia results from an inability to metabolize galactose secondary to a deficiency in one of the following enzymes: galactokinase, galactose-1-phosphate uridyl transferase (Gal-1-PUT), or uridine diphosphate galactose-4-epimease. Gal-1-PUT deficiency is the most common cause of galactosemia and results in the inability to metabolize galactose into glucose-1-phosphate. It is an autosomal recessive disorder with an incidence of 1:60,000 live births.[47]

Abnormal galactose metabolism results in the accumulation of toxic metabolites in the liver, brain, kidney, and eye lens. Classically, galactosemia presents within the first few weeks of life after infants ingest breast milk or milk-based formulas that contain lactose. Presenting symptoms may include failure to thrive, jaundice, vomiting, and diarrhea. Infants with galactosemia are at increased risk for gram-negative sepsis and hence may present acutely with sepsis and associated severe acidosis, jaundice, and coagulopathy. Additional clinical findings may include hepatomegaly, ascites, bleeding, hypotonia, edema, and bulging fontanelle.

Many state-mandated newborn screening tests identify variants of Gal-1-PUT–deficient disease. Although diagnosis can be suggested by the presence of reducing substances in the urine, this is only sensitive when affected individuals are still ingesting galactose. Definitive diagnosis requires demonstration of a complete absence of Gal-1-PUT activity via a quantitative red blood cell (RBC) assay; analysis post-RBC transfusion will give unreliable results.

Treatment of galactosemia centers on the immediate stabilization of the critically ill infant and removal of dietary galactose. Stabilization with intravenous glucose, vitamin K, antibiotics, and initiation of a soy-based (non-galactose-containing) formula when well enough is usually effective. Continued avoidance of lactose and galactose-containing foods is required throughout life. Despite treatment, many children will have some degree of developmental delay residual from the presenting illness.

Other Causes of Neonatal Cholestasis

Infections

Congenital or perinatal infections and sepsis are common causes of neonatal liver cholestasis. For ill-appearing infants with cholestasis, a rapid evaluation for bacterial infection (such as sepsis or urinary tract infection) is mandatory. Judicial selection of antimicrobials must be considered, because several are known to exacerbate cholestasis by displacing bilirubin from albumin (eg, Ceftriaxone), or may be potentially hepatotoxic (eg, fluconazole and acyclovir).[52] In addition to common bacterial infections,

TORCH infections (toxoplasmosis, rubella, cytomegalovirus, herpes, and syphilis) as well as hepatitis B, parvovirus B19, adenovirus, and echoviruses can result in neonatal cholestasis and hepatitis.

Parenteral nutrition-associated liver disease

Parenteral nutrition-associated liver disease (PNALD) is an important and common cause of cholestasis, hepatitis, and liver-related morbidity in the neonatal period. Several clinical risk factors have been identified that contribute to the development of PNALD, and these include prematurity, low birth weight, lack of enteral feeding, sepsis, short gut syndrome, and necrotizing enterocolitis.[53,54] An estimated 33% to 85% of premature infants who receive parenteral nutrition for more than 7 days develop PNALD.[55,56] When TPN is used for less than 2 weeks, any associated liver inflammation generally completely resolves. However, prolonged use increases the risk for irreversible liver disease that may ultimately result in liver fibrosis and failure.[57,58] The diagnosis of PNALD is suggested by the presence of a serum conjugated bilirubin level greater than 2 mg/dL, ALT greater than 2 times the upper limit of normal, and elevated GGT.

Minimization of PNALD requires early initiation and continuation of enteral feeding as possible, use of intralipids at a dose not more than 1 g/kg/d, and prevention of infection. Ursodiol at a dose of 20 to 30 mg/kg/d in divided doses may be additionally used to improve bile flow.[59–61] Use of omega-6 fatty acid or fish oil–based, rather than soy-based, lipid formulations has been shown to be effective at resolving cholestasis.[62] Aggressive prevention of PNALD and bowel rehabilitation when appropriate is critical in preventing irreversible liver damage.

Idiopathic neonatal hepatitis

Idiopathic neonatal hepatitis is a term historically applied to infants presenting with neonatal cholestasis or hepatitis in whom a specific cause could not be identified. Typically, liver biopsies in these infants demonstrated nonspecific intrahepatic cholestasis and giant cell transformation of hepatocytes[63] (**Fig. 7**). However, now it is recognized that multinucleated giant cells represent a stereotypical response by

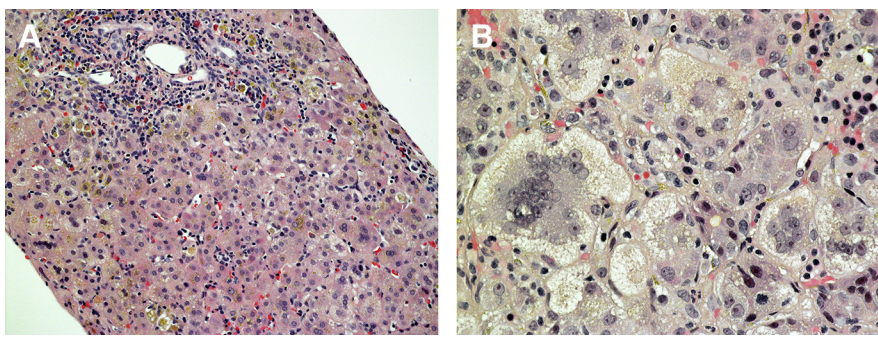

Fig. 7. Neonatal hepatitis histology. (*A*) Hematoxylin and eosin stain from a liver biopsy from a 6-week-old infant demonstrating hepatocyte ballooning and giant cell transformation. Extramedullary hematopoiesis is present, especially in the portal triad. Hepatocanalicular cholestasis is identified (original magnification ×200). (*B*) Higher power of a hematoxylin and eosin stain from a liver biopsy from a different 8-week-old infant highlights giant cell transformation of hepatocytes. Extramedullary hematopoiesis is present in the upper right (original magnification ×400). (*Courtesy of* Dr Karen Chisholm, Seattle Children's Hospital, Seattle, WA.)

the immature liver to many causes of hepatocyte injury, including infection, biliary obstruction, and metabolic disease. In addition, with advancements in next-generation DNA sequencing, the number of identifiable causes of neonatal cholestasis has increased, reducing the frequency of this nonspecific diagnosis.

Nutritional Support of the Cholestatic Infant

Nutritional support is critical and central to the medical management of infants with chronic cholestasis. Optimization of nutritional status can reverse, improve, and/or prevent complications of cholestasis, including fat-soluble vitamin (A, D, E, and K) deficiencies, bleeding secondary to progressive coagulopathy, and pathologic fractures (**Table 2**).[64] Growth failure in the cholestatic infant is common and occurs secondarily to malabsorption from inadequate bile flow and intestinal mucosal congestion from portal hypertension. In addition, infants with cholestasis often have increased caloric needs in the setting of chronic liver disease and may require a daily caloric intake exceeding 150% of those of healthy infants to achieve weight gain.

Enteral nutrition is the preferred modality, and when oral intake is inadequate, placement of a nasoenteric tube for supplemental feeding is recommended. Breast feeding is encouraged, but when growth is inadequate on breast milk alone, supplemental formula must be considered. The selection of formula should consider MCT content, because this fat source is directly absorbed into the portal venous system and does not require emulsification by bile acids or active transport, which is disrupted in cholestasis. Children with portal hypertension and ascites also benefit from sodium restriction; however, in the exclusively formula-fed infant, additional sodium restriction is unnecessary.

Table 2
Fat-soluble vitamin supplementation

Vitamin	Target Serum Level	Recommended Supplementation
Vitamin A (retinol)	19–77 μg/dL Retinol: RBP molar ratio >0.8	Dose in increments of 5000 IU (up to 25–50,000 IU/d) orally Or Monthly intramuscular administration of 50,000 IU Monitor serum levels very 1–2 mo
Vitamin D (25-hydroxy vitamin D)	>30 ng/mL	Serum 25(OH)D level 5–30 ng/mL: 1000–5000 IU daily for 3 mo Serum 25(OH)D level <5 ng/mL: 1000–8000 IU daily for 3 mo Or Calcitriol at 0.05–0.20 μg/kg daily Monitor serum levels every 1–3 mo
Vitamin E (α-tocopherol)	3.8–20.3 μg/mL Vitamin E: total serum lipids ratio >0.6 mg/g	Water-miscible vitamin E: 1 unit/kg daily Monitor serum levels every 1–2 mo
Vitamin K (phytonadione)	INR ≤1.2	Oral: 2.5–5 mg Or SQ, IM, IV: 1–10 mg/dose once INR may not correct with advanced liver failure

Abbreviations: IM, intramuscularly; IV, intravenously; RBP, retinol binding protein; SQ, subcutaneously.

Despite attempts at optimizing nutrition through enteral feeds, many infants with advanced liver disease may evolve to require parenteral nutrition in anticipation of liver transplantation.

Treatment of Pruritis in the Cholestatic Infant

Infants with chronic cholestasis often have significant discomfort from intractable pruritus secondary to abnormal retention and accumulation of bile salts in the skin. Treatment is largely aimed at symptomatic improvement, with resolution of symptoms only after definitive intervention of the underlying cause of the cholestasis. Pharmacologic treatments include antihistamines (diphenhydramine, hydroxyzine), ursodeoxycholic acid, cholestyramine, rifampin, and opioid antagonists.

SUMMARY

Although jaundice in the neonatal period is common and often physiologic, cholestasis is always pathologic and indicates hepatobiliary disease. A high level of suspicion and prompt investigation for all infants with early, persistent, or high levels of hyperbilirubinemia is required and warrants fractionating the bilirubin levels. If cholestasis is confirmed, urgent referral to a pediatric gastroenterologist or hepatologist is recommended to assist in diagnostic and therapeutic interventions to optimize clinical outcome.

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