Neonatal Cholestasis

Amy G. Feldman, MD,*
Ronald J. Sokol, MD†

Author Disclosure
Drs Feldman and Sokol have disclosed no financial relationships relevant to this article. This commentary does contain a discussion of an unapproved/investigative use of a commercial product/device.

Educational Gaps

1. Early diagnosis of neonatal cholestasis is potentially life-saving; however, delayed diagnosis remains a problem.
2. There are several key steps in evaluating the patient who has cholestasis, and following these steps in a timely manner is crucial to identifying the underlying etiology.
3. Biliary atresia (BA) is the most common cause of cholestasis, and although an effective BA screening program was created in Taiwan and is being initiated in many countries around the world, the program's success is not assured in the United States because there is no standard 1-month infant health provider visit, in spite of the public health benefit.

Abstract

Cholestatic jaundice is a common presenting feature of neonatal hepatobiliary and metabolic dysfunction. Any infant who remains jaundiced beyond age 2 to 3 weeks should have the serum bilirubin level fractionated into a conjugated (direct) and unconjugated (indirect) portion. Conjugated hyperbilirubinemia is never physiologic or normal. The differential diagnosis of cholestasis is extensive, and a step-wise approach based on the initial history and physical examination is useful to rapidly identify the underlying etiology. Early recognition of neonatal cholestasis is essential to ensure timely treatment and optimal prognosis. Even when specific treatment is not available, infants who have cholestasis benefit from early medical management and optimization of nutrition. Future studies are necessary to determine the most reliable and cost-effective method of universal screening for neonatal cholestasis.

Objectives

After completing this article, readers should be able to:

1. Understand when a jaundiced infant needs evaluation for cholestatic liver disease.
2. List the differential diagnosis for cholestatic liver disease of the neonate and identify those causes that are amenable to immediate medical or surgical intervention.
3. Describe the step-wise approach to evaluation of a cholestatic infant.
4. Understand the importance of early screening for cholestatic liver disease and be aware of new research suggesting the importance of early laboratory values in identifying cholestatic infants.

Abbreviations

A_1AT:	α_1-antitrypsin
BA:	biliary atresia
GGT:	γ-glutamyl transpeptidase
HPE:	hepatic portoenterostomy
INH:	idiopathic neonatal hepatitis
PFIC:	progressive familial intrahepatic cholestasis
PN:	parenteral nutrition
PNAC:	parenteral nutrition–associated cholestasis
SBS:	short bowel syndrome

Introduction

Jaundice, a yellow discoloration of the skin, sclera, mucous membranes, and bodily fluids, is a common clinical finding

*Fellow in Pediatric Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, University of Colorado School of Medicine, and Digestive Health Institute, Children's Hospital Colorado, CO.
†Professor and Vice Chair of Pediatrics, Chief of Section of Pediatric Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, and Director of Colorado Clinical and Translational Sciences Institute, University of Colorado Denver, and Digestive Health Institute, Children's Hospital Colorado, CO.

in the first 2 weeks after birth, occurring in 2.4% to 15% of newborns. (1) Most often, jaundice is of the indirect/unconjugated bilirubin variety and resolves spontaneously without intervention. However, persistent jaundice is abnormal and can be the presenting sign of serious hepatobiliary and metabolic dysfunction. When jaundice persists beyond age 2 weeks, cholestasis or conjugated hyperbilirubinemia must be considered in the differential diagnosis. Cholestasis represents an impairment in bile flow and may be caused by either an intrahepatic or extrahepatic disorder. To differentiate cholestasis from benign causes of jaundice, the serum bilirubin must be fractionated into conjugated (or direct) and unconjugated (or indirect) fractions. Conjugated hyperbilirubinemia is generally defined as a conjugated or direct bilirubin level greater than 1 mg/dL when the total bilirubin is less than 5 mg/dL or more than 20% of the total bilirubin if the total bilirubin is greater than 5 mg/dL. Conjugated hyperbilirubinemia is never physiologic or normal. Unconjugated hyperbilirubinemia, conversely, is a common finding and can result from physiologic jaundice, breastfeeding and human milk–associated jaundice, red blood cell hemolysis, hypothyroidism, Gilbert syndrome, or Crigler-Najjar syndrome. Clues to the diagnosis of cholestasis include hepatomegaly, diarrhea and poor weight gain, hypopigmented or acholic stools, and dark urine that may stain the diaper.

Any infant who remains jaundiced beyond age 2 to 3 weeks needs to be evaluated to first exclude neonatal cholestasis and, if present, to rapidly identify those causes of cholestasis that are amenable to medical or surgical treatment. Even when specific treatment is not available or curative, infants who have cholestasis benefit from early medical management and optimization of nutrition to prevent complications. Despite data showing that early diagnosis of cholestasis and its etiologies is potentially life-saving, (2) delayed diagnosis remains a problem. (3) Early hospital discharge of newborns, inadequate follow-up of persisting jaundice, false reassurance by the appearance of pigmented stool, fluctuating serum bilirubin levels, and misdiagnosis of human milk–associated jaundice are all cited as reasons for late referral for evaluation of cholestasis. (3)(4)(5)

Etiology

Cholestatic jaundice affects approximately 1 in every 2,500 infants and has a multitude of causes. (6) The number of unique disorders presenting with cholestasis in the neonatal period may be greater than at any other time in life and include infections, anatomic abnormalities of the biliary system, endocrinopathies, genetic disorders, metabolic abnormalities, toxin and drug exposures, vascular abnormalities, neoplastic processes, and other miscellaneous causes (Table 1). (7) Of the many conditions that cause neonatal cholestasis, the most commonly identifiable are biliary atresia (BA) (25%–35%), genetic disorders (25%), metabolic diseases (20%), and α_1-antitrypsin (A_1AT) deficiency (10%). (8) In older series, idiopathic neonatal hepatitis (INH) was the most common cause of neonatal cholestasis, with a reported incidence of 1 in 4,800 to 1 in 9,000 live births. (9) However, with the discovery of specific etiologies that share the phenotype of INH in addition to more advanced diagnostic methods, the incidence of INH has decreased substantially. In infants born prematurely and in those who have short bowel syndrome (SBS) or intestinal failure, parenteral nutrition–associated cholestasis (PNAC) commonly develops in those receiving parenteral nutrition (PN) for more than 2 to 4 weeks.

Clinical Features

The typical findings in an infant who has cholestasis are protracted jaundice, scleral icterus, acholic stools, dark yellow urine, and hepatomegaly. It should be noted that there may be a perception of decreasing jaundice over the first weeks after birth as the indirect bilirubin component (from human milk–associated jaundice) decreases, causing false reassurance that the jaundice is resolving and need not be evaluated further. Acholic stools in an infant should always prompt further evaluation. Some infants may have coagulopathy secondary to vitamin K malabsorption and deficiency and present with bleeding or bruising. Coagulopathy may also be caused by liver failure, indicating either severe metabolic derangement of the liver (as in respiratory chain deficiency disorders) or cirrhosis and end-stage liver disease (as in neonatal hemochromatosis). Splenomegaly can be observed in infants who have cirrhosis and portal hypertension, storage diseases, and hemolytic disorders. Neurologic abnormalities including irritability, lethargy, poor feeding, hypotonia, or seizures can indicate sepsis, intracranial hemorrhage, metabolic (including Zellweger syndrome) and mitochondrial disorders, or severe liver dysfunction resulting in hyperammonemia and encephalopathy. Low birth weight, thrombocytopenia, petechiae and purpura, and chorioretinitis are often associated with congenital infection. Facial dysmorphism may suggest a chromosomal abnormality or Alagille syndrome. A palpable mass in the right upper quadrant may indicate a choledochal

Table 1. Differential Diagnosis of Neonatal Cholestasis

Infectious	Genetic and Metabolic
Viral (adenovirus; cytomegalovirus; coxsackievirus; Epstein-Barr; echovirus; enterovirus; hepatitis A, B, or C; herpes simplex; human immunodeficiency virus; parvovirus; reovirus; rubella)	A_1AT deficiency
Bacterial (urinary tract infection, sepsis, listeriosis, tuberculosis)	Alagille syndrome
Spirochete (syphilis, leptospirosis)	Aagenaes syndrome
Parasites (toxoplasmosis, malaria, toxocariasis)	Arthrogryposis, renal dysfunction, and cholestasis syndrome
Histoplasmosis	Bile acid synthetic defects
	Cholestasis of North American Indians
	Cholesterol synthesis defects
	Citrin deficiency
	Cystic fibrosis
	Dubin-Johnson syndrome
	Fatty acid oxidation defects (SCAD, LCAD)
	Galactosemia
	Glycogen storage disease type 4
	GRACILE syndrome
	Hereditary fructose intolerance
	Indian childhood cirrhosis
	Mitochondrial respiratory chain disorders
	Neonatal iron storage disease
	Niemann-Pick disease type C
	Peroxisomal disorders (including Zellweger syndrome)
	PFIC 1, 2, and 3 (FIC1, BSEP, or MDR3 deficiency)
	Rotor syndrome
	Lipid storage diseases (eg, Wolman, Gaucher, Farber)
	Trisomy 13, 18, or 21; Turner syndrome
	Tyrosinemia
	Urea cycle defects, arginase deficiency
Endocrine	**Toxins**
Hypothyroidism	Drugs (ceftriaxone, chloral hydrate, erythromycin, ethanol, isoniazid, methotrexate, rifampin, sulfa-containing products, tetracycline)
Hypopituitarism (septo-optic dysplasia)	Total parenteral nutrition–associated cholestasis
McCune-Albright syndrome	Herbal products
Anatomic obstruction	**Other**
Biliary atresia	Cardiovascular abnormalities
Caroli disease	• Ischemia-reperfusion injury
Choledochal cyst or other congenital bile duct anomaly	• Perinatal asphyxia
Congenital hepatic fibrosis	• Extracorporeal membrane oxygenation
Gallstones or biliary sludge	• Budd-Chiari syndrome
Inspissated bile syndrome	• Veno-occlusive disease
Neonatal sclerosing cholangitis	Graft-versus-host disease
Nonsyndromic bile duct paucity	Hemophagocytic lymphohistiocytosis
Spontaneous perforation of the bile duct	Idiopathic neonatal hepatitis
Tumor/mass	Neonatal lupus erythematosus
	Malignancy (neonatal leukemia)

A_1AT=alpha$_1$-antitrypsin, LCAD=long chain acyl-CoA dehydrogenase, PFIC=progressive familial intrahepatic cholestasis, SCAD=short-chain acyl-CoA dehydrogenase, TPGS=D-α-tocopheryl polyethylene glycol 1,000 succinate.

cyst. A cardiac murmur increases the likelihood of Alagille syndrome or BA. Although 20% of BA patients will have other extrahepatic congenital malformations (including cardiac anomalies, situs inversus, intestinal malrotation, midline liver, and polysplenia or asplenia), the majority of patients who have BA are well appearing during the first month after birth, and there is no single historical or physical examination finding that uniquely suggests BA.

Evaluation of Neonatal Cholestasis

Evaluation of a jaundiced infant should begin with fractionation of serum bilirubin into total and direct (or conjugated) bilirubin. Infants who have cholestasis will generally have a direct (or conjugated) bilirubin greater than 2.0 mg/dL, which will be more than 20% of the total bilirubin concentration. Recent data suggest that in the first 4 days after birth, the cutoff for elevated direct bilirubin may be greater than 0.8 mg/dL and more than 8% to 10% of the total bilirubin. (10) Another recent study suggested that in the first 14 days after birth, the cutoff for elevated conjugated bilirubin may be greater than 0.5 mg/dL, and for direct bilirubin greater than 2 mg/dL. (11) Clearly, further careful study is needed to determine the normal distribution of direct and conjugated bilirubin levels and their percentage of total bilirubin, and to establish abnormal cutoffs based on day of age.

If cholestasis is present, further evaluation should be completed with a sense of urgency because patients who have BA have a better outcome if they undergo a Kasai hepatic portoenterostomy (HPE) before age 30 to 45 days, and other conditions (eg, hypothyroidism) require prompt treatment. Levels of liver enzymes, including alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, are usually elevated in a cholestatic infant but are poor predictors of etiology. γ-Glutamyl transpeptidase (GGT) is generally elevated during cholestasis (particularly in extrahepatic obstructive lesions and those involving intrahepatic bile ducts); however, a low or normal GGT out of proportion to the degree of cholestasis suggests the presence of progressive familial intrahepatic cholestasis (PFIC) type 1, PFIC type 2, an inborn error of bile acid synthesis or metabolism, or panhypopituitarism. GGT may be normal or elevated in PNAC. Baseline albumin, glucose, and prothrombin time/international normalized ratios are useful in assessing the degree of liver synthetic dysfunction. Severe coagulopathy that is unresponsive to parenteral vitamin K suggests synthetic liver failure, metabolic disease, or sepsis. A low serum albumin level may indicate liver synthetic failure, undernutrition, or protein loss from the kidney or intestine.

Depending on the clinical scenario, bacterial cultures from blood and urine may be indicated. The search for congenital viral infection may include a combination of cultures and serologies; immunoglobulin G–based serologies indicate transplacental transport of maternal immunoglobulin G rather than neonatal infection. The newborn screen can be helpful in identifying galactosemia and hypothyroidism, two treatable causes of cholestasis. An elevated immunoreactive trypsinogen on the newborn screen raises suspicion for cystic fibrosis and should be followed up with genetic testing and/or a sweat test to determine if the infant has cystic fibrosis. A low serum A_1AT level and an abnormal protease inhibitor phenotype (PIZZ and PISZ) are used to identify A_1AT deficiency. Other tests that are commonly used to establish a specific diagnosis include urinary-reducing substances or red blood cell galactose-1-phosphate uridyl transferase drawn before any blood transfusions (for galactosemia), urine succinylacetone (for hereditary tyrosinemia), sweat test (for cystic fibrosis), thyroid-stimulating hormone and thyroxine (for hypothyroidism), total serum bile acid level and urine bile acid profile (for disorders of bile acid synthesis), serum amino acids and urine organic and amino acids (for citrin deficiency, fatty acid oxidation defects, and other metabolic diseases), very long chain fatty acid levels (for peroxisomal disorders), and other infectious agent serologies as indicated. Genetic testing for Alagille syndrome, cystic fibrosis, A_1AT deficiency, three distinct forms of PFIC, and peroxisomal defects are commercially available. In the near future, next-generation DNA sequencing will allow for multiple genetic tests on small amounts of blood at a relatively low cost. (12)

An abdominal ultrasound examination should be obtained as part of the early evaluation of a cholestatic infant to assess liver structure, size, and composition; to evaluate for the presence of ascites; and to identify findings of an extrahepatic obstructive lesion (choledochal cyst, mass, gallstone, and sludge). Ultrasound findings suggestive of BA include a triangular cord sign (cone-shaped fibrotic mass cranial to the bifurcation of the portal vein) or absence of the gallbladder; however, these findings cannot be reliably used to diagnose BA as they are neither highly sensitive nor specific. (13)(14) Ultrasound can also detect polysplenia or asplenia, interrupted inferior vena cava, preduodenal portal vein, and situs inversus; all of these conditions would strongly suggest BA splenic malformation syndrome and other laterality defects. Common bile duct dilation is not seen in BA and suggests a distal obstruction or a forme fruste choledochal cyst.

If a cardiac murmur is appreciated on physical examination, an echocardiogram should be obtained to assess for cardiac anomalies. Up to 24% of patients who have Alagille syndrome and a subset of BA patients will have structural heart disease. A chest radiograph may reveal cardiomegaly or butterfly vertebrae in patients who have Alagille syndrome. A careful slit-lamp examination may reveal posterior embryotoxon or other anterior chamber abnormalities in an infant who has Alagille syndrome or chorioretinitis in an infant who has a congenital infection.

Hepatobiliary scintigraphy with a technetium-labeled iminodiacetic acid analogue can sometimes be of assistance in distinguishing obstructive from nonobstructive causes of cholestasis. In a healthy infant, injected radioisotype is taken up by the hepatocytes, secreted into the biliary system, and then excreted into the small intestine within 24 hours. Slow uptake of the injected radioisotope or nonvisualization of the liver with persistence of the cardiac pool suggests hepatocellular dysfunction, whereas nonvisualization of the radioisotope in the small intestine at 4 to 24 hours suggests either bile duct obstruction or the severe inability of the hepatocyte to secrete. The sensitivity of scintigraphy for BA is relatively high (83%–100%); however, its specificity is low (33%–80%), (15)(16) limiting its use to differentiate BA from other nonsurgical conditions. Pretreatment with phenobarbital may increase test sensitivity. Many centers do not routinely use this test in the evaluation of cholestatic infants because it may delay the diagnostic evaluation without providing definitive diagnostic information. At this time, endoscopic retrograde cholangiopancreatography and magnetic resonance cholangiopancreatography are of limited usefulness for the evaluation of neonatal cholestasis.

Percutaneous liver biopsy remains an important diagnostic tool in evaluating neonatal cholestasis and can be performed safely in even the smallest infants. In several single-center studies, a diagnosis of BA was correctly suggested by liver biopsy histologic findings in 90% to 95% of cases. (17) A more recent study suggests a somewhat lower predictive value of liver biopsy findings when examined in a multicenter research network. (18) Characteristic histologic findings of BA include bile plugs in the portal tract bile duct, bile ductular proliferation, and portal tract edema and fibrosis. Results of a liver biopsy can be helpful in establishing other causes of neonatal cholestasis, including A_1AT deficiency (periodic acid Schiff-positive, diastase-resistant intrahepatocytic globules), Alagille syndrome (bile duct paucity), neonatal sclerosing cholangitis (necroinflammatory duct lesions), viral infection (cytomegalovirus or herpes simplex virus inclusions), metabolic liver diseases (steatosis and pseudoacinar formation of hepatocytes), PFIC and storage diseases (electron microscopy findings), and INH (multinuclear giant cells, extramedullary hematopoiesis, and hepatocellular cholestasis). Liver histologic findings in PNAC may resemble all the features of BA and are not useful in differentiating between the two conditions. Repeat liver biopsies may occasionally be needed if the diagnosis is unclear; several of these diseases are dynamic and may not be diagnosable by using results of liver biopsy if performed early in the disease course.

In cases in which BA, choledochal cyst, or biliary tract stone disease is suspected, the infant should undergo intraoperative cholangiography through a mini-laparotomy to delineate the biliary anatomy and localize the area of obstruction. The surgeon should be prepared and capable of performing an HPE for BA or choledochal cyst–corrective surgery during the same surgical session if these lesions are found on cholangiography. The decision to pursue cholangiography in infants who have SBS with suspected PNAC but who develop acholic stools may be difficult and requires careful consideration of the surgical options if BA is found.

Specific Disorders Resulting in Neonatal Cholestasis

Biliary Atresia

BA occurs in 1 in 6,000 to 18,000 live births and is an idiopathic fibrosing cholangiopathy of unknown etiology that leads to complete obstruction of the extrahepatic bile duct during the first few months after birth, progressive biliary cirrhosis, and eventual death if left untreated. It is more common in Asians and African Americans, with a slight female predominance. BA is the leading cause of neonatal cholestasis and the most common reason for pediatric liver transplantation, accounting for 40% to 50% of children who undergo transplantation. The majority of children who have BA appear to be healthy thriving infants who develop or have persisting jaundice and acholic stools at approximately age 3 to 6 weeks. Up to 20% of infants who have BA have congenital malformations, including the BA splenic malformation syndrome (~8%) or other isolated major congenital malformations, the so-called fetal/embryonic form. These infants may appear jaundiced at birth and remain so. The remaining 80% of infants who have BA have isolated atresia without other congenital malformations and are labeled as having the perinatal or so-called acquired form.

At the time of diagnosis, an HPE procedure is performed during which a Roux-en-Y loop of intestine is anastomosed to a carefully dissected hilum of the liver to create a conduit for biliary drainage. The rate of success

in re-establishing bile flow is dependent on the age of the infant when the HPE is performed. There is up to an 80% success rate if the surgery takes place at less than age 30 to 45 days; however, fewer than 20% of patients who undergo HPE at older than 90 days achieve bile drainage. (2)(19)(20) If jaundice successfully clears after HPE, the 10-year transplant-free survival rate ranges from 75% to 90%; conversely, if jaundice (serum total bilirubin higher than 1.5–2.0 mg/dL) persists after HPE, the 3-year transplant-free survival rate is 20%. Eventually, the vast majority of patients who have BA have progressive disease, with at least 80% requiring liver transplantation by age 20 years. (21) Of those who survive into the third decade after birth, almost all have portal hypertension or other complications of cirrhosis.

α_1-Antitrypsin Deficiency

A_1AT deficiency is an autosomal recessive disorder, most common in those of Northern European descent and extremely unusual in Asians. It is the most common inherited cause of neonatal cholestasis, affecting approximately 1 in 2,000 live births. Affected individuals have a misfolded A_1AT protein that fails to be secreted normally by the hepatocyte, leading to decreased A_1AT activity in the blood and lungs and excess retention in hepatocytes. The circulating deficiency of A_1AT leads to a failure to neutralize neutrophil elastase in the lungs and premature emphysema in young adults. Forty percent to 50% of infants who have the PIZZ phenotype may have asymptomatic abnormal liver biochemical test results in the first year after birth, and 10% to 15% will develop neonatal cholestasis. However, less than 25% of those presenting with cholestasis will progress to end-stage liver disease during childhood. (22) Eight percent to 15% of patients will develop clinically significant liver disease during their lifetime. There is no specific treatment for A_1AT deficiency. Children who develop cirrhosis and liver failure may require liver transplantation.

Alagille Syndrome

Alagille syndrome is an autosomal dominant multisystem disorder characterized by a paucity of intralobular bile ducts and occurring in approximately 1 in 70,000 live births. Almost all patients have a mutation in the *JAGGED* 1 gene that encodes a ligand in the Notch signaling pathway. Patients who have Alagille syndrome have a combination of neonatal cholestasis and bile duct paucity, congenital heart disease (with peripheral pulmonary artery stenosis being the most common lesion), dysmorphic facies (triangular face, broad forehead and deep-set eyes, small pointed chin, and bulbous nose), butterfly vertebrae, ocular posterior embryotoxon, renal anomalies, vascular abnormalities (including intracranial lesions in up to 12% of patients), and short stature. (9)(22) The outcome of Alagille syndrome is largely dependent on the individual's particular clinical manifestations, especially the severity of the cardiac and renal lesions. For those presenting with cholestatic liver disease in infancy, 20% to 50% will require liver transplantation or succumb to cardiac or renal disease by age 20 years. (24)

Parenteral Nutrition–Associated Cholestasis

Overall, 18% to 67% of infants who receive prolonged courses of PN (longer than 14 days) develop liver injury and cholestasis. (25) The incidence of PNAC is correlated inversely with birthweight and directly with duration of PN therapy. (26) In a study of more than 1,300 infants, the incidence of PNAC increased from 14% in infants who received PN for 14 to 28 days to 86% in those infants who received PN for more than 100 days. Infants who have sepsis, bacterial overgrowth of the small intestine, and intestinal failure (secondary to necrotizing enterocolitis, gastroschisis, or intestinal atresia) are at increased risk for developing PNAC. (26)(27) The presence of cholestasis is the leading predictor of mortality in infants who have short bowel syndrome. (28)

The pathogenesis of PNAC is thought to be multifactorial. The soybean-based lipid emulsion component of PN has been implicated as a potential causative factor in PNAC. However, the lipid emulsion component of PN cannot be completely removed because it provides an energy-dense source of calories and essential fatty acids. There is evidence that restriction of the intravenous fat emulsion to 1 g/kg two to three times per week can reduce total bilirubin without causing growth failure or severe essential fatty acid deficiency. (29) Therapeutic lipid restriction (to 1–1.5 g/kg per day) is currently recommended for infants who have developed PNAC. (30) Omegaven® (Fresenius, Homburg, Germany), an investigational product in the United States, is a fish oil–based lipid emulsion composed of omega-3 fatty acids instead of omega-6 fatty acids, and is devoid of plant sterols. It has been used as a substitute for the standard soybean-based lipid emulsions, although only at doses of 1.5 g/kg per day. Several case series have reported that Omegaven seems to be safe and effective in reversing PNAC compared with historical controls receiving soy lipid–based lipid emulsions. A prospective clinical trial comparing Omegaven with a standard soybean oil lipid emulsion is underway. (31) Whether Omegaven will reverse the fibrotic component of PNAC and provide long-term benefit is not known. (32) At this time, Omegaven

is restricted to only compassionate use in the United States. Recently, a combination of soybean, medium-chain triglycerides, olive oil, and fish oil lipid emulsions (Fresenius) has been tested in infants who have PNAC. It has shown promising effects on decreasing bilirubin without causing essential fatty acid deficiency; however, further investigation of the effects of this combination in infants who have intestinal failure is needed. (30)(33)

Infants receiving PN should be started on enteral feedings as early as possible to stimulate bile flow, gallbladder contraction, and intestinal motility. Even trophic feeds have been shown to be beneficial in reducing the incidence and severity of PNAC. For patients who have PNAC who continue on PN, the manganese and copper in PN solutions should be reduced and plasma levels monitored because these metals can accumulate to toxic levels in the cholestatic liver. Fat-soluble vitamin levels should be closely monitored and total PN solutions adjusted accordingly. Ursodeoxycholic acid is theoretically of benefit by stimulating bile flow; however, there is no evidence of its efficacy in PNAC. High-dose oral erythromycin resulted in lower serum direct bilirubin in one large trial in preterm infants receiving total PN. (34)

Galactosemia

Galactosemia is an autosomal recessive disorder that occurs in 1 in 50,000 live births. A deficiency of the enzyme galactose-1-phosphate uridyl transferase results in defective metabolism of galactose. Newborn screening for galactosemia is performed in most countries, thus identifying the majority of infants before they become symptomatic. However, infants who have galactosemia may present with failure to thrive, vomiting, diarrhea, cataracts, *Escherichia coli* septicemia, jaundice and cholestasis, hepatomegaly, ascites, or hypoglycemia. Treatment of galactosemia involves dietary avoidance of all foods that contain galactose and lactose.

Progressive Familial Intrahepatic Cholestasis

PFIC is a group of at least three autosomal recessive hereditary disorders in which mutations in one of the genes involved in canalicular bile formation results in progressive cholestasis of hepatocellular origin. In PFIC type 1 (FIC1 [an aminophospholipid flippase]; Byler disease) and PFIC type 2 (BSEP [the ATP-dependent bile acid transporter] deficiency), patients typically have low or normal GGT levels and low cholesterol levels and develop early cholestasis. Patients who have PFIC 1 may also have severe diarrhea, pancreatitis, and hearing loss. Severe pruritus develops before age 1 year. Many of these patients respond to partial biliary diversion or ileal exclusion surgery. (35)(36) Unresponsive patients may require liver transplantation in the first decade after birth. Patients who have PFIC type 3 (MDR3 [canalicular phospholipid transporter] deficiency) have cholestasis with elevated GGT and low biliary phospholipids, bile duct inflammation, and proliferation on liver biopsy, and they develop biliary cirrhosis rather quickly during childhood. Pruritus is less severe than in the other forms of PFIC and is often responsive to ursodeoxycholic acid.

Treatment of Neonatal Cholestasis

It is crucial to rapidly identify infants who have medically treatable forms of cholestasis as well as those causes amenable to surgical intervention (Table 2). The timing of HPE in patients who have BA is critical. In a recent French study of 695 patients who have BA, survival with native liver was best in children who underwent the HPE procedure in the first 30 days after birth. (2)

Table 2. Causes of Cholestasis That Require Specific Medical or Surgical Intervention

Cause of Cholestasis	Intervention
Infection (bacterial or viral)	Antibiotic or antiviral agents
Galactosemia	Galactose-free diet
Tyrosinemia	Low tyrosine/phenylalanine diet, 2-(2-nitro-4-trifluoromethylbenzol)-1,3-cyclohexanedione
Hereditary fructose intolerance	Fructose- and sucrose-free diet
Hypothyroidism	Thyroid hormone replacement
Cystic fibrosis	Pancreatic enzymes, ursodeoxycholic acid
Hypopituitarism	Thyroid, growth hormone, cortisol replacement
Bile acid synthetic defect	Ursodeoxycholic or cholic acid supplementation
Biliary atresia	Hepatoportoenterostomy (Kasai procedure)
Choledochal cyst	Choledochoenterostomy
Spontaneous perforation of the common bile duct	Surgical drainage
Inspissated bile in the common bile duct	Biliary tract irrigation

Nutritional therapy is of utmost importance in cholestatic infants. Growth failure is common secondary to impaired absorption of fats, impaired metabolism of proteins and carbohydrates, and increased metabolic demand. Reduced delivery of bile acids to the small intestine leads to decreased mixed micelle formation and subsequent fat and fat-soluble vitamin malabsorption. Caloric intake should be approximately 125% of the recommended dietary allowance based on ideal body weight. Adequate protein intake of 2 to 3 g/kg per day should be delivered. Cholestatic infants should receive a formula containing medium-chain triglycerides, such as Pregestimil® (Mead Johnson & Company, Evansville, IN) or Alimentum® (Abbot Laboratories, Chicago, IL), because these triglycerides can be directly absorbed from the small intestine without requiring bile acids. Formulas can be concentrated or have additional carbohydrates or fats added to increase energy density. Oral feeding is the preferred route of formula intake; however, if patients are unable to ingest the needed calories, nasogastric tube drip feedings should be initiated, generally overnight. Fat-soluble vitamins should be supplemented in all cholestatic infants, and blood levels should be routinely monitored to guide dosing. No single multiple vitamin preparation is adequate for all cholestatic infants; most will need additional vitamins K and E, and many will need vitamins D and A beyond a multiple vitamin preparation (Table 3). Vitamin supplementation should be continued for at least 3 months after resolution of jaundice and blood levels checked once an infant has stopped taking the vitamins.

Screening and Prevention

BA is the most common cause of neonatal cholestasis and progresses to end-stage liver disease in up to 80% of patients within the first two decades after birth. Early identification and HPE are essential to establish bile flow and avoid liver transplantation within the first 2 years. (2) A loss of stool pigmentation (acholic stools) may be one of the earliest clinical indicators of BA and is not confounded by breastfeeding, as is relying solely on the presence of jaundice. Lai et al (37) found that 95% of infants who have BA had acholic stool in early infancy. In Taiwan, a national stool color screening system was implemented in 2004 through which an infant stool color card was placed into the child health booklet given to the mother of every newborn. (38) Mothers were to notify a care provider if the infant had an acholic stool before age 1 month and brought the stool color card into the 1-month health supervision visit to show the provider the color of the stools. This program reduced the average age at diagnosis of BA, increased the national rate of the HPE operation performed before age 60 days, increased the 3-month jaundice-free rate after HPE, and increased the 5-year overall survival rate. This program is being initiated in a number of countries; however, its success is not assured in the US health-care system in which there is no standard 1-month infant health provider visit. Pilot testing of a stool color card program would be of great interest and potential public health benefit.

In a recent study by Harpavat et al, (10) direct bilirubin and conjugated bilirubin levels that were obtained within the first 72 hours after birth were retrospectively reviewed from 34 infants who had BA and a number of controls. All direct or conjugated bilirubin levels in the BA infants exceeded laboratory norms and were significantly higher than those of the control subjects ($P < .0001$). However, total bilirubin remained below the American Academy of Pediatrics' recommended

Table 3. Fat-Soluble Vitamin Supplementation in the Cholestatic Infant

Vitamin	Laboratory Sign of Deficiency	Clinical Sign of Deficiency	Treatment/Prevention
Vitamin A	Retinol: retinol-binding protein <0.8 mol/mol	Xerophthalmia, keratomalacia	Vitamin A: 3,000–10,000 U/d
Vitamin D	25-Hydroxyvitamin D <14 ng/mL = deficiency; <30 ng/mL = insufficiency	Rickets, osteomalacia	Cholecalciferol: 800–5,000 IU/d; 1,25 OH_2 cholecalciferol: 0.05–0.2 μg/kg per d
Vitamin K	Prolonged prothrombin time, elevated protein in vitamin K absence	Coagulopathy	Phytonadione: 2.5–5 mg twice a week to every day
Vitamin E	Vitamin E: total serum lipid ratio <0.6 mg/g	Neurologic changes, hemolysis	TPGS: 15–25 U/kg per d; D-α tocopherol: up to 100 U/kg per d

TPGS=D-α-tocopheryl polyethylene glycol 1,000 succinate.

phototherapy levels, (39)(40) and the ratio of direct bilirubin:total bilirubin was less than 0.2, the current level at which the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition recommends further evaluation. (12) Additional studies will be needed to confirm these findings; however, this study suggests that if all newborns were to be screened for elevated direct bilirubin levels in the first 96 hours after birth regardless of clinical appearance, that it might be possible to identify those who have BA and cholestasis at a young age, potentially improving the outcomes for BA and possibly other conditions. Of course, a cost-effectiveness analysis would need to be conducted to determine the rate of false-positive findings and the costs of such a recommendation for essentially universal screening of total and direct or conjugated bilirubin levels before a newborn is discharged from the hospital. Currently, the American Academy of Pediatrics does recommend obtaining a total serum bilirubin or transcutaneous bilirubin level in all newborns before discharge from the hospital.

Conclusions

Cholestatic jaundice, defined as conjugated hyperbilirubinemia, must be considered in any infant presenting with prolonged jaundice longer than 2 weeks (or with hepatomegaly, failure to thrive, acholic stools, or dark urine before or after age 2 weeks) because it can be the first sign of liver disease. Early detection of cholestasis and subsequent prompt diagnostic evaluation by a pediatric hepatologist is essential to successful treatment and optimal prognosis. Delayed diagnosis of neonatal cholestasis (and particularly of BA) remains a problem. Further investigation and development of evidence will be necessary to determine if a reliable and cost-effective method of universal screening for neonatal cholestasis should be implemented in the United States.

American Board of Pediatrics Neonatal–Perinatal Content Specifications

- Recognize the association of cholestasis with total parenteral nutrition, know how to manage this, and understand how to diagnose other possible causes.
- Know the clinical manifestations, diagnostic features, and treatment of infants who have choledochal cysts.
- Know the pathogenesis and clinical manifestations of extrahepatic biliary atresia.
- Know the clinical, laboratory, and diagnostic features of extrahepatic biliary atresia that differentiate it from neonatal hepatitis and other causes of cholestasis in the neonate and know the approach to management of extrahepatic biliary atresia.
- Know the etiology, clinical manifestations, and differential diagnosis of metabolic and familial causes of cholestasis in the neonate.
- Know the laboratory and imaging features and management of metabolic and familial causes of cholestasis in the neonate.

FUNDING: This research was supported in part by National Institutes of Health grants T32 DK067009, U01DK062453, and UL1TR000154.

References

1. Kelly DA, Stanton A. Jaundice in babies: implications for community screening for biliary atresia. *BMJ*. 1995;310(6988):1172–1173
2. Serinet MO, Wildhaber BE, Broué P, et al. Impact of age at Kasai operation on its results in late childhood and adolescence: a rational basis for biliary atresia screening. *Pediatrics*. 2009;123(5):1280–1286
3. Hussein M, Howard ER, Mieli-Vergani G, Mowat AP. Jaundice at 14 days of age: exclude biliary atresia. *Arch Dis Child*. 1991;66(10):1177–1179
4. Mieli-Vergani G, Howard ER, Portman B, Mowat AP. Late referral for biliary atresia—missed opportunities for effective surgery. *Lancet*. 1989;1(8635):421–423
5. Lee WS. Pre-admission consultation and late referral in infants with neonatal cholestasis. *J Paediatr Child Health*. 2008;44(1–2):57–61
6. Balistreri WF. Neonatal cholestasis. *J Pediatr*. 1985;106(2):171–184
7. Suchy FJ. Neonatal cholestasis. *Pediatr Rev*. 2004;25(11):388–396
8. Balistreri WF, Bezerra JA. Whatever happened to "neonatal hepatitis"? *Clin Liver Dis*. 2006;10(1):27–53, v
9. Dick MC, Mowat AP. Hepatitis syndrome in infancy—an epidemiological survey with 10 year follow up. *Arch Dis Child*. 1985;60(6):512–516
10. Harpavat S, Finegold MJ, Karpen SJ. Patients with biliary atresia have elevated direct/conjugated bilirubin levels shortly after birth. *Pediatrics*. 2011;128(6):e1428–e1433
11. Davis AR, Rosenthal P, Escobar GJ, Newman TB. Interpreting conjugated bilirubin levels in newborns. *J Pediatr*. 2011;158(4):562.e1–565.e1
12. Bamshad MJ et al. Exome sequencing as a tool for Mendelian disease gene discovery. *Genetics*. 2011;12(11):745–755
13. Moyer V, Freese DK, Whitington PF, et al; North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. Guideline for the evaluation of cholestatic jaundice in infants: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr*. 2004;39(2):115–128
14. Nievelstein RA, Robben SG, Blickman JG. Hepatobiliary and pancreatic imaging in children—techniques and an overview of non-neoplastic disease entities. *Pediatr Radiol*. 2011;41(1):55–75
15. Gilmour SM, Hershkop M, Reifen R, Gilday D, Roberts EA. Outcome of hepatobiliary scanning in neonatal hepatitis syndrome. *J Nucl Med*. 1997;38(8):1279–1282
16. Esmaili J, Izadyar S, Karegar I, Gholamrezanezhad A. Biliary atresia in infants with prolonged cholestatic jaundice: diagnostic accuracy of hepatobiliary scintigraphy. *Abdom Imaging*. 2007;32(2):243–247

17. Zerbini MC, Gallucci SD, Maezono R, et al. Liver biopsy in neonatal cholestasis: a review on statistical grounds. *Mod Pathol*. 1997;10(8):793–799
18. Russo P, Magee JC, Boitnott J, et al. Design and validation of the biliary atresia research consortium histologic assessment system for cholestasis in infancy. *Clin Gastroenterol Hepatol*. 2011;9(4): 357.e2–362.e2
19. Schreiber RA, Barker CC, Roberts EA, et al. Biliary atresia: the Canadian experience. *J Pediatr*. 2007;151(6):659–665, 665.e1
20. Nio M, Ohi R, Miyano T, Saeki M, Shiraki K, Tanaka K; Japanese Biliary Atresia Registry. Five- and 10-year survival rates after surgery for biliary atresia: a report from the Japanese Biliary Atresia Registry. *J Pediatr Surg*. 2003;38(7):997–1000
21. Lykavieris P, Chardot C, Sokhn M, Gauthier F, Valayer J, Bernard O. Outcome in adulthood of biliary atresia: a study of 63 patients who survived for over 20 years with their native liver. *Hepatology*. 2005;41(2):366–371
22. Sveger T. Liver disease in alpha1-antitrypsin deficiency detected by screening of 200,000 infants. *N Engl J Med*. 1976;294 (24):1316–1321
23. Emerick KM, Rand EB, Goldmuntz E, Krantz ID, Spinner NB, Piccoli DA. Features of Alagille syndrome in 92 patients: frequency and relation to prognosis. *Hepatology*. 1999;29(3):822–829
24. Hoffenberg EJ, Narkewicz MR, Sondheimer JM, Smith DJ, Silverman A, Sokol RJ. Outcome of syndromic paucity of interlobular bile ducts (Alagille syndrome) with onset of cholestasis in infancy. *J Pediatr*. 1995;127(2):220–224
25. Javid PJ, Malone FR, Dick AA, et al. A contemporary analysis of parenteral nutrition-associated liver disease in surgical infants. *J Pediatr Surg*. 2011;46(10):1913–1917
26. Christensen RD, Henry E, Wiedmeier SE, Burnett J, Lambert DK. Identifying patients, on the first day of life, at high-risk of developing parenteral nutrition-associated liver disease. *J Perinatol*. 2007;27(5):284–290
27. Hoang V, Sills J, Chandler M, Busalani E, Clifton-Koeppel R, Modanlou HD. Percutaneously inserted central catheter for total parenteral nutrition in neonates: complications rates related to upper versus lower extremity insertion. *Pediatrics*. 2008;121(5):e1152–e1159
28. Spencer AU, Neaga A, West B, et al. Pediatric short bowel syndrome: redefining predictors of success. *Ann Surg*. 2005;242 (3):403–409, discussion 409–412
29. Cober MP, Killu G, Brattain A, Welch KB, Kunisaki SM, Teitelbaum DH. Intravenous fat emulsions reduction for patients with parenteral nutrition-associated liver disease. *J Pediatr*. 2012; 160(3):421–427
30. Rangel SJ, Calkins CM, Cowles RA, et al; 2011 American Pediatric Surgical Association Outcomes and Clinical Trials Committee. Parenteral nutrition-associated cholestasis: an American Pediatric Surgical Association Outcomes and Clinical Trials Committee systematic review. *J Pediatr Surg*. 2012;47(1):225–240
31. de Meijer VE, Gura KM, Le HD, Meisel JA, Puder M. Fish oil-based lipid emulsions prevent and reverse parenteral nutrition-associated liver disease: the Boston experience. *JPEN J Parenter Enteral Nutr*. 2009;33(5):541–547
32. Soden JS, Lovell MA, Brown K, et al. Failure of resolution of portal fibrosis during omega-3 fatty acid lipid emulsion therapy in two patients with irreversible intestinal failure. *J Pediatr*. 2010;156 (2):327–331
33. Goulet O, Antébi H, Wolf C, et al. A new intravenous fat emulsion containing soybean oil, medium-chain triglycerides, olive oil, and fish oil: a single-center, double-blind randomized study on efficacy and safety in pediatric patients receiving home parenteral nutrition. *JPEN J Parenter Enteral Nutr*. 2010;34(5):485–495
34. Ng PC, Lee CH, Wong SP, et al. High-dose oral erythromycin decreased the incidence of parenteral nutrition-associated cholestasis in preterm infants. *Gastroenterology*. 2007;132(5):1726–1739
35. Kurbegov AC, Setchell KD, Haas JE, et al. Biliary diversion for progressive familial intrahepatic cholestasis: improved liver morphology and bile acid profile. *Gastroenterology*. 2003;125(4):1227–1234
36. Whitington PF, Whitington GL. Partial external diversion of bile for the treatment of intractable pruritus associated with intrahepatic cholestasis. *Gastroenterology*. 1988;95(1):130–136
37. Lai MW, Chang MH, Hsu SC, et al. Differential diagnosis of extrahepatic biliary atresia from neonatal hepatitis: a prospective study. *J Pediatr Gastroenterol Nutr*. 1994;18(2):121–127
38. Lien TH, Chang MH, Wu JF, et al; Taiwan Infant Stool Color Card Study Group. Effects of the infant stool color card screening program on 5-year outcome of biliary atresia in Taiwan. *Hepatology*. 2011;53(1):202–208
39. American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2004;114(1):297–316
40. US Preventive Services Task Force. Screening of infants for hyperbilirubinemia to prevent chronic bilirubin encephalopathy: US Preventive Services Task Force recommendation statement. *Pediatrics*. 2009;124(4):1172–1177

NeoReviews Quiz
New minimum performance level requirements

Per the 2010 revision of the American Medical Association (AMA) Physician's Recognition Award (PRA) and credit system, a minimum performance level must be established on enduring material and journal-based CME activities that are certified for *AMA PRA Category 1 Credit*™. In order to successfully complete 2013 *NeoReviews* articles for *AMA PRA Category 1 Credit*™, learners must demonstrate a minimum performance level of 60% or higher on this assessment, which measures achievement of the educational purpose and/or objectives of this activity.

In *NeoReviews*, *AMA PRA Category 1 Credit*™ can be claimed only if 60% or more of the questions are answered correctly. If you score less than 60% on the assessment, you will be given additional opportunities to answer questions until an overall 60% or greater score is achieved.

1. **A previously well infant presents with jaundice at age 3 weeks. Results of a blood test reveal a direct bilirubin level of 4 mg/dL and a total bilirubin level of 8 mg/dL. Which of the following is most likely to be true?**
   - **A. Because the infant is just 3 weeks old, the first step can be to discontinue human milk and monitor the patient closely for several days to see if the skin color improves.**

B. Direct hyperbilirubinemia at this age may be due to infectious, metabolic, or anatomic abnormalities, and depending on the clinical context, a comprehensive diagnostic evaluation should be performed expeditiously.
C. In virtually all cases, surgery will be necessary. If the infant is growing well and developmentally normal, surgery should be delayed until 6 months to reduce the risks of anesthesia.
D. Metabolic conditions should be ruled out before diagnostic tests for anatomic conditions, because a well-appearing patient is unlikely to have biliary atresia.
E. The first step should be intensive phototherapy, after which further diagnostic tests can be performed.

2. A 2-month-old male infant born at term is diagnosed with biliary atresia. You are counseling the parents about the condition. Which of the following is true regarding long-term prognosis for biliary atresia?
   A. Although liver transplantation may be necessary for ~50% of patients, it will most likely be in the third or fourth decade after birth.
   B. If the child undergoes hepatic portoenterostomy at this age, he is unlikely to have any long-term complications.
   C. More than 80% of patients who have biliary atresia have other congenital anomalies, which may have more of an impact on survival and disability than liver disease.
   D. The level of jaundice after hepatic portoenterostomy may help to guide the necessity and timing of future liver transplantation.
   E. Watchful waiting is an alternative to surgery because some patients may develop a tolerance for conjugated bilirubin.

3. An 8-week-old female presents with jaundice and acholic stools. On further evaluation, the patient is noted to have peripheral pulmonic stenosis on echocardiogram and posterior embryotoxon on eye examination. Which of the following tests is likely to have a positive result in this patient?
   A. Abnormal sweat chloride test.
   B. Absence of spleen found on abdominal ultrasound.
   C. Abnormal protease inhibitor phenotype.
   D. Mutation in the *JAGGED* 1 gene.
   E. Normal γ-glutamyl transpeptidase levels.

4. A 28-weeks'-gestational-age male is now 3 weeks old. Due to necrotizing enterocolitis, his nutrition has primarily been by parenteral nutrition, which he continues to receive via a peripherally inserted central catheter line. On routine laboratory testing, he is noted to have conjugated hyperbilirubinemia with a direct bilirubin level of 3.4 mg/dL. Which of the following is one of the facets of optimal nutrition therapy for this patient at this time?
   A. Continue full parenteral nutrition and start phenobarbital intravenously daily, and monitor direct bilirubin level twice weekly.
   B. Continue parenteral nutrition but do not give intravenous lipids until the direct bilirubin level decreases to less than 2 mg/dL.
   C. Discontinue parenteral nutrition and give intravenous fluids with only dextrose, sodium chloride, and potassium chloride until full enteral feeds are achieved.
   D. Reduce manganese and copper in the parenteral nutrition solution and monitor levels.
   E. Switch to a soybean oil lipid emulsion.

5. The 28-weeks'-gestational age male who has a history of necrotizing enterocolitis and parenteral nutrition–associated cholestasis is now 2 months old and receiving full enteral feedings but continues to have a direct bilirubin level of 3.4 mg/dL. Which of the following is an appropriate aspect of his current nutrition regimen?
   A. An infant formula with no lipids should be given every other day.
   B. Caloric intake should be increased to ~110% of the typically recommended allowance.
   C. He will require additional vitamins A, D, E, and K to meet nutritional needs.
   D. If the infant receives vitamin supplementation, he should stop supplementation ~1 week after jaundice is resolved.
   E. Vitamins should be avoided until the direct bilirubin level is less than 2 mg/dL.