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Guideline for the Evaluation of Cholestatic Jaundice in Infants: Recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition

Abstract

For the primary care provider, cholestatic jaundice in infancy, defined as jaundice caused by an elevated conjugated bilirubin, is an uncommon but potentially serious problem that indicates hepatobiliary dysfunction. Early detection of cholestatic jaundice by the primary care physician and timely, accurate diagnosis by the pediatric gastroenterologist are important for successful treatment and a favorable prognosis. The Cholestasis Guideline Committee of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition has formulated a clinical practice guideline for the diagnostic evaluation of cholestatic jaundice in the infant. The Cholestasis Guideline Committee, consisting of a primary care pediatrician, a clinical epidemiologist (who also practices primary care pediatrics), and five pediatric gastroenterologists, based its recommendations on a comprehensive and systematic review of the medical literature integrated with expert opinion. Consensus was achieved through the Nominal Group Technique, a structured quantitative method.

The Committee examined the value of diagnostic tests commonly used for the evaluation of cholestatic jaundice and how those interventions can be applied to clinical situations in the infant. The guideline provides recommendations for management by the primary care provider, indications for consultation by a pediatric gastroenterologist, and recommendations for management by the pediatric gastroenterologist.

The Cholestasis Guideline Committee recommends that any infant noted to be jaundiced at 2 weeks of age be evaluated for cholestasis with measurement of total and direct serum bilirubin. However, breast-fed infants who can be reliably monitored and who have an otherwise normal history (no dark urine or light stools) and physical examination may be asked to return at 3 weeks of age and, if jaundice persists, have measurement of total and direct serum bilirubin at that time.

This document represents the official recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition on the evaluation of cholestatic jaundice in infants. The American Academy of Pediatrics has also endorsed these recommendations. These recommendations are a general guideline and are not intended as a substitute for clinical judgment or as a protocol for the care of all patients with this problem.

BACKGROUND

Cholestatic jaundice, characterized by elevation of serum *conjugated* bilirubin, is an uncommon but potentially serious condition that indicates hepatobiliary dysfunction. Early detection of cholestatic jaundice by the primary care provider and timely, accurate diagnosis by the pediatric gastroenterologist are important for successful treatment and a favorable prognosis. In contrast, *physiologic jaundice* and *breast milk jaundice*, common causes of jaundice in the first weeks of life, are caused by an elevation of serum *unconjugated* bilirubin. Both are self-limited maturational disorders observed in many infants in the first weeks of life.

Cholestatic jaundice affects approximately 1 in every 2,500 infants (1,2), and is thus infrequently seen by most providers of medical care to infants. However, distinguishing jaundice caused by cholestasis from noncholestatic conditions is critical because cholestatic jaundice is much more likely to have a serious etiology that needs prompt diagnosis and therapy. The most common causes of cholestatic jaundice in the first months of life are biliary atresia and neonatal hepatitis, which account for most cases. Neonatal hepatitis has referred to a histologic appearance of widespread giant cell transformation. Although giant cell transformation is recognized to be nonspecific and may be associated with infectious, metabolic, and syndromic disorders, this term is used to be consistent with the older literature reviewed for this guideline. Alpha-1 antitrypsin deficiency causes another 5% to 15% of cases (1,2). The remaining cases are caused by a variety of other disorders, including extrahepatic obstruction from common duct gallstone or choledochal cyst; metabolic disorders such as tyrosinemia, galactosemia, and hypothyroidism; inborn errors of bile acid metabolism; Alagille syndrome; infection; and other rare disorders (Table 1).

Infants with cholestatic jaundice caused by bacterial sepsis, galactosemia, hypopituitarism, or gallstone often appear acutely ill. These disorders require early diagnosis and urgent treatment. However, many infants with cholestatic jaundice appear otherwise healthy and grow normally. The benign appearance of such an infant may lull the parents or physician into believing that the jaundice is physiologic or caused by breast-feeding, when in fact it may be caused by biliary atresia. Biliary atresia occurs in 1 in 10,000 to 19,000 infants (3–6) (Elliott EJ, Australian Pediatric Surveillance Unit, Extra-hepatic Biliary Atresia Study Group, personal communication,

TABLE 1. *Most likely causes of cholestasis in the younger-than 2-month-old infant*

Obstructive cholestasis
Biliary atresia
Choledochal cyst
Gallstones or biliary sludge
Alagille syndrome
Inspissated bile
Cystic fibrosis
Neonatal sclerosing cholangitis
Congenital hepatic fibrosis/Caroli's disease
Hepatocellular cholestasis
Idiopathic neonatal hepatitis
Viral infection
Cytomegalovirus
HIV
Bacterial infection
Urinary tract infection
Sepsis
Syphilis
Genetic/metabolic disorders
α1-antitrypsin deficiency
Tyrosinemia
Galactosemia
Hypothyroidism
Progressive familial intrahepatic cholestasis (PFIC)
Cystic fibrosis
Panhypopituitarism
Toxic/secondary
Parenteral nutrition-associated cholestasis

2001). Substantial observational evidence suggests that earlier diagnosis and surgical repair lead to better outcomes for this disorder (7). The Kasai portoenterostomy appears to have the greatest likelihood of re-establishment of bile flow and the longest term survival of the infant's native liver when performed before the age of 45 to 60 days (7–10). Early diagnosis of many of the other conditions that cause cholestasis may also lead to better outcomes because better support of the infant may avoid complications of liver disease (11).

Despite these data showing that early diagnosis is potentially life saving, referral for evaluation of cholestatic jaundice frequently occurs after 45 to 60 days of age (12). In recognition of this, and noting that no evidence-based guideline for its evaluation currently exists, the Cholestasis Guideline Committee was formed by the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) to develop a clinical practice guideline for the diagnostic evaluation of cholestasis in infants.

METHODS

The Cholestasis Guideline Committee consisted of a general pediatrician, a clinical epidemiologist who also practices general pediatrics, and five pediatric gastroenterologists. The team addressed the problem of jaundice caused by conjugated hyperbilirubinemia in the 2- to 8-week-old infant. The guideline is intended to assist primary care pediatricians, neonatologists, family practitioners, nurse practitioners, physician's assistants, pediatric gastroenterologists, and pediatric surgeons with the process of diagnosis of infants with cholestatic jaundice, and is specifically intended to decrease the time from initial presentation to diagnosis because of the importance of early diagnosis and treatment of biliary atresia. The targeted settings include primary care outpatient settings, as well as specialty referral facilities. This guideline is not intended for the care of the ill premature infant in the intensive care setting.

Cholestasis was defined as reduced bile formation or flow resulting in the retention of substances normally excreted into bile. Conjugated hyperbilirubinemia is the most common marker; issues regarding the specifics of bilirubin measurement are detailed below. A desirable outcome was defined as optimal treatment of the underlying conditions. Optimal treatment requires timely diagnosis of the cause of the jaundice, minimizing unnecessary diagnostic testing and consultation, and minimizing delay in diagnosis of time-sensitive conditions, such as biliary atresia.

The guideline development process was intended to incorporate the best available evidence from the medical literature, in combination with expert consensus when available evidence was limited. Using expert opinion, an initial diagnostic algorithm was developed, including a set of potentially useful diagnostic tests for infants with cholestatic jaundice. To develop evidence-based estimates of the sensitivity, specificity, and accuracy of the diagnostic tests under consideration, Medline (1966–2002) was searched without language restriction using the terms listed in Table 2. Unpublished literature was not sought.

Abstracts for articles found in each search were reviewed for relevance. After editorials, letters, and review articles were eliminated, original studies that appeared to address the accuracy and reliability of the tests in question for the diagnosis of biliary atresia were retrieved in full and independently reviewed by two committee members, using previously published criteria for the validity of studies of diagnostic tests (13) (Table 3). The key criteria include an independent, blind comparison of the diagnostic test to a criterion standard, performed in non-selected, consecutive patients at risk for the condition of

TABLE 2. *Search strategies*

Biopsy: biliary atresia/ti,ab,sh and (biopsy or biopsy, needle)
GGT: biliary atresia/ti,ab,sh and gamma glutamyl transferase
Radionuclide scan: biliary atresia/RI
ERCP: biliary atresia/ti,ab,sh and explode cholangiopancreatography, endoscopic retrograde
Lipoprotein X: biliary atresia/ti,ab,sh and lipoprotein X
Ultrasound: biliary atresia/ti,ab,sh and (ultrasound or sonography or ultrasonography)
Duodenal aspirate: biliary atresia/ti,ab,sh and (intestinal secretions/sh or intubation, gastrointestinal)

TABLE 3. *Validity criteria for studies of diagnostic tests*

1. Was the diagnostic standard adequate?
2. Was the test compared independently and blindly to the diagnostic ("criterion") standard?
3. Were the referral pattern and patient population described?
4. Was there an adequate spectrum of disease in tested patients?
5. Was the test clearly described?
6. Was observer variation in test results addressed?

interest. Studies were considered to meet minimum criteria for validity if the test was performed in consecutive patients and did not appear to influence the determination of the final diagnosis. Each article was assigned a score based on these criteria, and the quality of evidence underlying each of the recommendations made by the Cholestasis Guideline Committee was determined according to the scheme shown in Table 4.

The final algorithm (Fig. 1) is based on the evidence found for each test, combined with expert opinion and consensus when the published evidence was insufficient. Recommendations are summarized in Table 5. Consensus was achieved through the nominal group technique (14).

The guidelines were then reviewed by primary care physicians in community and academic practices. In addition, the guidelines were distributed to the NASPGHAN membership for review and comment and were officially endorsed by the NASPGHAN Executive Council. This document represents the official recommendations of NASPHGAN on the evaluation of cholestatic jaundice of the infant. The American Academy of Pediatrics has also endorsed these recommendations. This review and recommendations are a general guideline and are not intended as a substitute for clinical judgment or as a protocol for the care of all patients with this problem.

TABLE 4. *Coding scheme for quality of evidence*

Level of evidence	Criterion for this level
Level A	Recommendation based on 2 or more studies that compared the test to a criterion standard in an independent, blind manner in an unselected population of infants similar to those addressed in the guideline.
Level B	Recommendation based on a single study that compared the test to a criterion standard in an independent, blind manner in an unselected population of infants similar to those addressed in the guideline.
Level C	Recommendation based on lower quality studies or studies for which inadequate information is provided to assess quality, together with expert opinion and consensus of the committee.
Level D	No studies available; recommendation based on expert opinion and consensus of the committee.

Table 5. *Recommendations*

Recommendation	Level of evidence
It is recommended that any infant noted to be jaundiced at 2 weeks of age be clinically evaluated for cholestasis with measurement of total and direct serum bilirubin. However, breast-fed infants who can be reliably monitored and who have an otherwise normal history (no dark urine or light stools) and physical examination may be asked to return at 3 weeks of age and, if jaundice persists, have measurement of total and direct serum bilirubin at that time.	C
Retest any infant with an acute condition or other explanation for jaundice whose jaundice does not resolve with appropriate management of the diagnosed condition.	D
Ultrasound is recommended for infants with cholestasis of unknown etiology.	A
Liver biopsy is recommended for most infants with cholestasis of unknown etiology.	A
GGTP and lipoprotein X are not routinely recommended in the evaluation of cholestasis in young infants.	C
Scintigraphy and duodenal aspirate are not routinely recommended but may be useful in situations in which other tests are not readily available.	A
MRCP and ERCP are not routinely recommended, although ERCP may be useful in experienced hands.	C

INITIAL EVALUATION OF THE JAUNDICED INFANT

The initial recognition and evaluation of any infant with possible cholestasis depends upon physicians and other providers of medical care to infants. The age limits of 2 weeks to 8 weeks of age used in this guideline were selected because they represent times when primary care providers typically examine healthy infants. As the evaluation proceeds, the tests to accurately diagnose cholestasis fall increasingly in the province of the referral center and pediatric gastroenterologist. Different care models or differing relationships between primary physicians and referral centers may alter the point at which the responsibility for the evaluation shifts from primary care to specialist. The goal remains the early detection and efficient diagnostic evaluation of cholestasis in infants.

No screening test can predict which infant will experience cholestasis (15). Thus, the detection of cholestasis rests on the clinical recognition of jaundice, pale stools, and/or dark urine by the parent or primary care provider. Each of these findings is an imperfect method of detecting cholestasis. Jaundice at 2 weeks of age is a relatively common finding, observed in 2.4% to 15% of newborns (16,17). Most such infants have unconjugated hyperbilirubinemia because of breast milk jaundice, a benign condition, although a recent report documents occasional kernicterus in otherwise healthy-appearing infants (18). In one study, jaundice was found in 9% of breast-fed infants at 4 weeks of age, but in fewer than 1 in 1,000

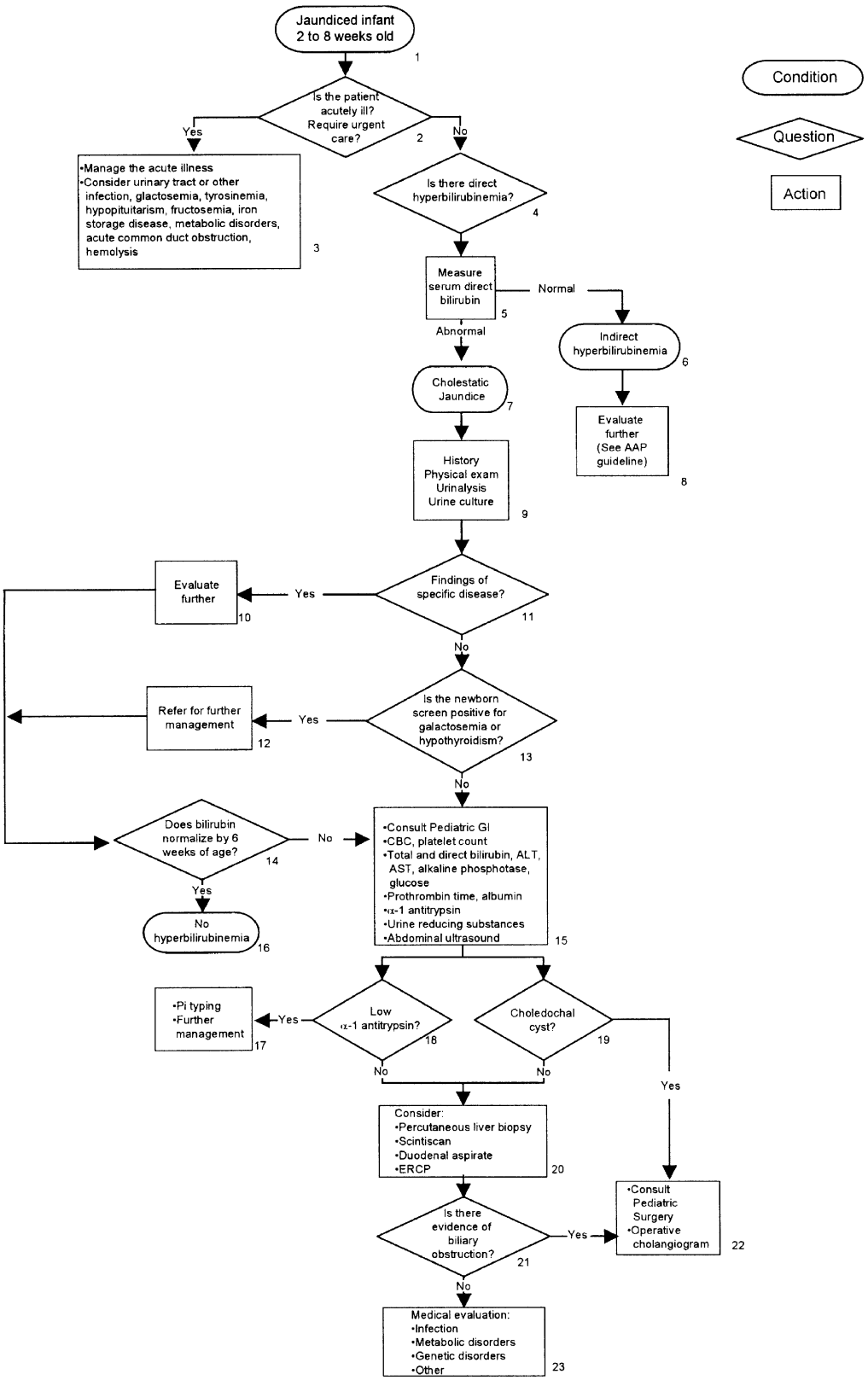

FIG. 1. Cholestasis clinical practice guideline. Algorithm for a 2- to 8-week-old infant. (North American Society for Pediatric Gastroenterology, Hepatology & Nutrition Cholestasis Guideline Committee.)

bottle-fed infants (19). Testing of all jaundiced newborns at the 2-week visit will detect cholestasis in relatively few infants (for every 60 to 375 infants with visible jaundice, 1 will have cholestasis). However, the next scheduled health maintenance visit generally does not occur until 2 months of age, beyond the optimal time for intervention in biliary atresia.

The report of pale stools by the parent or observation of clay colored stool by the physician raises the suspicion of cholestasis. In one study of 3,629 infants, 4 passed pale stools during the first 4 weeks of life, none more than three times. None of these infants had liver disease. The high specificity of persistent pale stools means that this finding almost certainly indicates disease when it is present. However, infants with biliary atresia have been noted to have pigmented stools at presentation, and stool color is variable in other conditions causing cholestasis (20). Parents do not appear to be reliable observers of stool color: even when the specific question "What color is your baby's stool?" was added to a booklet received by all parents for well care, the average age for definitive care for infants with biliary atresia was not improved (4). A scoring card displaying color photographs of normal and abnormal stools modestly improved case finding in a subsequent program (21).

Dark urine is also a nonspecific indicator of increased conjugated bilirubin. No studies could be found that addressed the utility of this sign for case finding. Literature review did not identify any studies that defined specificity or sensitivity for the findings of jaundice, pale stools, or dark urine in combination. Thus, the clinician must continue to test for cholestasis when these findings are present, despite the low yield. The American Academy of Pediatrics practice parameter on the management of hyperbilirubinemia in the healthy term newborn recommends testing for conjugated hyperbilirubinemia if jaundice is accompanied by dark urine or light stool or if it persists beyond 3 weeks (22).

The Cholestasis Guideline Committee recommends that any infant noted to be jaundiced at 2 weeks of age be evaluated for cholestasis with measurement of total and direct serum bilirubin. However, breast-fed infants who can be reliably monitored and who have an otherwise normal history (no dark urine or light stools) and physical examination (Table 6) may be asked to return at 3 weeks of age and, if jaundice persists, have measurement of total and direct serum bilirubin at that time.

Bacterial infections, including urinary tract infection, are a well known cause of concomitant conjugated hyperbilirubinemia, probably because of the effect of bacterial endotoxin on bile formation (23,24). Otherwise asymptomatic jaundiced infants may have urinary tract infection, particularly if the onset of jaundice occurs after 8 days of age (25). Jaundice caused by an acute infection resolves when the acute illness has been addressed. Because jaundice is more likely to be related to the underlying illness, rather than primary liver disease, in the acutely ill infant, management is oriented to the underlying acute illness.

Because either conjugated or unconjugated hyperbilirubinemia may be present in a jaundiced infant who does not appear acutely ill, measurement of serum bilirubin that includes both total and direct (conjugated) bilirubin is recommended. The most commonly used laboratory determination (the diazo or van den Bergh method) does not specifically measure conjugated bilirubin but reports direct bilirubin. For methodological reasons, the higher the total bilirubin (even if it is all unconjugated), the higher the reported direct bilirubin (26–28). Measurements of direct bilirubin may vary significantly both within and between laboratories (29–32). The methods used may also influence the measurement of conjugated bilirubin (33–35). A specific measurement of conjugated bilirubin, such as that obtained with the Ektachem system, is optimal. Because canalicular excretion of bilirubin can be rate limiting to overall clearance, infants with high nonconjugated bilirubin may retain some conjugated bilirubin. Therefore, in the presence of elevated total bilirubin, conjugated bilirubin levels are considered abnormal when values greater than 1.0 mg/dL are reported (36). Practitioners whose laboratories use the Ektachem system could use a value of conjugated bilirubin greater than 1.0 to define cholestasis, regardless of total bilirubin. For this guideline, we defined an abnormal direct bilirubin as a value greater than 1.0 mg/dL if the total bilirubin is less than 5 mg/dL, or a value of direct bilirubin that represents more than 20% of the total bilirubin if the total bilirubin is greater than 5 mg/dL. It is important to be aware of the potential for error in these measurements and consult with the laboratory if the measurements are inconsistent with the appearance of the patient. Urine testing has also been used to diagnose cholestasis: assay of sulfated bile acids in urine was found in a single study to distinguish control infants from those with cholestasis, but this test is not generally available (37).

INITIAL EVALUATION OF THE INFANT WITH CONJUGATED HYPERBILIRUBINEMIA

For infants with only unconjugated hyperbilirubinemia, practitioners can refer to the previously published guideline on management of unconjugated hyperbilirubinemia from the American Academy of Pediatrics (22). If the conjugated serum bilirubin is elevated, cholestasis is present. The further evaluation of an infant with cholestasis is a matter of some urgency. Because biliary atresia is one of the possible causes of cholestasis, the goal of management is to complete the diagnostic evaluation, or at least to exclude biliary atresia, by 45 to 60 days of age. History and physical examination can help guide the diagnostic process for the infant with cholestasis. Some findings on history or physical examination

TABLE 6. *History and physical findings to consider for the differential diagnosis of infants with conjugated hyperbilirubinemia (see Box 9 of Figure 1)*

Topic	Specific question	Implication
History	Similar problem with parents or among siblings	Occurrence in other family members implies autosomal dominant inheritance, and occurrence in sibs implies genetic disease or nongenetic recurrence pattern: e.g., α-1AT deficiency, progressive familial intrahepatic cholestasis (PFIC), Alagille's, cystic fibrosis
	Consanguinity	Risk for autosomal recessive inheritance
	Maternal infection that can affect baby	TORCH infections, occasionally HBV, others
	Cholestasis of pregnancy	May be seen in PFIC
	Fetal ultrasound (yes/no; findings)	Choledochal cyst and bowel anomalies (e.g., duplication cysts) that can cause jaundice
	Past ABO or Rh disease, or Rh negative	Hemolysis
	Birth weight	SGA implies fetal involvement and weighs against biliary atresia
	Neonatal infection, including UTI, sepsis and viral infection	Often associated with conjugated hyperbilirubinemia
	Feeding history and history of weight gain	Neonatal hepatitis can cause FTT; metabolic disease (e.g., galactosemia and hereditary fructose intolerance) can cause anorexia, FTT, and jaundice. Panhypopituitarism
	Bowel history—vomiting, stooling	Vomiting—metabolic disease, pyloric stenosis, bowel obstruction (atresia, annular pancreas); delayed stooling—CF, hypothyroidism; diarrhea—infection, metabolic disease, PFIC1, CF; clay colored stool—biliary obstruction
	Source of nutrition	Breast or formula; composition of formula: galactose containing—galactosemia, fructose or sucrose containing—hereditary fructose intolerance
	Disposition	Irritable: may be associated with some metabolic disorders; "great baby, sleeps all the time, never awake," lethargic—hypothyroidism or panhypopituitarism
	Urine color—preferably directly observed	Dark urine—conjugated hyperbilirubinemia
	Stool color (useful to have reference such as yellow paint strip from paint dealer); preferably directly observed	Pale or clay colored; cholestasis, rule out obstruction
	Excessive bleeding	Coagulopathy; vitamin K deficiency
Physical findings	Vital signs; weight, length, OFC; weight for length	
	Global assessment of general health	
	Global assessment of nutritional status	Acute illness
	HEENT	Dysmorphic findings—Alagille's syndrome, scleral icterus (qualitatively different in cholestasis versus nonconjugated jaundice), fundoscopic (intrauterine infection)
		Slit-lamp findings (Alagille syndrome, cataracts) snuffles
	Chest/heart	Evidence of pneumonia—neonatal infection
		Evidence of heart failure—Congestive hepatopathy
		Murmurs or other evidence of congenital heart disease—biliary atresia, Alagille's syndrome
	Abdomen	Distention
		Ascites
		Abdominal wall vasculature
		Liver size (exactly measured)
		Liver consistency
		Spleen size (below costal margin)
		Spleen consistency
		Masses
		Umbilical hernia
	Diaper exam	Dark urine—conjugated hyperbilirubinemia
		Pale or clay colored stool—cholestasis, rule out obstruction
	Skin—bruising, petechiae, rashes	
	Neurologic—general assessment of vigor, tone, and symmetry	

will lead the clinician to other diagnostic possibilities. For example, 3% of infants with severe ABO hemolytic disease have conjugated hyperbilirubinemia that may persist until 2 weeks of age; thus, further observation may suffice for these infants (38). Table 6 describes abnormalities that may be present on history and physical examination and their potential significance. Abnormalities suggestive of a particular disease entity would lead

to more focused diagnostic evaluation, rather than further investigation of cholestasis. It is important to evaluate or repeat the newborn screen for galactosemia and hypothyroidism because these conditions can present with conjugated hyperbilirubinemia and require urgent management to prevent serious sequelae (39–42). Keeping in mind that the diagnosis of one disease does not preclude the presence of another, additional evaluation is necessary if the jaundice does not resolve with treatment of these specific conditions.

Consultation with a pediatric gastroenterologist is essential for infants with conjugated hyperbilirubinemia of unknown cause. The nature of this consultation may vary among practice settings. A telephone consultation may be particularly useful for practitioners at some distance from specialty care. Of the tests listed in Box 15 of the algorithm (Fig. 1), which should be ordered and where they should be performed is likely to vary, depending on which of the tests are readily available to the referring physician, and on whether examination of the patient by the specialist will be delayed. If the level of serum alpha-1 antitrypsin is found to be low, Pi typing is indicated. If an obvious extrinsic obstruction (such as choledochal cyst) is present, referral for surgery is warranted. Of the tests listed at this decision point, the ultrasound is the most operator dependent, and thus may be most appropriately performed at the referral center by more experienced personnel. In many practice settings, all of the listed tests would be best obtained by immediate referral of the patient for evaluation by the pediatric gastroenterologist. In the evaluation for infants with possible biliary atresia, it is optimal for an experienced pediatric surgeon to also be involved.

Ultrasonography is useful to identify anatomic abnormalities such as choledochal cyst. The finding of a small or absent gallbladder may suggest extrahepatic biliary atresia, but reported sensitivities as low as 73% indicate that ultrasound cannot be used to rule out this diagnosis. Several reports of high sensitivity and specificity of the "triangular cord" sign on ultrasound suggest that this test may be useful in the diagnosis of extrahepatic biliary atresia.(43–45) Again, this appears to be operator dependent. Despite these limitations, we recommend ultrasonography for the evaluation of the infant with cholestasis of unknown etiology.

Gamma glutamyl transpeptidase (GGT) has been used in the past to distinguish biliary atresia from neonatal hepatitis, but wide variability in levels makes interpretation of test results difficult. Especially in the older infant with cholestasis, a very low GGT level may be useful to exclude obstruction and, in conjunction with an elevated alkaline phosphatase level, suggests genetic and metabolic causes of intracellular cholestasis. The degree of elevation of GGT is not useful in discriminating the etiology of the cholestasis. The evidence supporting the use of ultrasound and GGT is summarized in Table 7.

FURTHER EVALUATION OF THE INFANT WITH CHOLESTASIS

Once it is established that cholestasis is present, the principal diagnostic concern is the differentiation of hepatocellular from obstructive cholestasis, of disorders of physiology from disorders of anatomy, and of disease that is managed medically from disease that is managed surgically. The tests that have been used to make this differentiation and about which we were able to find at least some evidence regarding their value were percutaneous liver biopsy, hepatobiliary radionuclide scan, and duodenal aspirate. Evidence and citations for the use of these tests are summarized in Table 7.

Percutaneous Liver Biopsy

Most studies of percutaneous liver biopsy are retrospective analyses using as a gold standard the clinical course or surgical or autopsy results. Biopsy interpretation is pathologist dependent, and requires experience that many general pathologists lack. Review of all of the studies of biopsy revealed that 50% to 99% of patients with biliary atresia are correctly identified with biopsy. Biliary atresia is incorrectly suspected from the biopsy in 0% to 46%.

In 1974, Brough and Bernstein (46) demonstrated the diagnostic usefulness of the percutaneous liver biopsy and established the diagnostic criteria that are in current use. They compared the original pathologic diagnosis in 181 consecutive patients to the ultimate diagnosis based on surgical findings and long-term follow-up. The original diagnosis was correct in 148 patients (93.7%), a high level of accuracy. More importantly, the type of error seen in the 10 patients with incorrect diagnoses would not lead to missed diagnosis of biliary atresia. In only one patient with biliary atresia was the original biopsy interpreted as hepatitis, which would lead to delay in the diagnosis and loss of important time before surgical correction. The remaining nine were suspected of having biliary atresia but actually had hepatitis. In this report, liver biopsy had a very high sensitivity (99%) and specificity (92%) for the diagnosis of biliary atresia, with somewhat less specificity for the diagnosis of neonatal hepatitis. Unfortunately, 23 patients were lost to follow-up, which reduces the value of this study. The highest quality subsequent studies performed using the Brough and Bernstein criteria demonstrate very good sensitivity and specificity for biliary atresia.

The interpretation of a single liver biopsy in a child with neonatal cholestasis is also limited by the dynamics of disease. Many cholestatic conditions express themselves differently with time. Liver biopsy specimens obtained early in the course of biliary atresia may be indistinguishable from hepatitis (47). In addition to being able to visualize the hepatocanalicular cholestasis and injury,

TABLE 7. *Diagnostic tests to distinguish EHBA from other causes of cholestasis (Box 21 of Figure 1)*

Procedure	Quality of evidence	Sensitivity* for obstruction	Specificity* for obstruction	Likelihood ratio* for obstruction	Likelihood ratio* for nonobstructive cholestasis	Time delay	Risk	Comments—other diagnoses, issues relevant to this test
Ultrasound 44–46, 51–71	A	73%–100% for small or absent GB 83%–100% for "triangular cord" sign	67%–100% for small or absent GB 98%–100% for "triangular cord" sign	2.2–infinite 41.5–infinite	2.5–infinite 5.8–infinite	Minimal	Minimal	Procedure is operator dependent; primarily useful to rule out anatomic abnormalities such as choledochal cyst, rather than make a diagnosis of EHBA.
GGTP 47, 56, 72–81	C	78%–86%	67%–100%	2.4–infinite	3.0–7.1	Minimal	Minimal	Variety of cut-off values used, no study with clearly independent comparison; most studies show considerable overlap.
Percutaneous biopsy 47, 56, 62, 69, 74, 82–89	A	89%–99%	82.5%–98%	5.2–49.5	7.5–98	1–3 days	Very low	Paucity of intrahepatic bile ducts, giant cell transformation, metabolic and storage diseases, PFIC, infection, neonatal sclerosing cholangitis can be diagnosed with this test. Most patients will require this test; should be interpreted by a pathologist experienced with pediatric liver disease. Sensitivity may be higher in expert hands.
Radionuclide scanning 53, 54, 56, 62, 65, 69, 74, 82, 86, 89–109	A	83%–100%	33%–100%	1.3–infinite	2–infinite	3–5 days for phenobarbital priming	Minimal	Most studies with sensitivity of 100%, so obstruction extremely unlikely if excretion found. Disadvantage of test is time delay and cost.
Duodenal aspirate 62, 82, 88, 97, 110–114	A	91%–100%	43%–100%	1.6–infinite	4.8–infinite	none	Minimal	Obstruction extremely unlikely if bile found in duodenal fluid. May require fluoroscopy for tube placement; test is invasive.
MRCP 71, 115–119	C	100% 2 studies	60% 1 study	2.5 (imprecise)	Infinite (imprecise)		Minimal	Requires deep sedation or general anesthesia; very few adequate studies.
ERCP 88, 120–127	C	100% 1 study	100% 1 study	Infinite	Infinite	?	Unknown	Requires sophisticated instrumentation and expertise not currently widely available; numbers based on a single study with a single endoscopist.
Lipoprotein X 79, 128–136	Not discussed	30%–88%	81%–100%	1.5–infinite	2.7–8.3		Minimal	Test no longer available, most studies very small with poor description of methods.

Sensitivity, range of values from highest quality studies.
Specificity, range of values from highest quality studies.
LR, the ratio of the likelihood of a test result in patients who have a disease compared with the likelihood of the same test result in those without the disease; can be calculated as sensitivity (1-specificity). See Jaeschke et al[14] for details. In this table, the range is based on the sensitivity and specificity from highest quality studies.

the liver biopsy also can provide disease-specific findings. Examples include PAS-positive granules in alpha-1 antitrypsin deficiency, ductal paucity in Alagille syndrome, necroinflammatory duct lesions in sclerosing cholangitis, and other findings that are relatively specific for metabolic and storage diseases.

The evidence indicates that liver biopsy can be performed safely and expeditiously in young infants and is useful in establishing specific diagnoses (48). The Cholestasis Guideline Committee recommends that a liver biopsy be performed in most infants with undiagnosed cholestasis, to be interpreted by a pathologist with expertise in pediatric liver disease. A percutaneous liver biopsy is recommended before performing a surgical procedure to diagnose biliary atresia. If the biopsy is done early in the course of the disease (before 6 weeks of age), the biopsy may have to be repeated if the results are equivocal.

Scintigraphy

Injected radioactive material is normally excreted into the intestine within a predictable period of time. Nonvisualization of radioactivity within the intestine (in the scanning field comprising the intestines) 24 hours after injection is considered to be an abnormal result, indicating biliary obstruction or hepatocellular dysfunction. In the studies reviewed, a variety of radiolabeled scintigraphic agents were used, the diagnostic criteria varied greatly, and no blinded comparisons were found between scintigraphy and a gold standard for diagnosis. Although it is thought that the precision of the test can be improved by administering phenobarbital for several days before imaging, no studies are available to confirm or refute this hypothesis. In the available studies, the sensitivity of scintigraphy for the diagnosis of biliary atresia is high; virtually all patients with complete biliary obstruction showed no excretion on scintiscan. A few patients with a negative test (tracer evident in the intestine) later experienced biliary atresia, presumably because of disease evolving from incomplete to complete obstruction. Specificity of scintigraphy for biliary atresia or other obstructive processes is low; many patients without anatomic obstruction will not excrete tracer. Although the high sensitivity for biliary atresia makes this a fairly good single test for detecting disease, it is time consuming and expensive and does have significant false-positive and false-negative results. The Cholestasis Guideline Committee concludes that hepatobiliary scintigraphy generally adds little to the routine evaluation of the cholestatic infant but may be of value if other means for excluding biliary obstruction are not available.

Duodenal Aspirate

Limited data indicate that duodenal aspirate analysis for bilirubin concentration can identify patients with biliary obstruction with a sensitivity similar to that of scintiscan. In this test, fluid is obtained from the duodenum, either by placing a tube or a string in the duodenum, and analyzed for bilirubin concentration. A positive test for obstruction is one in which the bilirubin concentration of the aspirate is no greater than serum concentration. This would appear to be a low-tech, inexpensive alternative to scintiscan, yet it is not commonly used, probably because it is time and labor intensive, invasive, and inconvenient. The Cholestasis Guideline Committee concludes that the duodenal aspirate test may be useful in situations in which other tests to detect biliary obstruction are not available.

Magnetic Resonance Cholangiopancreatography

The few reports available to date have studied a very small number of patients, and although the results are encouraging, firm conclusions are not possible. Magnetic resonance cholangiopancreatography (MRCP) requires deep sedation or general anesthesia. Additional technical advancement and clinical experience are necessary before MRCP can be used in the evaluation of cholestatic infants. The Cholestasis Guideline Committee concludes that this test cannot be routinely recommended based on the currently available data.

Endoscopic Retrograde Cholangiopancreatography

Endoscopic retrograde cholangiopancreatography (ERCP) is an evolving technology that has been increasingly used in some tertiary referral centers to diagnose the cause of cholestasis in young infants. ERCP involves endoscopic intubation of the biliary (and pancreatic) ducts via the ampulla of Vater with a small tapered catheter and injection of contrast material to facilitate radiologic visualization of the ductal systems. A small pediatric side-viewing duodenoscope has enhanced the capability to perform this procedure in young infants. Most endoscopists prefer to perform this procedure with the patient under general anesthesia.

The data supporting the use of ERCP for the evaluation of the cholestatic infant are sparse. Many of the reports simply document the use of the prototype smaller side-viewing instrument for these patients. Operators report mixed success with the procedure to distinguish biliary atresia from neonatal hepatitis and other forms of nonoperative cholestasis. Most tertiary centers use ERCP to sort as surgical or nonsurgical cases that remain equivocal after liver biopsy. No controlled studies have been conducted to compare endoscopic techniques. In larger studies, sensitivity and specificity are excellent, but failed cannulation and/or visualization are reported in more than 10%. Patient populations vary from young infants to older infants (before or after 60 to 90 days of age). No controlled study has been done to demonstrate

that ERCP will alter the final diagnosis. A cost-benefit analysis of ERCP has not been performed, but it is possible that the procedure could obviate the need for surgical exploration in some infants. ERCP in pediatric patients has been recommended only at facilities with appropriate support staff and specialists with expertise in this procedure in young infants (49).

The Cholestasis Guideline Committee concludes that ERCP is not frequently used because of the cost of instrumentation and the need for technical expertise. The usefulness of ERCP appears to be center and operator dependent. Clinicians with the appropriate expertise and instrumentation will find the test a useful adjunct in the evaluation of the difficult-to-diagnose cause of cholestasis in an infant in whom laparotomy is being contemplated. The Committee recommends that a liver biopsy be obtained before subjecting an infant to ERCP. Under selected circumstances, ERCP can clarify the cause of neonatal cholestasis and obviate the need for laparotomy. ERCP can be recommended in selected cases at facilities with appropriate support staff and specialists with expertise in this procedure in young infants.

Lipoprotein X

This test is no longer widely available; the few studies on this subject are of poor quality and suggest poor sensitivity and specificity for differentiating biliary atresia from neonatal hepatitis. The Cholestasis Guideline Committee recommends that this test not be performed.

CONCLUSION

The rapid and effective diagnosis of the cause of cholestasis in an infant is challenging. The initial detection of these infants remains in the domain of the primary care provider and depends on the recognition of jaundice past the age of 2 weeks or recognition of abnormal stool or urine color. Laboratory testing for cholestasis should include direct (conjugated) bilirubin. A direct bilirubin value greater than 1.0 mg/dL if the total bilirubin is less than 5 mg/dL or a direct bilirubin more than 20% of the total bilirubin if the total bilirubin is greater than 5 mg/dL is considered abnormal. The relative rarity of cholestatic jaundice in contrast to unconjugated hyperbilirubinemia in this age range dictates that many jaundiced infants will be tested to find a few with elevated direct bilirubin. It is recommended that primary care providers continue to screen for cholestasis, despite its rarity, because of the gravity of the consequences of missed diagnosis.

The Cholestasis Guideline Committee recommends that any infant noted to be jaundiced at 2 weeks of age be evaluated for cholestasis with measurement of total and direct serum bilirubin. However, breast-fed infants who can be reliably monitored and who have an otherwise normal history (no dark urine or light stools) and physical examination may be asked to return at 3 weeks of age and, if jaundice persists, have measurement of total and direct serum bilirubin at that time.

Identification of cholestasis warrants a prompt effort to accurately diagnose the cause. In an ill appearing infant, the primary cause of the acute problem should be sought and treated. Cholestasis that does not resolve with resolution of the acute problem requires thorough evaluation. In a well appearing infant, cholestasis should be evaluated without delay because this condition has etiologies that are both serious and treatable. Patients with extrahepatic biliary atresia often appear well at initial presentation, and in these patients there is evidence that early diagnosis and surgical bile drainage is associated with longer survival of the native liver.

Most of the diagnostic tests that are used to determine the etiology of cholestasis in infants are operator dependent and variable in utility, and most are not commonly performed outside of referral centers. Although specific circumstances may support the performance of diagnostic testing at a referring hospital, most practitioners will need to use the diagnostic expertise of a referral center. Diagnostic workup by referring hospitals may be most useful to quickly exclude some of the many causes of cholestasis.

For all diagnostic tests reviewed, the sensitivity and specificity varied widely. Percutaneous liver biopsy had the greatest diagnostic accuracy in published studies. However, because of the dynamic and progressive nature of extrahepatic biliary atresia, even this test can be misleading. Scintigraphy appears to be primarily useful in excluding extrahepatic obstruction, consistent with the diagnosis of extrahepatic biliary atresia. Sonographic evaluation is currently useful for excluding anatomic abnormalities but may have a greater value in the future if descriptions of new findings (such as the "triangular cord" sign) are corroborated. ERCP may have a role in centers with pediatric gastroenterologists experienced in its use. Too little experience with MRCP has been reported to analyze its utility. Duodenal aspirate or string test may be useful in remote sites or if other testing is not readily available.

In summary, the diagnosis of neonatal cholestasis is an urgent matter. This guideline and algorithm have been developed to assist in this process. No single pathway appears to be clearly superior for the diagnosis of conditions leading to cholestasis. A guide is provided here for clinicians who are challenged with the prospect of detecting and evaluating the rare infant with cholestasis. Vigilance is crucial in detecting these patients, selecting appropriate and timely diagnostic testing based on the resources available in their own institutions, and referring the infant to a pediatric gastroenterologist who can provide the essential diagnostic and treatment modalities for optimal outcome. The scarcity and relatively low

quality of research found in this area indicate that further research is needed.

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