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Guideline for the Evaluation of Cholestatic Jaundice in Infants:

Joint Recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN)

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ABSTRACT

Cholestatic jaundice in infancy affects approximately 1 in every 2500 term infants and is infrequently recognized by primary providers in the setting of physiologic jaundice. Cholestatic jaundice is always pathologic and indicates hepatobiliary dysfunction. Early detection by the primary care physician and timely referrals to the pediatric gastroenterologist/hepatologist are important contributors to optimal treatment and prognosis. The most common causes of cholestatic jaundice in the first months of life are biliary atresia (BA, 25- 40%) followed by an expanding list of monogenic disorders (25%), plus many unknown or multifactorial (e.g., parenteral nutrition related) causes, each of which may have time-sensitive and distinct treatment plans. Thus, these Guidelines can have an essential role for the evaluation of neonatal cholestasis to optimize care. The recommendations from this clinical practice guideline are based upon review and analysis of published literature as well as the combined experience of the authors. The Committee recommends that any infant noted to be jaundiced after 2 weeks of age be evaluated for cholestasis with measurement of total and direct serum bilirubin, and that an elevated serum direct bilirubin level (direct bilirubin levels >1.0 mg/dl or >17 μmol/L) warrants timely consideration for evaluation and referral to a pediatric gastroenterologist or hepatologist. Of note, current differential diagnostic plans now incorporate consideration of modern broad-based next generation DNA sequencing technologies in the proper clinical context. 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 infants with cholestasis. Broad implementation of these recommendations is expected to reduce the time to the diagnosis of pediatric liver diseases, including BA, leading to improved outcomes.

PREAMBLE

Cholestatic jaundice in infancy 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 an optimal prognosis. The Cholestasis Guideline Committee consisted of 11 members of two professional societies--the North American Society for Gastroenterology, Hepatology and Nutrition, (NASPGHAN) and the European Society for Gastroenterology, Hepatology and Nutrition (ESPGHAN). This Committee has responded to a need in pediatrics and developed an updated clinical practice guideline for the diagnostic evaluation of cholestatic jaundice in the infant. There is an obligate focus upon identifying infants with cholestasis due to biliary atresia (BA), but also incorporating the recognition that most forms of cholestasis in this age group are due to non-BA causes. Thus a structured and broad-based diagnostic approach is required. The recommendations presented in this clinical practice guideline are based on review and analysis of published literature as well as the experience of the authors and colleagues. The quality of evidence supporting the recommendations is based on the Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) workgroup. Each recommendation is assigned a Class (reflecting benefit versus risk) and Level (assessing strength or certainty). Utilizing these approaches, the recommendations presented herein provide an approach to diagnose infants with cholestasis. These guidelines are intended to be flexible and

tailored to the individual patient and local practice and are not meant to determine standards of care for all infants. This guideline has been approved both by NASPGHAN and ESPGHAN after extensive review.

LITERATURE SEARCH

A systematic literature search was performed using accessible databases of relevance: PubMed, MEDLINE from 2002 until 2014 for targeted topics and keywords (see Supplementary Digital Content 1, Table, *http://links.lww.com/MPG/A733*). The search involved only papers published in English and involving human subjects.

GRADES OF EVIDENCE

Grades of evidence for each statement were based on the grading of the literature and were assigned using the AASLD Practice Guidelines method: Grading of Recommendation Assessment, Development, and Evaluation (GRADE) workgroup with minor modifications[1]. The strength of recommendations in the Grading of Recommendation Assessment, Development, and Evaluation system was classified as outlined in Supplementary Digital Content 2, Table, *http://links.lww.com/MPG/A734*.

BACKGROUND

Cholestasis is defined as reduced bile formation or flow resulting in the retention of biliary substances within the liver normally excreted into bile and destined for elimination into the intestinal lumen. Cholestasis is generally recognized by evaluation of serum studies, with elevation of serum conjugated (or direct) bilirubin and bile acids as central readily-identified

features of hepatobiliary dysfunction. While cholestasis and hyperbilirubinemia are not synonymous, during cholestasis normal bile acid flux and conjugated bilirubin excretion into bile are both impaired and frequently linked. Hence, a central feature of conjugated (or direct) hyperbilirubinemia is a practical clinical marker and surrogate of cholestasis. Distinguishing jaundice caused by cholestasis from non-cholestatic conditions (such as physiological jaundice of the newborn) is critical because cholestatic jaundice is likely pathological and therefore patients with cholestatic jaundice will benefit from prompt diagnosis and institution of specific therapy. Cholestasis can be classified into biliary (obstructive, large extrahepatic or small intrahepatic bile ducts) or hepatocellular (defect in membrane transport, embryogenesis or metabolic dysfunction) **i**n origin.

Cholestatic jaundice affects approximately 1 in every 2,500 term infants and is thus infrequently seen by most providers taking care of infants[2]. The most common causes of cholestatic jaundice in the first months of life are biliary atresia (25- 40%) and an array of individually uncommon genetic disorders (25%). Often, however, the etiology is unknown. It may be associated with prematurity or intravenous soy lipid infusions (see below)[3]. The rate of patients designated by the descriptive term, "idiopathic neonatal hepatitis" as the cause of neonatal cholestasis continues to decline with advancements in diagnostic evaluation and discovery of new etiologies, now clinically discoverable with utilization of available next generation DNA sequencing technologies (see below). Other causes of neonatal cholestasis include extrahepatic obstruction from common duct gallstones or choledochal cyst; metabolic disorders such as tyrosinemia type I, galactosemia and inborn errors of bile acid metabolism; panhypopituitarism; Alagille syndrome; infection; parenteral nutrition associated liver disease

and a broad array of generally rare disorders (see Table 1)[4]. The common clinical feature of impaired bile flow resulting from either biliary obstruction or hepatocellular metabolic derangements requires a broad-minded approach to the individual cholestatic infant—without which opportunities for providing effective therapeutic interventions may be overlooked.

The incidence of neonatal cholestasis is increased in premature infants, more so in those born at the limits of viability than those born closer to term. Parenteral nutrition (PN)-related cholestasis is present in up to one-fifth of neonates receiving PN for more than two weeks[5]. Longer duration of PN and intestinal failure are independent risk factors for the development of PN cholestasis in infants and has led to the consideration for reducing exposure to soy lipids where appropriate[5, 6]. In addition, small for gestational age is a strong independent risk factor for neonatal cholestasis[7]. This clinical guideline is not meant to address cholestasis in the preterm infant on parenteral nutrition, but close follow up and serial measurements of fractionated bilirubin levels early in life are important, alongside monitoring growth and tolerance of enteral feedings. However persistent cholestasis in any infant should be considered pathological and identifiable causes of cholestasis, including BA, should be ruled out in a timely fashion, since another cholestatic condition can certainly be present in an infant who requires PN. It should be noted the incidence of BA or genetic forms of cholestasis is the same in premature as in term infants—thus, premature infants warrant consideration for the same evaluation of neonatal cholestasis as do full-term infants. Several studies demonstrate a higher incidence of BA in preterm infants compared with term infants, and delayed diagnosis results in poorer outcome[8, 9].

Biliary Atresia (BA)

BA is the most frequent identifiable cause of obstructive jaundice in the first 3 months of life. The prevalence of BA varies according to location around the globe: ~1 in 6,000 live births in Taiwan, 1 in 12,000 in the United States, 1 in 19,000 in Canada and 1 in 18,000 in Europe[10-12]. There are three classifications of BA- the nonsyndromic form (84%) which is the most common, BA with at least one malformation but without laterality (e.g., situs inversus) defects (6%) and the syndromic BA with laterality defects (10%). The latter two groups have other associated anomalies predominantly in the cardiovascular (16%) and gastrointestinal (14%) systems but the group without laterality defects has more frequent genitourinary anomalies. BA patients with laterality defects more commonly have splenic anomalies[13]. The etiology of BA is unknown and theories of pathogenesis include genetic contributions to bile duct dysmorphogenesis, viral infection, toxins, chronic inflammatory or autoimmune-mediated bile duct injury[14-17]. Direct hyperbilirubinemia is identified sooner after birth in BA patients compared with normal (control, non-cholestatic) infants, suggesting that the initiation of the biliary injury occurs prior to, or very soon after birth (i.e. perhaps due to intrauterine insult or genetic etiology), thus minimizing the likelihood of biliary tract disease acquired after birth[18]. Timely diagnosis is important to optimize the response to a Kasai hepatic portoenterostomy (HPE) aimed at re-establishing bile flow[19]. If the HPE is performed within the first 60 days of life, ~70% of patients will establish bile flow; after 90 days of life less than 25% of patients will have bile flow[3]. Late diagnosis of BA however remains a problem worldwide for a variety of reasons including the obligate visual overlap with normal physiologic jaundice and the lack of a readily applicable newborn screening. The average age at HPE in the US is 61 days and 44% of patients still undergo HPE after 60 days of life[20]. In Europe, late diagnosis is also a challenge

and average age at HPE has been reported between 57 and 68 days[21-23]. In the largest outcome series from Canada, medium age at HPE was 55 days but late referral was still problematic[11]. Although not systematically evaluated, surgical outcome has been associated with the expertise in performing HPE in Europe, with improved outcome seen with centralized care models[11, 24, 25]. In the United States this might be more challenging, but referral to a specialized center with expertise in performing HPE remains crucial.

The optimal management of infants with delayed presentation of BA remains controversial. Some series report successful HPE drainage beyond 90 days of life reaching 13-35 %[11, 19, 26] . In a large series that examined outcomes in 743 infants with BA, 2-, 5-, 10-, and 15-year survival rates with native liver were 57.1%, 37.9%, 32.4%, and 28.5%, survival rates with native liver decreased when age at surgery increased (< or =30, 31-45, 46-60, 61-75, and 76-90 days). The investigators in this study estimated that if every patient with BA underwent the Kasai operation before 46 days of age, 5.7% of all liver transplantations performed annually in France in patients younger than 16 years could be avoided. These studies highlight the importance of early detection of cholestasis by providers that can improve outcomes[19]. These also indicate a need for unbiased screening for cholestasis and BA, perhaps via yet-to-be discovered newborn screening or the application of stool color cards as successfully employed in Taiwan[27].

Non-BA etiologies of neonatal cholestasis

Treatable conditions that can present with cholestatic jaundice include bacterial sepsis, galactosemia, tyrosinemia, panhypopituitarism, bile acid synthetic defects or obstructive gallstones. These infants often appear acutely ill and early diagnosis will enable timely initiation

of directed treatment. Conversely, infants with BA usually appear otherwise healthy and grow normally which might deceive the parent or physician into believing that the jaundice is physiologic or caused by breast-feeding[4]. It is important to note that medical management and optimization of nutrition to prevent complications of neonatal cholestasis is beneficial even when specific treatment is not available or curative. The differential diagnoses include a variety of anatomic, infectious, autoimmune, genetic, metabolic and congenital conditions. This list is not meant to be exhaustive but rather an overview to help orient the reader (see Table 1).

EVALUATION OF THE JAUNDICED INFANT

Jaundice or icterus is clinically evident when the total serum bilirubin level exceeds 2.5-3.0 mg/dL (42- 51 μmol/L). Visual determinations of bilirubin levels are inherently problematic. Several studies confirm the inability of even experienced caregivers to accurately estimate an infant's total serum bilirubin level[28] and this visual assessment cannot determine if the jaundice is due to indirect, or direct hyperbilirubinemia. The most important initial step in evaluating a jaundiced infant is measuring serum total and direct (or conjugated) bilirubin. Jaundice at 2 weeks of age is a relatively common finding, observed in 2.4% to 15% of newborns[29, 30], however it should alert the provider of the possibility of cholestasis, although testing of all jaundiced newborns at the 2-week visit will detect cholestasis in relatively few[4]. Providers have several options: the most direct is to test serum for total and direct bilirubin at the 2 week visit (or if concerned at any age), but in the absence of any significant "red Flags", the infants can follow one of several paths. These "Flags" are detailed in Tables 2 and 3.

If the 2 week old infant is breastfed and has a normal physical exam, no history of dark urine or acholic stool another option is to see the infant back for follow up in 1 week according to local practice and caregiver/parental comfort with the plan. If this course is taken, and the jaundice persists at 3 weeks of age, laboratory evaluation is recommended[4]. If a 2 week old icteric infant is bottle fed then fractionation of bilirubin is recommended. If the infant's first visit is at 4 weeks of age as is common practice in many European countries, then any jaundiced infant should be investigated promptly by measurement of total and direct bilirubin. The actual age of the infant when measurement of a fractionated bilirubin is performed is dependent upon several factors and not meant to be proscribed—but practically the measurement should coincide with the clinical status of the infant in the context of accepted local practice. However, the earlier measurements of fractionated bilirubin are performed, the earlier a diagnosis of cholestasis can be made or excluded and thus help direct optimal and timely clinical care plans.

The most commonly used laboratory determination, the diazo or van den Bergh method, does not specifically measure conjugated bilirubin but reports direct bilirubin which includes both conjugated bilirubin and delta bilirubin (conjugated bilirubin covalently bound to albumin). For methodological reasons, the higher the total bilirubin (even if nearly all unconjugated bilirubin) the higher the reported direct bilirubin, hence specific measurements of conjugated bilirubin are optimal if available.[31, 32]. Because canalicular excretion of bilirubin can be rate-limiting to overall clearance, infants with elevated unconjugated bilirubin may retain some conjugated bilirubin, but this is variable due to the distinct canalicular transporters for conjugated bilirubin (Abcc2) and bile acids (Abcb11), and their differential expression in the setting of cholestasis

and age. Therefore, in the presence of elevated total bilirubin, direct/conjugated bilirubin levels are considered abnormal when values are greater than 1.0 mg/dL (17 μmol/L) regardless of the total bilirubin[33]. Thus, for this guideline, an abnormal direct/conjugated bilirubin is defined as a serum value greater than 1.0 mg/dL (17 μmol/L), since it is physiologically and clinically complex to consider incorporating consideration of whether or not the direct fraction exceeds 20% of the total bilirubin level as mentioned in some publications[4, 34].

In a healthy newborn baby with indirect/unconjugated hyperbilirubinemia the most common causes of jaundice are physiologic jaundice and breast milk jaundice. Both are self-limited maturational disorders characterized by an elevation of serum indirect/unconjugated bilirubin. Infants who are breast-fed are more susceptible to neonatal jaundice since maternal milk contains beta-glucuronidase that breaks down conjugated bilirubin to form unconjugated bilirubin and hence increases the enterohepatic circulation of bilirubin[4, 35, 36]. Expressed breast milk also contains factors which may inhibit the conjugating enzyme in hepatocytes[37]. Please refer to the American Academy of Pediatrics guidelines for the management of unconjugated hyperbilirubinemia in the newborn infant 35 or more weeks of gestation[38].

Recommendations:

1. ***Any formula-fed infant noted to be jaundiced after 2 weeks of age should be evaluated for cholestasis with measurement of total and conjugated (direct) serum bilirubin (1A). Depending upon local practice, breast-fed babies that appear otherwise well may be followed clinically until 3 weeks of age, at which time if they appear icteric should then undergo serum evaluation of total and conjugated (direct) serum bilirubin.***

2. ***Measurements of serum bilirubin should always be fractionated into unconjugated (indirect) or conjugated (direct) hyperbilirubinemia (1A).***
3. ***Conjugated (direct) hyperbilirubinemia (> 1.0 mg/dl, 17umol/L) is considered pathological and warrants diagnostic evaluation. (1A)***

HISTORY

Obtaining a detailed prenatal and infant history is fundamental and should include details of the neonatal screening and any medication including vitamin K supplementation. Details of feeding history should be noted as well as the timing of the first bowel movement, since delayed passage of meconium can be seen in patients with cystic fibrosis. The history should systematically collect information about the onset of jaundice, changes in stool pigmentation and urine color. It is important to identify history of pale or acholic stools and it is highly recommended to observe the stool pigment (see below). It is well recognized that parents and health care professionals assess stool pigmentation subjectively and abnormally pale stools are frequently misinterpreted. Acholic stools were correctly identified only by 63% of health care providers[39]. Stool color charts may be helpful in review of history and ascertaining lack of pigmentation of stools in children with suspected liver disease. In Taiwan, use of a stool color card proved to be effective with 95.2% sensitivity for pale stools[40]. A large prospective cohort study utilizing home based screening for BA with a stool card proved cost effective in Canada[41]. Use of the stool card has been piloted in some European countries, such as Switzerland[42] but has not been systematically implemented across the US or Europe. Many efforts are being investigated to increase awareness and recognition of acholic stool.

In addition, the common intersection of prematurity, inability to advance enteral feedings and use of prolonged soy lipid-based parenteral nutrition leads to cholestasis quite commonly—known as Parenteral Nutrition Associated Cholestasis (PNAC)[43]. This is a major confounder in the evaluation of the cholestatic infant, and it is often worthwhile for caregivers to note the timing and initiation of parenteral nutrition in relation to serial measurements of fractionated bilirubin levels, especially if direct hyperbilirubinemia precedes the initiation of parenteral nutrition.

Details in the family history including previous and current pregnancy such as miscarriages, pruritus or overt liver dysfunction in maternal history should be noted; history of maternal fever, rash, adenopathy or medication intake can be helpful. The family history should not only focus on known liver conditions but also on hemolysis and /or cardiac and vascular anomalies. A detailed overview of noteworthy features is given in Tables 2 and 3.

PHYSICAL EXAM

The clinician performing a physical exam should not only focus on the abdomen but should also consider extrahepatic signs, such as: dysmorphic features, poor growth, dermatologic, neurologic or pulmonary symptoms (Table 3). Palpation of the abdomen may reveal firm hepatomegaly suspicious for the diagnosis of BA, often with a prominent middle or left lobe. Splenomegaly in BA appears after the newborn period, and if present at a young age of 2-4 weeks should point towards other diseases such as storage or hematological disorders. Cardiac examination is key as discovery of a murmur might suggest Alagille syndrome (ALGS) or cardiac anomalies

associated with BA (e.g., septal defects). For a variety of causes, right heart failure may lead to impaired hepatic venous outflow, hepatomegaly, and cholestasis. Hypoplastic (male) genitalia may be a feature of panhypopituitarism but normal genitalia does not exclude this condition. Confirming whether the infant can visually fix and follow is helpful to rule out septo-optic dysplasia, but often cross-sectional brain imaging is required for this diagnosis[44, 45]. Direct observation of urine color, and most importantly stool color, is a necessary component of the assessment of the jaundiced infant, as acholic stools and dark urine often indicates the presence of cholestasis and conjugated hyperbilirubinemia. It is important to note that there are no findings obtained by a careful history or a detailed physical exam that are unique to BA patients.

Recommendations:

4. ***A thorough physical examination is crucial to the proper evaluation of the jaundiced infant. Attention to hepatomegaly, splenomegaly and ill appearance warrants special considerations. (1A)***
5. ***Direct visualization of stool pigment is a key aspect of a complete evaluation of the jaundiced infant. (1A)***

DIAGNOSTIC EVALUATION

This section is devoted to the diagnostic approach to the infant with cholestasis. In addition to laboratory studies, imaging and liver histopathology are important to evaluate for bile duct patency since cholestatic infants must be evaluated promptly to exclude treatable surgical conditions. As noted above, performance of the Kasai HPE for BA is much less likely to benefit

infants if performed after 3-months of age[46] hence the importance of an expedient and efficient evaluation.

LABORATORY EVALUATION

During the evaluation of the infant with cholestasis, laboratory investigations will help define the etiology, the severity of the liver disease and detect treatable conditions.

A critical and important initial blood test is the measurement of serum conjugated (direct) bilirubin (DB), which, if elevated, is a reliable laboratory indicator of cholestasis at this age. Accompanying evaluation of DB levels are standard biochemical and synthetic liver tests to assess the severity of the liver disease to include total bilirubin (TB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma glutamyl transpeptidase (GGTP), prothrombin time (PT) with the International Normalized Ratio (INR), glucose and albumin. An elevated serum AST without substantial increase in ALT, TB or DB may point to a hematologic or muscular process, since AST is an enzyme present in red blood cells and myocytes. GGTP value is typically higher in neonates than older children[47] and is generally elevated during cholestasis[48]. However, some cholestatic diseases present with normal or low GGTP, including progressive familial intrahepatic cholestasis (PFIC) type 1 (ATP8B1 deficiency) and 2 (ABCB11 deficiency), bile acid synthesis disorders and TJP2 deficiency[49, 50]. Other conditions including ALGS, PFIC3 (due to ABCB4 deficiency) and often, but not always, BA frequently present with a high GGTP. Serum AP levels are generally less helpful than serum GGTP in the evaluation of cholestatic infants since the normal range of serum AP levels varies greatly in growing infants. Bacterial cultures of blood, urine and other fluids should be obtained as dictated by the clinical assessment. Severe coagulopathy

unresponsive to parenteral vitamin K administration and out of proportion to the liver injury may indicate gestational alloimmune liver disease, metabolic disease or sepsis. When evaluating a patient with cholestasis it is crucial to review the standard local newborn screening since many diseases that cause cholestasis are tested such as hypothyroidism, galactosemia, tyrosinemia and cystic fibrosis. Some countries have extended newborn screens that can be performed upon request.

The minimum evaluation for any health care professional encountering an infant with jaundice present after the age of 14 days should include a full history including family history and gestational history of the mother, physical examination, inspection of stool color, and obtaining a fractionated bilirubin measurement. When cholestasis is suspected, expedited focused investigations (Table 4, Tier 1) are recommended. A disciplined and stepwise approach to the infant with confirmed cholestasis in concert with a pediatric gastroenterologist can then follow in the ordering of laboratory tests appropriate in each situation, and enabling a targeted work-up (Table 4, Tier 2). Some local variation is unavoidable due to available expertise (Table 4). "Red flags" which mandate evaluation for BA include acholic stools, high GGT cholestasis without alternative etiology and abnormal or absence of gallbladder on ultrasound. Conditions that mimic BA such as alpha-1-antitrypsin, CF, ALGS and others, should be excluded early on in the evaluation process.

DIAGNOSTIC IMAGING

A fasting abdominal ultrasound is an easy and non-invasive first diagnostic imaging investigation to assess for visible obstructing lesions of the biliary tree or identification of

\choledochal cyst, and to assess for signs of advanced liver disease or vascular and or splenic abnormalities[51]. Several hepatic sonographic parameters such as the triangular cord sign, abnormal gall bladder morphology, lack of gall bladder contraction after oral feeding, non-visualization of the common bile duct, hepatic artery diameter and hepatic artery diameter to portal vein diameter ratio, subcapsular blood flow, have been suggested to aid in the diagnosis of BA[52-56], although none can singularly confirm a diagnosis of BA. It is useful, however, to know that many, but not all, infants with BA have a small or undetectable gall bladder[57]. In addition, findings such as abdominal heterotaxy, midline liver, polysplenia, asplenia and pre-duodenal portal vein increase the concern for BA with malformations. However, it is imperative to remember that a normal US does not rule out non-syndromic BA.

Hepatobiliary scintigraphy (HBS) has been used to confirm biliary tract patency, but can be limited by its low specificity (range 68.5% - 72.2%), and a non-diagnostic result when bile flow is limited due to a wide variety of etiologies[58]. Patients with interlobular bile duct paucity, idiopathic neonatal hepatitis, low birth weight and those on parenteral nutrition may have non-excreting scans[59] This limited accuracy of HBS in differentiating idiopathic neonatal hepatitis from BA was demonstrated in a study by Yang et al[60] where magnetic resonance cholangiopancreatography (MRCP), ultrasonography (US), technetium 99m-iminodiacetic acid HBS, HBS single photon emission computed tomography (HBS SPECT) and liver biopsy were compared. The goal of this study of 69 infants with cholestatic jaundice and a final diagnosis of idiopathic neonatal hepatitis and BA was to determine which modality may help distinguish between these two diagnoses. All 69 infants underwent MRCP, US, HBS, SPECT and liver biopsy. HBS had sensitivity and a specificity of 88.24% and 45.71% for detecting BA,

respectively with an accuracy of 66.67%. Scintigraphy adds little to the routine evaluation of the cholestatic infant, but may be of value in determining patency of the biliary tract, thereby excluding BA. In this study, liver biopsy had the highest sensitivity in detecting BA at 100%, a specificity of 94.29% and an accuracy rate of 96.9%.

A recent meta-analysis addressing the utility of HBS yielded a pooled sensitivity of 98.7% (range 98-1-99-2%) and a specificity of 70.4%(range 68.5-72.2%) of a non-draining HBS for excluding BA. This shows that false negative results (excretion of the tracer into the bowel despite BA) are extremely rare[58]. Limited reports describe infants with apparently initially excreting HBS and a subsequent diagnosis of BA, although the technical limitations of the study may have been a factor in its utility[62, 63].

Many clinicians and radiologists administer phenobarbital for 5-days prior to the study, in an attempt to enhance biliary excretion of the isotope and increase its discriminatory value[61], which often unnecessarily delays the diagnosis of BA and the necessary hepatoportoenterostomy[51][19]. Further work is necessary to assess the utility of premedication for HBS[62, 63].

Despite the use of the diagnostic tests described above, it is still not easy to discriminate between BA and other causes of neonatal cholestasis. As detection of patency of the extrahepatic biliary tree is the primary goal of diagnostic evaluations in infants with cholestasis, the role of endoscopic retrograde cholangiopancreatography (ERCP) in the diagnosis of BA has been studied by various groups[64, 65]. While ERCP has proved effective with high positive and

negative predictive values for BA (sensitivity 86% - 100%, specificity 87% - 94%, positive predictive value 88% - 96%, negative predictive value 100%)[64, 66], ERCP requires an experienced endoscopist, specific infant endoscopy equipment not readily available at many centers, and a general anesthetic. The superiority of ERCP compared with other types of cholangiograms has not been demonstrated[67].

A few reports have suggested that magnetic resonance cholangiopancreatography (MRCP) is a well-established non-invasive modality for visualizing the biliary system, including the first order branches of the intrahepatic bile ducts, extrahepatic bile ducts and gallbladder[68]. The diagnostic value of three-dimensional MRCP for BA in a large cohort of cholestatic infants and neonates was therefore recently evaluated, with a reported specificity of 36% and sensitivity of 99%[69].

Recent case series have documented the technique and feasibility of percutaneous transhepatic cholecysto-cholangiography (PTCC) to exclude BA[70, 71]. In the largest series reported[71], PTCC was performed in combination with simultaneous liver biopsy. While this was reported to effectively exclude BA with a lower negative laparotomy rate, there is a genuine concern that PTCC may be utilized unnecessarily in infants in whom a liver biopsy alone would have excluded biliary obstruction. Moreover, a PTCC may not be able to demonstrate retrograde patency of the biliary tree into the liver and may miss proximal obstruction, thus obviating a surgical cholangiogram in a patient who may have BA. Importantly, the specificity of liver biopsy in diagnosing biliary obstruction in this case series was much lower than frequently

reported values[51]. Taken together, the use of MRCP, ERCP, and PTCC has a limited role in the general guidance to caregivers towards diagnosing BA in the present era.

HISTOPATHOLOGY

Liver biopsy often remains the cornerstone of the diagnostic work up of infants with cholestatic jaundice as interpretation by an experienced pathologist will provide the correct diagnosis in 90% to 95% of cases and avoid unnecessary surgery in patients with intrahepatic disease[51,72,73]. Pathologists participating in the National Institutes of Health (NIH) supported Biliary Atresia Research Consortium (BARC, currently the ChiLDReN consortium[childrennetwork.org]) have developed and evaluated a standardized system for reporting of liver biopsies from infants with cholestasis. Overall, the pathologists diagnosis of obstruction in clinically proven cases of BA ranged from 79% to 98%, with a positive predictive value of 90.7%. The group diagnosed BA with a high level of sensitivity and identified infants with biliary obstruction with reasonable inter-observer agreement[72]. Of note, a diagnosis of BA or obstruction other than BA was made in 14 of 15 cases of parenteral nutrition associated liver disease and all three cases of alpha 1 antitrypsin deficiency. Conversely, a majority of the pathologists' favored a diagnosis of no obstruction in the three cases of progressive familial intrahepatic cholestasis and one case of bile acid synthetic disorder. In cases of idiopathic neonatal hepatitis, the percentage of cases read by each pathologist as no obstruction ranged from 57% – 93%. The classic histologic features of biliary obstruction are bile duct proliferation, bile plugs, portal or perilobular fibrosis and edema, with preservation of the basic hepatic lobular architecture (Figure 1)**.** In neonatal hepatitis, lobular disarray and inflammatory cells are seen within the portal areas, and the bile ductules show little or no alteration (Figure 2). Giant cell

transformation can be seen in 20% - 50% of patients with BA[74,75]; however, it is not as prominent as that seen in neonatal hepatitis[73]. Some disorders that can mimic BA histologically are parenteral nutrition associated cholestasis, cystic fibrosis and alpha-1-antitrypsin deficiency. They may show variable ductular reaction and may be impossible to distinguish from BA without clinical data[72,73]. It is however important to recognize that the earliest histologic changes of BA may be relatively non-specific, and biopsies performed too early in the course of the disease may result in a falsely negative diagnosis[72,73,76].

The proper utilization of liver biopsy therefore remains a central component of the diagnostic evaluation of infants with cholestatic jaundice as the differential diagnosis is perhaps the broadest of any age group and encompasses obstructive as well as, more commonly, non-obstructive disorders. In addition to its role in diagnosis, the liver biopsy may also reveal histological features of significant prognostic value, such as the degree of fibrosis, which may help predict outcome following HPE and the decision to proceed with HPE[77,78]. Although sonography-guided percutaneous core liver biopsy is considered to be a safe and effective procedure in children with a low complication rate of 1.7%[79], the overall complication rate in infants, even in the hands of an experienced physician, was reported in one small series to be 4.6% (3/65 infants had a bleeding event that required an intervention)[80].

Recommendations:

6. ***The abdominal ultrasound is useful in excluding choledochal cyst or gallstone disease causing extrahepatic bile duct obstruction. It may demonstrate an absent or abnormal gallbladder, or other features suggestive, but not diagnostic, of BA (1A).***

7. Limited specificity precludes the use of the HBS scan as a stand-alone test in making a definitive diagnosis of BA. (1B) **Definitively-demonstrated bile flow by selective use of HBS may be of value in excluding BA (1B).**

8. Limited specificity of MRCP, ERCP, PTCC has a limited role in the general guidance to caregivers towards diagnosing BA in the present era.

9 In the hands of an experienced pediatric pathologist, histopathological findings of bile duct proliferation, bile plugs and fibrosis in an appropriately timed liver biopsy is the most supportive test in the evaluation of the infant with protracted conjugated hyperbilirubinemia (1B). Diseases other than BA that cause cholestasis can be determined via histologic examination of the liver.

INTRAOPERATIVE CHOLANGIOGRAM

The intraoperative cholangiogram and histological examination of the duct remnant is considered the gold standard to diagnose biliary atresia.[51,81,82]. Interestingly, in up to 20% of cases, even a cholangiogram can suggest an incorrect diagnosis—cases with a hypoplastic biliary tree, ALGS and cystic fibrosis (CF) being confounding diagnostic conditions[83]. Hence, preoperative testing for CF and ALGS is very helpful in assisting in the interpretation of the cholangiogram and decreasing false positive results. Intraoperative cholangiogram is typically performed after biliary obstruction is suggested in a liver biopsy or if sufficient clinical indications suggest direct referral to the surgeon for the procedure. If BA is confirmed (i.e. non-visualization of a patent extrahepatic biliary tree), a (Kasai) HPE is usually performed immediately, unless there are considerations made by the team that it would be in the best interest of the infant to proceed to transplant evaluation and not undergo the HPE. No effective

diagnostic tools currently determine whether a patient should proceed to HPE, and it is up to the team to the specialists to determine whether the patient would be better served without the HPE.

It is important to note that diagnostic evaluation to rule out BA should be expedited especially when the infant is above 6 weeks of age. The younger the age at diagnosis of BA, the more likely that the HPE will be successful (at least in the short term, see above).

Recommendation:

10. Evaluation by intraoperative cholangiogram and histological examination of the duct remnant is considered the gold standard to diagnose biliary atresia (1A).

OTHER CAUSES OF NEONATAL CHOLESTASIS

Structural abnormalities:

Choledochal Cyst: Patients with choledochal cysts present with laboratory findings suggestive of cholestasis. Sometimes patients have cholangitis and present with fever, elevation of the GGTP and direct hyperbilirubinemia. Ultrasonography can often differentiate between choledochal cyst and BA as the bile ducts are typically dilated or cystic and the gallbladder is not atretic[84]. However, a diagnosis of choledochal cyst in a cholestatic neonate should always prompt careful evaluation for BA (atresia of the distal common bile duct accompanied by cystic dilation: type 1 BA). In a few studies, cyst size appeared to decrease between prenatal diagnosis and birth in patients with BA but did not change in patients with choledochal cyst[85,86]. Moreover, choledochal cysts can coincide with BA.

Select Genetic/Metabolic disorders:

Alagille Syndrome: Alagille syndrome (ALGS) is an autosomal dominant multisystem disorder characterized by paucity of interlobular ducts. It is the most common form of familial intrahepatic cholestasis occurring in 1 in 30,000live births. Diagnosis is usually made by the clinical findings, laboratory and diagnostic evaluation, and confirmed by sequencing of *JAG1* and *NOTCH2* genes, with mutations found in 95% and 5% of patients with ALGS, respectively[87]. Clinical criteria for the diagnosis of ALGS includes ductopenia on liver biopsy as well as a characteristic Alagille facies (broad forehead, small pointy chin, but is often difficult to recognize in the neonatal period), posterior embryotoxon, butterfly vertebrae, renal disease and a variety of developmental cardiac defects (most commonly peripheral pulmonic stenosis)[88] or tetralogy of Fallot. Direct hyperbilirubinemia and occasional acholic stool that may improve with age can be present[88]. Serum ALT and bile acids are usually elevated. The GGTP is an important test in orienting the practitioner towards this disorder since it is often disproportionately elevated; sometimes up to 20 times the normal value. Practitioners are encouraged to evaluate for associated clinical abnormalities found in patients with ALGS when the diagnosis is suspected either on liver biopsy or by characteristic facies, prior to proceeding with IOC.

Cystic Fibrosis (CF): Some CF infants present with abnormal liver tests suggestive of biliary obstruction due to the presence of abnormal bile with plugging of the common bile duct[89]. Checking the newborn screen for immunoreactive trypsinogen is helpful. The gold standard remains sequencing of the CFTR gene or a positive sweat chloride test, but this is sometimes not possible as infants may not produce enough sweat[90].

Progressive Familial Intrahepatic Cholestasis (PFIC): PFIC is a group of unrelated monogenic disorders in which mutations in one of the genes involved in canalicular hepatobiliary transport results in progressive cholestasis and liver injury (Table 1). Patients with PFIC1-3 have a significant elevation of the total serum bile acids. A very important clinical finding in individuals with PFIC type 1 and 2 (due to ATP8B1 and ABCB11 gene deficiencies, respectively) is the presence of a normal or low GGTP out of proportion to the degree of cholestasis[91], associated with normal or low serum cholesterol. Some of the patients with normal GGTP PFIC were found to have a mutation in the tight junction protein 2 gene **(***TJP2*) which causes failure of protein localization and disruption of tight-junction structure, leading to severe cholestatic liver disease that can present very early in life[49]. Patients with PFIC type 3 (ABCB4 deficiency) have elevated GGTP and a variable degree of cholestasis—typically presenting later in infancy or in early childhood[92,93].

Alpha-1-Antitrypsin Deficiency (A1ATD): This is the most common cause of inherited neonatal cholestasis. Approximately 10-15% of neonates with this condition will present with cholestasis and a combined picture of hepatocellular injury and obstruction with elevation of the ALT, AST, GGTP and alkaline phosphatase. The cholestasis is usually severe and the presence of acholic stools may present a challenge because of the resemblance to BA. Although some patients may develop cirrhosis early on, jaundice clears in most patients by 4 months of age[94]. The diagnosis is made based on the phenotype (normal: MM; abnormal: ZZ or SZ; heterozygous: MZ, MS)[95]. It is important to note that neonates with ZZ phenotype may have no biliary excretion on scintigraphic studies[96] and liver biopsy may appear obstructive[72]. Hence obtaining the phenotype early in the evaluation of cholestasis could avoid unnecessary biopsy in this condition. Checking for serum levels of alpha-1-antitrypsin could be helpful if

used along with the phenotype to distinguish patients who are homozygous for the Z allele or SZ compound heterozygotes, both of whom may develop liver disease. Patients with MZ, MS, SZ or homozygous SS A1 phenotypes do not present with neonatal cholestasis unless associated with another cause[97]. Serum alpha-1-antitrypsin concentrations alone are an insufficient test since alpha-1-antitrypsin is an acute phase reactant and during illnesses may be elevated[98,99]. Of note there have been few case reports of the concurrence of alpha-1-antitrypsin deficiency and BA[100,101].

Bile Acid Synthesis Disorders (BASD): More than 14 enzymes are involved in the synthesis of bile acids from cholesterol precursor molecules. BASDs are rare, but in many cases, are treatable forms of cholestasis. Not all of the infants with the genetic abnormalities leading to BASD present with cholestasis and jaundice; some may have a more indolent presentation later during childhood. These conditions often present with normal or low GGTP. Total serum bile acids are usually low, in contrast to other cholestatic disorders. Fast atom bombardment mass spectrometry of urine should be considered as a screening tool before starting ursodeoxycholic acid; it is possible to perform rapid diagnosis of potential inborn errors in bile acid synthesis from urinary bile acid analysis. Molecular techniques then identify the specific mutations in genes encoding the enzymes responsible for bile acid synthesis[102-104]. Treatment with the end-products of bile acid synthesis, cholic acid and chenodeoxycholic acid, is often curative for several of the BASDs, prompting directed evaluations.

Select Inborn Errors of Metabolism (IEM): A group of metabolic conditions classified as IEM can present with cholestasis, and as in the other cholestatic disorders, the practitioner has to

have a high index of suspicion. The initial laboratory testing recommended when the clinical picture is compatible with an IEM includes: blood gases, electrolytes, glucose, ammonia, uric acid, lactic acid, pyruvic acid (L:P ratio), ketone bodies; and in urine, ketone bodies, 2-keto acids, reducing substances, acylglycines, pH and sulfites[105-108]. Newborn screening for galactosemia and tyrosinemia are performed in some countries to identify infants before they are symptomatic. Infants with tyrosinemia can present with mild cholestasis, although more typically present with coagulopathy disproportionate to other biochemical abnormalities[109]. Diagnosis via serum fumarylacetoacetate hydrolase enzyme determination or urine succinylacetone is vital in this treatable but life-threatening condition. With the incorporation of gene panels and exome sequencing, these disorders may have more precise and timely means of genetic investigations in the near future.

Infections:

Cytomegalovirus (CMV): CMV the most common congenital infection, affects 1-2% of newborns. Most infected newborns are asymptomatic; unfortunately 5-10% of the patients have a myriad of clinical symptoms that include low birth weight, microcephaly, periventricular calcifications, chorioretinitis, and deafness. Hepatosplenomegaly and direct hyperbilirubinemia are the most prominent liver-related problems[110,111]. The diagnosis of congenital CMV is confirmed by culture or PCR from the nasopharynx, saliva, blood or urine soon after birth. Urine CMV culture or CMV-DNA detection by PCR is presently used for the diagnosis[112,113]. The IgM CMV-specific antibodies can be monitored but are of limited value, and may be less

sensitive. Evidence of recent CMV infection at the time of diagnosis of BA has been reported by multiple investigators, but a role for CMV in the etiology of BA remains unproven[114-116].

Viral hepatitis A, B and C: In general these viruses do not cause neonatal cholestasis. Single case reports document special circumstances where these infections present with neonatal cholestasis. Specific studies for these infectious agents in the evaluation of neonatal cholestasis are generally unwarranted.

Other infections: Syphilis, Rubella, Toxoplasmosis and Herpes-virus can present with neonatal cholestasis, coagulopathy and growth restriction. Obtaining a good maternal history and discussing with the obstetrician and neonatal intensive care team about placental abnormalities can help with directing the work up for an infection. Typically infants with these infections present with jaundice within first 24 hours of life. Congenital syphilis incidence is rising in the US[117]. Urinary tract infections present with cholestasis in the neonatal period and a urine culture should be obtained early on in the diagnostic evaluation of cholestasis[118].

Endocrine Disorders:

Thyroid disorders: Few reports in the pediatric literature describe cholestatic liver disease in infants born to mothers with Graves' disease[119] The newborn screen is designed to detect high levels of TSH hence in cases of central hypothyroidism this can be missed and repeating a blood TSH, free T4, and T3 is helpful[120,121].

Panhypopituitarism: The pituitary hormones are involved in the regulation of bile synthesis and excretion and bile flow. The neonates with this condition present with elevation of the total and direct bilirubin; they may have hypoglycemia and even shock from adrenal insufficiency. Some

infants have associated septo-optic dysplasia and on physical exam will lack the ability to focus or track. Diagnostic evaluation includes: TSH, total and free T4, early morning cortisol level and a brain MRI. In these patients a nonfasting ultrasound should be requested as prolonged fasting can lead to devastating complications related to severe hypoglycemia The cholestasis resolves with the correction of the pituitary hormone insufficiency[44,45,122-124].

Rare diseases and Idiopathic neonatal cholestasis:

The neonatal cholestasis "black box" of unidentified etiologies continues to shrink, but is still a substantial group of disorders. The identifiable causes of neonatal cholestasis have grown more numerous largely due to application of modern techniques of clinical genetics. Reducing costs and easy access to genetic testing, including exome and genome sequencing, and targeted gene panels, have facilitated diagnosis. Among the most studied causes of neonatal cholestasis in recent years are metabolic diseases and disorders of bile transport and bile acid synthesis. However, it is important to stress that the meaning of a gene mutation or polymorphism is dependent on clinical context. Advanced sequencing methods promise to further increase the diagnostic yield of genetic approaches. Table 1 summarizes known genetic findings presenting as neonatal cholestasis.

Conclusion

Cholestatic jaundice in an infant is a typical presenting feature of neonatal liver disease and is frequently clinically confused with the more common prolonged unconjugated hyperbilirubinemia. Identification of infants with cholestasis remains crucial and is in the domain

of the primary care provider, generally uncovered with measurement of a serum fractionated bilirubin. Hence a careful history, thorough physical exam and fractionation of serum bilirubin are recommended in any infant with jaundice seen after 2 weeks of life. The relative rarity of cholestatic jaundice in contrast to unconjugated hyperbilirubinemia in this age range dictates that many jaundiced infants will be tested to detect those with elevated direct bilirubin levels. However, investigation for neonatal cholestasis in these settings is highly beneficial, despite its rarity, because of the gravity of the consequences of missing BA and monogenic diagnoses that have specific, and often life-saving, interventions. This guideline has been developed to assist in this process and is not intended as a substitute for clinical judgment or as a protocol for the care of all infants with cholestasis. Vigilance is crucial in detecting these infants and referring them to a pediatric gastroenterologist or hepatologist who can provide the essential diagnostic and treatment modalities to optimize outcome.

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Table 1. Anatomic and monogenic disorders of neonatal cholestasis

Disease	Presentation	Radiology	Gene(s)	Gene function	Reference
Multisystem disease					
Alagille syndrome (ALGS)	GGTP, cholesterol often elevated, eye & cardiac findings, LB not always clearly diagnostic when performed early in life	Vertebral anomalies	*JAG1* *NOTCH2*	Signaling ligand Receptor for Jagged 1	[127, 128]
ARC syndrome	Lax skin, limb contractures, renal tubular acidosis		*VPS33B* *VIPAR*	Membrane protein recycling Basolateral sorting of canalicular proteins involved in bile secretion	[129-131]
Congenital disorders of glycosylation	Multisystemic		Numerous genes coding for glycosylation enzymes	N- and O- protein glycosylation leading to impaired function	[134, 135]
Cystic fibrosis	Elevated sweat chloride ; possible ductular proliferation on LB		Cystic-fibrosis trans-membrane receptor (CFTR)	Chloride channel	[125, 126]
Mitochondrial disorders	Multisystemic		Nuclear genes Mitochondrial genes	May impact mtDNA replication, protein translation, electron transport	[136-138]
Neonatal Ichthyosis Sclerosing Cholangitis Syndrome	Hypotrichosis, alopecia, cholestasis		*CLDN1*	Claudin-1: tight junctions	[132]
Panhypopituitarism	LB: duct paucity, low pituitary hormones on stimulation, adrenal insufficiency	MRI may reveal microadenoma or absent sella			

Trisomy 21	Typical stigmata		Unknown	Unknown	[133]
Extrahepatic bile duct abnormalities					
Biliary atresia (BA)	LB diagnostic of obstruction with bile duct proliferation and bile duct plugs. Acholic stools	Situs or vascular anomalies in 5-10%; possible absence of gallbladder			
Choledochal cyst	Abdominal mass plus features that overlap with BA (see below)	Cyst seen by US			
Choledocholithiasis	Acholic stools	US and IOC diagnostic	*ABCB4*	Multidrug resistance P-glycoprotein, MDR3	[139]
Congenital perforation of the common bile duct	Ascites without liver disease	Echogenic ascites			
Neonatal sclerosing cholangitis	GGTP often >800 IU/L; LB shows small duct destruction	IOC shows pruning of small bile ducts			
Hepatocellular diseases					
Alpha-1-antitrypsin deficiency	GGTP often very high, α-1-antitrypsin level low (neonate may have false low), Pi type ZZ or SZ		*SERPINA1*	Anti-protease	[144-147]
Bile acid synthesis defects	GGTP normal, FABMS of urinary bile acids, may present with cirrhosis, fat soluble vitamin deficiencies		*CYP7B1* *AKR1D1 (SRD5B1)* *HSD3B7*	Oxysterol 7α-hydroxylase Δ4-3-oxosteroid-5β-reductase deficiency 3β-hydroxy-Δ5-C27-steroid dehydrogenase deficiency	[148] [103, 104]
Bile acid conjugation defects	FABMS of urinary bile acids		*BAAT* *BAL*	Absence of conjugation	[149, 150]
PFIC1	GGTP low or normal; diarrhea		*ATP8B1*	FIC1 translocates phospholipids from	[140]

	and FTT; LB/EM helpful			outer to inner canalicular membrane (floppase)—also expressed in intestine → considered multisystem disease	
PFIC2	Low or normal GGTP; LB/EM helpful		*ABCB11*	Bile salt export pump	[141]
PFIC3	Elevated GGTP		*ABCB4*	Phospholipid flippase responsible for phosphatidylcholine transport into bile	[142]
Tight junction protein 2 mutations	Severe cholestasis		*TJP2*	Failure of tight junctions and protein localization	[49]
Transient neonatal cholestasis (neonatal hepatitis)	GGTP and AP 200-400 IU/L, ALT and AST 80-200 IU/L, LB negative for obstruction		*ATP8B1* *ABCB11* *ABCB4*	FIC1 polymorphisms MDR3 polymorphisms	[139, 143]
Inborn errors of metabolism					
Urea cycle defects					
Citrin deficiency	Normal LFTs or slightly elevated		*SLC25A13*	Mitochondrial aspartate-glutamate carrier	[151, 152]
Ornithine trans-carbamylase deficiency	Neonatal hyperammonemia with/ without cholestasis and w/w-out liver failure		*OTC*	Mitochondrial enzyme of urea cycle	[153]
Carbohydrate metabolism					
Galactosemia	Cholestasis and liver dysfunction		*GALT*	Galactose-1-phosphate uridyltransferase	[154-156]
Amino-acid metabolism					
Tyrosinemia-type 1	May present with liver failure, Fanconi-related nephropathy, or		*FAH*	Fumarylacetoacetate hydrolase	[157]

	seizures				
Lipid metabolism					
Niemann-Pick type C	Splenomegaly		*NPC1*	acid sphingomyelinase	[158]
Lysosomal acid lipase deficiency (Wolman's disease)	Hepatomegaly, features suggesting NAFLD (Neonatal liver failure)	Hyper-echoic liver	*LIPA*	Lysosomal acid lipase	[159]

When multiple mutations have been identified, the original paper is referenced. This list is not exhaustive; rather it is an overview of the most characterized genetic diseases and congenital conditions which may present as neonatal cholestasis. † In clinical practice, protein phenotyping by electrophoresis is used instead of gene sequencing. US: Ultrasound, IOC: intraoperative cholangiogram, LB: liver biopsy, GGTP: gamma-glutamyl transferase, MRI: magnetic resonance imaging, CT: computed tomography, FABMS: fast atom bombardment mass spectroscopy, FTT: failure to thrive; EM: electron microscopy. NAFLD: non-alcoholic fatty liver disease

Table 2. Parameters of clinical interest in the history of the cholestatic infant

Family History	
Consanguinity	Increased risk of autosomal recessive disorders
Neonatal cholestasis in the parents or siblings	Cystic fibrosis, alpha-1 antitrypsin deficiency, progressive familial intrahepatic cholestasis, Alagille syndrome are all genetic conditions causing neonatal cholestasis
History of repeated fetal loss or early demise	Neonatal hemochromatosis/ gestational alloimmune liver disease
Spherocytosis and other hemolytic diseases	Known to aggravate conjugated hyperbilirubinemia
Prenatal History	
Prenatal ultrasonography findings	Presence of choledochal cyst, cholelithiasis, bowel anomalies or concern for syndrome
Cholestasis of pregnancy	May be seen in heterozygotes for PFIC gene mutations; mitochondrial disorder
Acute fatty liver of pregnancy	Neonatal long-chain 3-hydroxyacyl-coenzyme A dehydrogenase (LCHAD) deficiency
Maternal infections	TORCH infections
Infant History	
Gestational age	Prematurity as a risk factor for neonatal hepatitis
SGA	Increased risk of neonatal cholestasis, congenital infections
Alloimmune hemolysis; glucose-6-P-dehydrogenase deficiency; hydrops fetalis	Increased risk of neonatal cholestasis
Neonatal infection	Urinary tract infection, sepsis related cholestasis, CMV, HIV, syphilis, etc
Newborn screen	Panhypopituitarism galactosemia, fatty acid oxidation defects, cystic fibrosis

Source of nutrition: breast milk, formula, PN	Galactosemia, hereditary fructose intolerance, PN associated liver disease
Growth	Genetic and metabolic disease
Vision	Septo-optic dysplasia
Hearing	PFIC1, TJP2
Vomiting	metabolic disease, bowel obstruction, and pyloric stenosis
Stooling	Delayed stooling: CF, panhypopituitarism Diarrhea: infection, metabolic disease
Stool Color	**Acholic stools:** cholestasis, biliary obstruction
Urine characteristics: smell and color	Dark urine (conjugated hyperbilirubinemia), metabolic disease
Excessive bleeding	May indicate coagulopathy, vitamin K deficiency
Disposition: irritability, lethargy	Metabolic disease or sepsis, panhypopituitarism
Abdominal surgery	Necrotizing enterocolitis, intestinal atresia

Table 3. Physical findings in children with neonatal cholestasis

Assessment of general health	Ill-appearance may indicate infection or metabolic disease, infants with biliary atresia typically appear well
General appearance	Dysmorphic features: Alagille syndrome in the neonate rarely exhibits characteristic facial appearance with a broad nasal bridge, triangular facies, and deep set eyes. Typical facial features may appear at around 6 months of age, but are often non-specific [160]
Vision/slit lamp examination	Congenital infection, storage disease, septo-optic dysplasia, posterior embryotoxon, cataracts
Hearing	Congenital Infections, PFIC1, TJP2, mitochondrial
Cardiac exam: murmur, signs of heart failure	Congenital heart disease: Alagille syndrome, biliary atresia splenic malformation syndrome
Abdominal examination	Presence of ascites; abdominal wall veins, **liver size and consistency**, spleen size and consistency (or absence thereof), abdominal masses, umbilical hernia
Stool exam **(Crucial—the primary physician should make every effort to view stool pigment)**	**Acholic or hypopigmented stools** suggest cholestasis or biliary obstruction
Neurologic	Note overall vigor and tone

Table 4: Targeted Investigations of the Persistently Cholestatic Infant

Tier 1: *Aim to evaluate after cholestasis has been established in order to both identify treatable disorder as well as to define the severity of the liver involvement.*

- Blood – CBC + differential, INR, AST, ALT, AP, GGTP, TB, DB (or conjugated bilirubin), albumin and glucose. Check alpha-1-antitryspin phenotype (Pi typing) and level, TSH, T4 if newborn screen results not readily available.
- Urine – urinalysis, culture, reducing substances (rule out galactosemia)
- Consider bacterial cultures of blood, urine and other fluids especially if infant is clinically ill.
- Verify results of treatable disorders (such as galactosemia and hypothyroidism) from newborn screen.
- Obtain fasting ultrasound

Tier 2: *Aim to complete a targeted evaluation in concert with pediatric gastroenterologist/hepatologist*

- General – TSH and T4 values, serum bile acids, cortisol
- Consideration of specific etiologies
    - Metabolic – serum ammonia, lactate level, cholesterol, red blood cell galactose-1-phosphate uridyltransferase, urine for succinylacetone and organic acids. Consider urine for bile salt species profiling.
    - ID – direct nucleic acid testing via PCR for CMV, HSV, Listeria.
    - Genetics – in discussion with pediatric gastroenterologist/hepatologist, with a low threshold for Gene Panels or Exome Sequencing
- Sweat chloride analysis (serum immunoreactive trypsinogen level or CFTR genetic testing) as appropriate
- Imaging
    - CXR – lung and heart disease
    - Spine – spinal abnormalities (such as butterfly vertebrae)
    - Echocardiogram – evaluating for cardiac anomalies seen in Alagille Syndrome
    - Cholangiogram
- Liver biopsy (timing and approach will vary according to institution and expertise)
- Consideration for consultations
    - Ophthalmology
    - Metabolic/Genetic (consider when to involve, especially when there is consideration for gene panels or whole exome sequencing)
    - Cardiology/ECHO (if murmur present or has hypoxia, poor cardiac function)
    - General Pediatric Surgery
    - Nutrition/Dietician

Figure 1: Liver biopsies from 2 individuals with BA. A.) H& E stain of a 3 month old infant with BA, highlighting peribiliary fibrosis, ductular proliferation, bile duct plugs. B.) High power view of A. emphasizing bile duct plugs and damaged cholangioles. C.) Liver biopsy from a 6 week old infant with BA, highlighting peribiliary fibrosis, disordered cholangiocyte profiles and scattered inflammatory infiltrate.

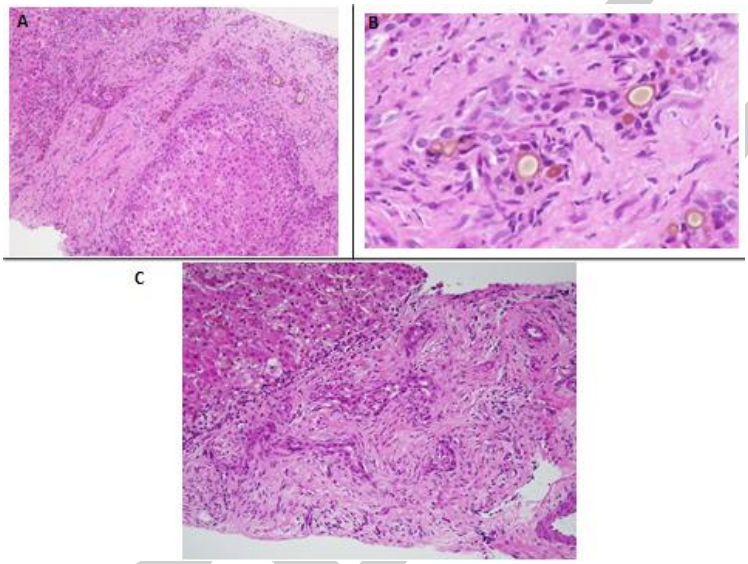

Figure 2: Idiopathic Neonatal Hepatitis: Lobular disarray with giant cell transformation.