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Review

Management options for cholestatic liver disease in children

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Abstract

Introduction: Due to a peculiar age-dependent increased susceptibility, neonatal cholestasis affects the liver of approximately 1 in every 2500 term infants. A high index of suspicion is the key to an early diagnosis, and to implement timely, often life-saving treatments. Even when specific treatment is not available or curative, prompt medical management and optimization of nutrition are of paramount importance to survival and avoidance of complications.

Areas covered: The present article will prominently focus on a series of newer diagnostic and therapeutic options of cholestasis in neonates and infants blended with consolidated established paradigms. The overview of strategies for the management reported here is based on a systematic literature search published in English using accessible databases (PubMed, MEDLINE) with the keywords biliary atresia, choleretics and neonatal cholestasis. References lists from retrieved articles were also reviewed.

Expert commentary: A large number of uncommon and rare hepatobiliary disorders may present with cholestasis during the neonatal and infantile period. Potentially life-saving disease-specific pharmacological and surgical therapeutic approaches are currently available. Advances in hepatobiliary transport mechanisms have started clarifying fundamental aspects of inherited and acquired cholestasis, laying the foundation for the development of possibly more effective specific therapies.

Keywords: biliary atresia, choleretics, diagnosis, jaundice, management, neonatal cholestasis, treatment, ursodeoxycholic acid

1. Introduction

Cholestasis is defined as reduced bile formation or flow resulting in the retention within the liver of biliary substances normally excreted into bile and destined for elimination into the intestinal lumen [1]. In the neonatal period this is a rather frequent condition that affects the liver of approximately 1 in every 2500 term infants and may represent an extraordinary clinical challenge [2-6].

From a laboratory point of view, cholestasis is generally recognized by evaluation of serum studies, with elevation of conjugated (so called “direct”) bilirubin and bile acids as central readily-identified features of hepatobiliary dysfunction. Conjugated hyperbilirubinemia is defined as a direct reacting bilirubin level > 1 mg/dL when the total bilirubin is < 5 mg/dL, or > 20% of the total bilirubin if the total bilirubin is > 5 mg/dL [5]. During cholestasis, normal bile acid flux and direct bilirubin excretion into bile are both impaired and frequently linked [1].

Any infant with prolonged jaundice (arbitrarily defined as jaundice longer than 14 days) should be rapidly evaluated, referred to a pediatric gastroenterologist/hepatologist, to promptly identify the etiology of jaundice and initiate an appropriate medical or surgical treatment. Even when specific treatment is not available or curative, infants may benefit from early medical management and optimized nutrition to prevent cholestasis complications and enhance patient growth and development.

Numerous disorders may be associated with cholestasis in the neonatal period [7,8]. The most commonly identifiable are biliary atresia (BA) (25%–40%), several increasingly recognized monogenic disorders (25%), and multifactorial causes [1]. In a recent European study, biliary atresia was the most common diagnosis (41%), followed by

idiopathic cases (13%), progressive familial intrahepatic cholestasis (PFIC, 10%), cholestasis in preterm infants ± parenteral nutrition (10%), mitochondrial, genetic and endocrine disorders (9%), Alagille syndrome (ALGS, 2%), infections (1%) and miscellaneous (14%)[9].The latter group included individual cases of anatomical abnormalities of the biliary or vascular system, sepsis, endocrinopathies, drug exposure and neoplastic processes. In preterm infants, parenteral nutrition–associated cholestasis (PNAC) is most commonly encountered [3] due to a combination of concomitant events (**Table 1**) (i.e. immaturity of the biliary excretory system, absence of oral feeding, bacterial overgrowth or sepsis and cumulative high intake of amino acids and lipids). In consequence of the advances in diagnostic evaluation and of the broadening of new causes of neonatal cholestasis, the percentage of patients previously designated as "idiopathic neonatal hepatitis" continues to decline [1].

The present article will prominently focus on newer diagnostic and therapeutic options of cholestasis in neonates and infants blended with consolidated established principles. The overview of strategies for the management reported here is based on a systematic search of most recent literature published in English using accessible databases (PubMed, MEDLINE) and the keywords biliary atresia, choleretics, neonatal cholestasis. Reference lists from retrieved articles were also reviewed.

2. Clinical and laboratory diagnosis

A detailed pre-, peri -, and postnatal history is an essential first step in the diagnostic investigation. The obstetric history may reveal maternal infection Toxoplasmosis, Rubella, Cytomegalovirus, Herpes simplex, Syphilis and Listeriosis), or cholestasis

during pregnancy, which may be associated with PFIC [10]. A history of neonatal asphyxia may suggest hypoxic neonatal cholestasis [11] as delayed meconium emission suggests cystic fibrosis.

Results of newborn screenings with the evaluation of clinical features and physical examination may help to recognize cystic fibrosis, galactosemia or hypothyroidism as possible causes of medically amenable cholestasis. Additional rare causes (e.g. fatty acid mitochondrial disorders/ beta oxidation defects) may also be screened in the neonatal period depending on the existing national/local screening program and on the technology used. In recent times, advances in laboratory technology with tandem mass spectrometry has enhanced the identification of newborns with an inherited metabolic disease [12].

A history of consanguinity may suggest a genetic or metabolic disorder. The family history should be exhaustive and include information about familiar hepatic conditions, blood disorders (e.g. hemolysis) or cardiac and vascular anomalies as well.

Further information gathered from the history should include the timing of the onset of jaundice, changes in stool pigmentation and urine color. Delays in referral and diagnosis can be secondary to a possible jaundice decrease over the first weeks of life as the indirect bilirubin declines, giving therefore a false impression that the jaundice is resolving.

Coded tables for comparison of stool color (“stool color card”) [13], may improve communication between examiner and parents. Employment of such tables in Taiwan to diagnose BA in the first 60 days revealed a sensitivity, specificity and predictive value of 89.7, 99.9 % and 28.6 %, respectively [14,15]. In the early course of BA, stools however

may appear normally or intermittently pigmented, hence, stool color should be serially recorded. Whenever possible, urine color and stools should be directly observed by an experienced clinician for a correct and timely clinical assessment.

As shown in **Table 2**, the physical exam should also notice extrahepatic signs like dysmorphic features, birth weight, poor growth, heterotaxy (i.e. the internal thoraco-abdominal organs demonstrate abnormal arrangement across the left-right axis of the body), dermatologic, neurologic, ophthalmic, cardiac, and hematologic signs and symptoms. Palpation of the abdomen should focus on the presence of hepatomegaly, registering organ size and consistency. Should early splenomegaly be present, storage or hematological disorders rather than portal hypertension need to be ruled out. Cardiac murmurs may suggest ALGS or other cardiac anomalies associated with liver disorders, and an ophthalmological evaluation may disclose signs found in certain types of intrahepatic cholestasis. Heterotaxy of abdominal or thoracic organs and malrotation of gut is a condition observed in some forms of BA associated with the polysplenia. Neurologic abnormalities, including irritability, lethargy, poor feeding, hypotonia, or seizures may suggest metabolic and mitochondrial disorders, sepsis or severe liver dysfunction.

The laboratory investigation typically reveals elevated levels of direct (DB) and total bilirubin (TB). Other standard liver tests assessing the presence and severity of the liver disease include hepatobiliary enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma glutamyltranspeptidase (GGT)]. ALT and AST are sensitive indicators of hepatocellular injury but are neither specific nor of prognostic value [2]. However normal values might be suggestive of

neonatal hemochromatosis [so called gestational alloimmune liver disease (GALD)] (see below, section “expert commentary”). Serum AP levels are generally less helpful than serum GGT in the evaluation of cholestatic infants since their normal range levels varies greatly in growing infants. Being AP also a marker for osteoblastic activity, growing children have higher levels than fully grown individuals.

Some forms of PFIC (type 1 and 2) and some other rare cholestatic conditions can have a normal or low GGT. Total serum bile acids are usually elevated except in bile acid synthesis defects which also have low GGT values (see **Figure 2**).

Albumin, and Prothrombin time (PT) ± the International Normalized Ratio (INR) are useful to test the liver's ability to synthesize proteins and/or the severity of vitamin K malabsorption and deficiency secondary to cholestasis. Prolonged PT may cause bleeding which requires prompt intensive management especially in case of cerebral hemorrhage. Neonates presenting with severe coagulopathy should be evaluated especially for galactosemia, hereditary fructose intolerance, tyrosinemia or GALD. Septic work up is also indicated since infection may cause disseminated intravascular coagulopathy.

In **Figure 2** we propose a comprehensive synoptic chart with a workflow for the diagnostic approach to neonatal cholestasis. This takes into account history, physical findings and a limited number of simple old and new aspects of clinical practice. GGT is typically highly elevated in extrahepatic obstructive conditions such as BA or neonatal sclerosing cholangitis (NSC) [5]. In a large series of infants with cholestasis, preoperative levels of GGT were significantly higher in the BA group. The diagnostic value of GGT declined in infants whose age exceeded 121 days. At a cut-off >303 IU/L in 61-90 day

old infants, GGT exhibited 82.8% sensitivity and 81.6% specificity for BA [16]. El-Guindi et al. found that GGT at a cutoff of >286 IU/L has 76.7% sensitivity and 80% specificity for the discrimination of BA [17].

A *high level* of GGT may suggest a diagnosis of several conditions including PFIC type 3, α1-antitrypsin deficiency (A1ATD), ALGS, cystic fibrosis, endocrinopathies, hypoxic neonatal cholestasis. A *low or normal* GGT in the presence of cholestasis is consistent with PFIC types 1 and 2, or an inborn error of bile acid synthesis or metabolism (PFIC 4), particularly when total serum bile acid values are within normal limits. Other rare conditions include Smith Lemli Opitz syndrome, Arthrogriposis-renal dysfunction and cholestasis (ARC syndrome), Transaldolase Deficiency and the more recently described mutations in the tight junction protein 2 gene (*TJP2*) which cause failure of protein localization [18], and microvillus inclusion disease (see below, section “Metabolic and inherited disorders”).

3. **Extrahepatic *vs.* intrahepatic cholestasis**

3.1 **Biliary Atresia**

Biliary atresia is an idiopathic fibrosing cholangiopathy, occurring in 1 in 6,000 to 18,000 live births. It represents the most frequent cause of obstructive jaundice in the first 3 months of life [19-21] and the most common indication for pediatric liver transplantation [5].

Usually, infants with BA appear healthy and well thriving, with persistent jaundice and acholic stools. Their unremarkable clinical condition may sometimes falsely suggest physiologic or breast-feeding jaundice unless a direct bilirubin level is measured. Slightly

higher serum values of direct bilirubin are a possible warning of BA [22]. Up to 20% display associated congenital malformations, including heterotaxy with a/poly-splenia, situs inversus, vena cava agenesis, and anomalous liver vascularity which may help to orientate the correct early diagnosis.

The rate of success in establishing bile drainage in infants with BA is a function of the age of the infant when the hepatoportoenterostomy (HPE) intervention (Kasai procedure) is performed, yielding up to 80% success rate if the procedure is performed below age 30 to 45 days, about 70% if the procedure is carried out within the first 60 days of life and fewer than 20% in those operated when older than 90 days. [1,20,23,24,25,26]. In general, the percentage of success is f jaundice persists after HPE, the 3-year transplant-free survival rate goes down to 20% [27].

Regarding prediction of post-Kasai short-term outcomes, total bilirubin seems to be a predictive biomarker for clinical sequelae of liver disease in the first 2 years of life after surgery. Transplant-free survival at 2 years seems to be significantly higher in patients who have total bilirubin <2.0 mg/dl. Infants with highest values have greater probability of developing early disease progression (ascites, hypoalbuminemia and coagulopathy) suggesting they should be considered for liver transplantation [28].

3.2 **Intrahepatic neonatal cholestasis**

3.2.1**Clinical**

Several conditions may feature cholestasis as a pathological manifestation. These infants often appear acutely ill. Early diagnosis and prompt initiation of a specific therapeutic regimen can improve the prognosis dramatically. Appropriate medical

management and optimization of nutrition are also important, because these measures may prevent complications even when specific treatment is not available.

The differential diagnosis includes anatomic abnormalities, infection, genetic, metabolic and congenital disorders.

Low birth weight, presence of poor (floppy) tone, petechiae or purpura, thrombocytopenia and chorioretinitis are often associated with intrauterine infection. Inability to suck and swallow normally may be a clue to mitochondrial disorders.

Neurologic abnormalities including irritability, lethargy, poor feeding, hypotonia, or seizures can indicate sepsis, intracranial hemorrhage, metabolic (including Zellweger syndrome) and mitochondrial disorders, or severe liver dysfunction resulting in hyperammonemia and encephalopathy (e.g. Ornithine transcarbamylase Deficiency)

When a cardiac murmur is found on physical examination, an echocardiogram should be performed. Structural heart disease is present in about one fourth of patients with ALGS, who generally present peripheral pulmonic stenosis, but also tetralogy of Fallot, pulmonary atresia and truncus arteriosus have been reported [2].

Distinctive facial features (a broad, prominent forehead; deep-set eyes; and a small, pointed chin) may be noted in ALGS, especially post-infancy, and at some extent in Zellweger Syndrome too **(Table 2)**.

In ALGS a careful slit lamp examination may reveal posterior embryotoxon or other anterior chamber abnormalities.In addition to embriotoxon, other ocular manifestations such as chorioretinitis (e.g. Cytomegalovirus infection), cataracts (e.g. galactosemia), "cherry red spots" (e.g. Niemann Pick) or hypoplasia of the optic nerve (e.g. pituitary problems) may be found in various congenital syndromes, in infections or

genetic/metabolic disorders.

A palpable mass in the right upper quadrant may indicate a choledochal cyst.

3.2.2 **Laboratory**

A laboratory work-up for cholestasis caused by a metabolic disorder should also include blood gases, pH, α1-antitrypsin (A1AT), ammonia, lactic acid, ketone bodies, glucose values, plasma and urinary aminoacids levels including tyrosine diagnostic metabolite succinylacetone, acylcarnitine profile, urinary organic acids and reducing agents. Very long chain fatty acids (VLCFA) testing and a peripheral and/or a bone marrow smear should be ordered in suspected cases of Zellweger syndrome and Niemann Pick disease, respectively. Total serum bile acid levels and a urine bile acid profile can identify several rare disorders of bile acid synthesis. A low serum A1AT level requires a phenotype test for protease inhibitor characterization and eventually genotyping [29]. Additional diagnostic tests include a sweat test to detect cystic fibrosis, and a thyroid hormone profile to exclude hypothyroidism (in the context of a pituitary gland dysfunction). Genetic tests for a number of metabolic/genetic conditions, such as ALGS, cystic fibrosis, A1ATD, distinct forms of PFIC, and peroxisomal defects are now commercially available. Currently, DNA sequencing technology allow multiple genetic tests, and it is hoped that in the near future this method will allow to further optimize tests on small amounts of blood at relatively lower cost [30].

3.2.3 **Imaging and biopsy data**

In addition to history, physical examination and laboratory tests, imaging and liver

histopathology are important tools in the evaluation of bile duct patency. Advantages, risks, and timing still remain debated. An abdominal ultrasound examination should be obtained as part of the initial evaluation. This procedure offers a non-invasive means to assess liver structure and size, and to detect an obstructive lesion of the biliary tree or a choledocal cyst [7,31]. Ultrasound findings suggestive of BA include the triangular cord sign (a cone-shaped fibrotic mass above the bifurcation of the portal vein), absence or abnormal gall bladder morphology, lack of gall bladder contraction after oral feeding, non-visualization of the common bile duct, subcapsular hepatic flow. However, these findings cannot be used to establish a definitive diagnosis of BA as they are not sufficiently sensitive or specific [22-39]. Hepatobiliary scintigraphy has been used to confirm biliary tract patency, but its specificity is low (range: 68.5% - 72.2%), limiting therefore its use in differentiating BA from other non-surgical conditions [40-42]. Pre-treatment with phenobarbital for 5 days before the study may increase test sensitivity but it is time-consuming and appears not to add new information to the clinical examination of stools color [43].

The development of a pediatric side-viewing endoscope in the late eighties [44] provided an opportunity to investigate the usefulness of endoscopic retrograde cholangio-pancreatography (ERCP) for the relatively noninvasive study of biliary tree in infants with persistent cryptogenic cholestasis, showing high positive and negative predictive values for BA [9]. This procedure however requires an expert operator with infant endoscopy equipment which is not easily available in many centers. The superiority of ERCP compared with other types of cholangiograms has not been demonstrated.

Magnetic resonance cholangiopancreatography (MRCP) sensitivity and specificity for the

diagnosis of BA are still not fully elucidated [45]. In a recent study with three-dimensional MRCP in infants and neonates sensitivity was excellent (99%) but the specificity was not as high as described in previous reports. This suggests that MRCP can be an effective screening method but should be combined with other modalities to identify BA and distinguish it from other causes of infant jaundice [46].

Globally these tests appear to have little room in the diagnosis of cholestasis at this age. An intra-operative cholangiogram and histological examination is considered the gold standard to diagnose BA [1].

Percutaneous liver biopsy remains an important diagnostic tool in BA. In several single center studies, a diagnosis of BA was correctly suggested by liver biopsy histologic findings in90% to 95% of cases [47,48] based on the findings of bile duct proliferation, bile plugs in portal tract, portal tract edema and fibrosis [5].Histopathological findings in an appropriately timed liver biopsy is the most supportive test in the evaluation of the infant with protracted conjugated hyperbilirubinemia [1] and can also assist in establishing several other causes of neonatal cholestasis. These include A1ATD, ALGS, neonatal sclerosing cholangitis, viral infection, metabolic liver diseases, including PFIC and some storage diseases [49]. Liver tissue may also be used for specific immunohistochemistry to detect A1AT and, more recently, PFIC. Here it can indicate mild or absent Bile Salt Export Anion Pump (BSEP) antibodies and canalicular staining with Multi Drug Resistance 3 (MDR3) in PFIC2 and PFIC3, respectively. However, normal immunostaining does not exclude the diagnosis of PFIC as certain mutations are associated with defects in protein which is otherwise normal in synthesis and expression [50,51].

4. Treatment options

The first objective in the management of infants with cholestasisis prompt implementation of general measures while actively pursuing the recognition of causative diseases which may be successfully treated with medical measuresor surgical intervention [10].

The choleretic effect of ursodeoxycholicacid (UDCA)at a dose of 10-30 mg/kg/day has been found to bebeneficial in many cases of cholestasis independently from their etiologies [2,10,52].

UDCA may also be added toa specific etiological treatment to improve the biliary flow. New prototypical compounds, such as FXR ligands (e.g. obeticholic acid), have not yet been approved for use in pediatric patients [53]. Higher doses of UDCA are occasionally used in cholestasis associated with parenteral nutrition (so called PNAC), although the preferred treatment should be prevention with use of lipid emulsions based on fish oil and administration of UDCA [54,55].

Several conditions have specific dietary treatments (e.g. exclusion of the offending metabolitein galactosemia, hereditary fructose intolerance,and tyrosinemia). In addition to dietary restriction of tyrosine and phenylalanine, tyrosinemia is currently treated with Nitisinone, which acts as an inhibitor of 4-hydroxyphenylpyruvate dioxygenase and prevents the formation of toxic metabolites such as succinyl-acetoacetate in the liver. This treatment has dramatically decreased progression towards cirrhosis and chronic renal disease [56].

In patients with citrin deficiency, who display an unexpected aversion to carbohydrates in contrast with other urea cycle defects, intake of foods rich in proteins and lipids should be

encouraged. In addition to dietary treatment, administration of sodium pyruvate may improve growth [57].

Oral treatment with specific primary bile acids (cholic acid, not UDCA) leads to normalization of liver functions in most patients with defects of bile acid synthesis [58].

Patients affected by Cholesterol Ester Storage Disease (CESD) and Wolman disease might benefit from human recombinant lysosomal acid lipase (LAL), a recently available enzyme-replacement therapy to slow disease progression and improve long-term survival [59]. In Niemann-Pick typeC, therapy with Miglustat (a small imino-sugar molecule that reversibly inhibits glycosphingolipid synthesis) has been approved for the management of associated neurologic manifestations in several countries [60].

Urinary tract infection (UTI) discovered as part of an evaluation for neonatal sepsis may display jaundice, fever and failure to thrive. According to clinical findings, laboratory tests and cultures, antibiotic therapy should be considered, and is often curative [61].

When pituitary insufficiency is suspected, the diagnosis should be confirmed, and identified hormonal abnormalities treated with appropriate hormonal replacement [62].

GALD (previously denominated Neonatal hemochromatosis) is a severe neonatal liver disease which is accompanied by extrahepatic siderosis caused, in most cases, by a complement-mediated fetal liver injury, triggered by maternal IgG antibodies directed against fetal hepatocytes. The treatment of GALD, based until recently on anti-oxidants and chelation therapy, is being replaced with much more effective exchange transfusions and intravenous immunoglobulin therapies [63]. Identification of infants with GALD is important because maternal high-dose Immunoglobulin injections are highly effective in preventing the recurrence of the disease in subsequent pregnancies (see below, section

“expert commentary”) [5].

4.1 **Pruritus**

The cause of pruritus in cholestasis is unclear but decreasing the levels of bile acids in blood has shown improvement of symptoms.

Treatment with UDCA at the dose of 10-30 mg/kg/day [52] or 600 mg/m^2/day [64] promotes excretion of bile acid and consequently reduces the severity of pruritus.

Its anti-cholestatic properties mainly depend on choleretic effects obtained by stimulating hepatocellular secretion of bile acids and organic anions post-translationally, and by inducing a bicarbonate-rich protection alongside the biliary tree [2,10,54,55]. Anti-apoptotic and anti-inflammatory actions may contribute to support its beneficial anticholestatic action.

In cases in which UDCA therapy proves ineffective,enzymatic inducers [rifampicin (5 mg/kg/day, progressively increasing doses up to 10 and 20 mg/Kg/day) and phenobarbital (3-10 mg/kg/day)] [2,10,65,52], chelating bile acid resins [cholestyramine at a dose of 240 mg/kg/day (initial dose usually limited to ≈1 g daily) in three divided doses, adjusted to a maximum dosage of 4 g daily in children ≤ age 10 years and a maximum of 8 g daily in children > 10 year] [10,65,66], antihistamines (e.g. hydroxyzine 2 mg/kg/day), and opioids antagonists (naltrexone, 1-2 mg/kg/day) [65] should be considered. Albeit all these medications can have a symptomatic benefit, they do have possible side effects as well.

Finally, preliminary data showed that serotonin reuptake inhibitor sertraline (1-4 mg/kg//day) is useful also in pediatric refractory cholestatic pruritus [67]

Early surgical partial/total biliary diversion may represent an option in severely cholestatic children with PFIC and ALGS to avoid liver transplantation. Interrupting the enterohepatic circulation of bile acids has the potential to reduce the total amount of bile acids in an individual's body resulting useful as a treatment for the pruritus. Surgical interruption of the enterohepatic circulation using partial external biliary diversion(PEBD) has been a mainstay of treatment for cholestatic syndromes, including ALGS and PFIC disease, provided it is performed before cirrhotic evolution. Symptomatically these patients experienced less pruritus. The biliary losses caused by the PEBD can lead to hydrolectrolytic disturbances which require a compensation by a solution rich in bicarbonate and sodium. Malabsorption and deficiency in fat-soluble vitamins have also been reported, requiring careful monitoring and adaptation of supplementation [68].
In spite of these measures, pruritus may still be uncontrollable and in this case liver transplantation may be required also when liver function is still acceptable if quality of life is impaired.

4.2 **Nutrition**

Infants with neonatal cholestasis often exhibit growth failure due to impaired absorption of fats, impaired metabolism of proteins and carbohydrates, and increased metabolic demand. In the presence of direct-reacting serum bilirubin levels greater than 2 mg/dl, the diet should be adequately supplemented with fat-soluble vitamins (FSV) [28]. Vitamin A at 3000-10000 IU/day [5], vitamin D (cholecalciferol)at 800-5000 IU/day or 1,25 $(OH)_2$cholecalciferolat 0.05–0.2 µg /kg/day [5], Vitamin E at 15-25 IU/kg/day, Vitamin K at 2.5 - 5 mg/day from twice a week to every day [2,5]. Serum vitamin and prothrombin

levels should be monitored to allow proper adjustment of dosages to the specific needs of the patient [69,70]. Possible side effects should be treated promptly. Vitamin A requires close monitoring during supplementation because of its potential for neurologic and hepatic toxicity.

Co-administration of micellar vitamin E or aqueous vitamin E formulations may improve vitamin E absorption in infants and children with cholestasis. In turn, absorption of vitamin E can be improved with an orally bioavailable source, e.g. α-tocopheryl polyethylene glycol 1000 succinate (TPGS). TPGS restores and/or maintains adequate serum vitamin E levels in the majority of children with cholestasis, avoiding the need for intramuscular injections [71]. In spite of their monitored administration as FSV preparation made with TPGS in one study, the prevalence of multiple FSV deficiency in infants with persistent hyperbilirubinemia six months after HPE, was 100%, 79%, 50%, and 46%, respectively, for vitamins A, D, E and K [72]. Advances in this field were recently reviewed [68-70].

The role of serum bile acid as a surrogate marker to guide monitoring of FSV deficiency in chronic intra hepatic cholestasis is still undefined. In infants with BA total serum bilirubin instead appears a better predictor of FSV deficiency than serum bile acids, although it is still imperfect [73].

In infants with cholestasis, an adequate caloric intake should be approximately 125% - 140% higher than the recommended diet for healthy babies, based on ideal body weight. Calories may be supplemented with glucose polymers. Unless hepatic encephalopathy is present, protein intake may be > 2-4 g/kg/day. To counteract cholestasis and liver failure, a source of lipids such as medium-chain triglycerides (MCT) should be given to improve

fat intestinal absorption[71].Ready-to-use commercial preparations with the above characteristics enriched with branched-chain amino acids are available, however, are costly.

In patients with chronic unresolving cholestasis, when oral feedings do not provide an adequate caloric intake, night-time/continuous drip nasogastric feedings using an enteral pump, or parenteral feeding may be necessary to maintain optimal nutritional status. This is important in candidates for a liver transplant, since preoperative nutritional status has been shown to be an important prognostic factor [65,74].

Ascites, impairment of the coagulation system and portosystemic collateral circulation due to portal hypertension have been reported as contraindications to PEG placement. [74] Newer approaches such as low profile gastrostomy are still scarcely documented in this category of pediatric patients [75].

The use of weight-for-age and weight-for-height measurements in children with chronic liver disease can be inaccurate for nutritional evaluation due to visceromegaly, subclinical edema and/or ascites. Serial triceps skinfold thickness and midarm circumference measurements compared with age- and height-matched normal values are used to estimate body fat and muscle bulk, respectively. With malnutrition, these measurements decline before changes in weight or height are apparent [74]

Patients with intractable cholestasis, should also receive earlier or accelerated pre-transplant vaccinations to protect against infection during intensive post-transplantation immunosuppressive therapy. Pre-transplantation immunization is effective in preventing serious infections, especially during the initial 6 months following transplantation. Live vaccines (Measles, Mumps, Rubella, and Varicella) should be administered if

transplantation is not anticipated within 4 weeks [76]. Post-transplantation live-vaccine administration is effective and safe in patients who receive modest immunosuppressive treatment [77].

5. Conclusions

In conclusion, many hepatobiliary disorders, the most common being BA, may present with cholestasis during the neonatal period. Cholestasis and its serious complications pose a serious threat to affected newborns and infants. Cholestasis must be suspected, and thoroughly investigated to initiate timely treatment. Potentially life-saving/disease-specific pharmacological and surgical therapeutic approaches are currently available. Advances in understanding of bile acids synthesis and hepatobiliary transport mechanisms have started clarifying fundamental aspects of inherited and acquired cholestasis, laying the foundation for the development of highly effective specific therapies.

6. Expert commentary

Neonatal cholestasis is a relatively uncommon, potentially serious condition. Early detection by the primary care provider and a timely diagnosis by the pediatric gastroenterologist are therefore important pre-conditions for optimal treatment and favorable prognosis. Clinical findings and standard laboratory tests need to be critically evaluated as the initial steps in the differential diagnostic process.

- General acceptance and implementation of recent NASPGHAN/ESPGHAN joint recommendations [1] is expected to further reduce the time to diagnosis of pediatric liver

diseases, leading to improved outcomes. The flow-chart we propose in our expert review article is inspired to these recommendations and may help guide the reader to the suggested clinical approach.

Among the many rare conditions cited, we would like to focus here on two forms that have presented new opportunities of diagnosis and treatment.

- Neonatal sclerosing cholangitis, a severe form of cholestasis associated with ichthyosis (so called “neonatal ichthyosis and sclerosing cholangitis” (NISCH) syndrome), is caused by mutations of the CLDN1 gene encoding for claudin-1, a tight-junction protein, with increased para-cellular permeability and bile regurgitation leading to hepatocellular damage [78]. Most recently, mutations in a gene encoding for DCDC2, a signaling and structural protein in the primary cilia of cholangiocytes, were identified in a substantial proportion of patients with NSC disease not associated with ichthyosis. Accordingly, NSC represents now a novel liver-based ciliopathy to be taken in consideration when evaluating children with high serum GGT chronic cholestasis [79].

- Neonatal hemochromatosis (NH) is a disorder with high perinatal mortality and morbidity rates. As previously outlined, advances in understanding its underlying pathogenetic mechanisms have profoundly changed the management and outcome of NH. The disorder is now regarded as GALD**.** Antenatal maternal administration of intravenous immunoglobulins starting at 14thweek gestation has been shown to prevent the development of NH in subsequent pregnancies. Postnatal treatment with relatively ineffective anti-oxidants and chelation agents to reduce oxidative (extra-)hepatic injury due to iron overload, is being increasingly and successfully replaced by exchange transfusions and intravenous immunoglobulin therapy [63]. This field is still making

significant progress [80].

7. Five-year view

7.1 New and prospective diagnostic options

7.1.1 Biliary atresia

The pathogenesis of BA remains a mystery ranging from genetic, to infectious, autoimmune, and inflammatory, but no single etiologic factor has been hitherto identified. A most recent report suggests that differentially expressed miRNAs may play critical roles by regulating their target genes, and at least two may become BA diagnostic markers [84].

Due to the importance of early recognition and surgical treatment, a number of diagnostic tests have been proposed.

Stool color cards have been shown to be helpful in making the diagnosis [35,81,82], but problems with interpretation remain [83]. Recently proposed use of images of stool obtained with digital cameras appear to have higher predictive accuracy compared with the standard stool color card, suggesting that they may represent a useful tool for correct detection of cholestasis in infants [85].

The investigation of diagnostic performance of different clinical and laboratory parameters have permitted to individuate a twelve-point scoring system based on clinical, laboratory, ultrasonographic, and histopathological parameters to simplify the diagnostic path in case of cholestasis. This scoring system seems to be able to accurately separate infants with BA and those with non-BA [17]. Finally, the quantitative measurement of

urinary urobilinogen (significantly lower in the BA patients) has been suggested as a cheap and non-invasive test to discriminate BA from other causes of neonatal cholestatic, especially if combined with measurement of GGT [86].

7.1.2 **Metabolic and inherited disorders**

A thorough clinical examination and history, and a small number of laboratory tests may already suggest diagnosis in most instances. “Red flags” that require early intervention are failure to thrive, poor feeding, lethargy, hypoglycemia (especially non-ketotic) and hyperammonemia. Nevertheless, diagnosis of metabolic and inherited disorders can be challenging. For example, diagnosing Niemann-Pick type C as a cause of cholestasis may be hampered by the variable and non-specific nature of signs and symptoms. Two oxysterols (cholestane-3β,5α,6β-triol and 7-ketocholesterol) recently proposed as biomarkers might facilitate the diagnosis [87].

Metabolic-genetic disorders associated with large gene sizes and lack of mutational hot-spots may impede an easy survey for disease-causing mutations in clinical practice. As stated in the recent joint recommendations [1], current diagnostic approaches should consider DNA sequencing in the proper clinical context. The Jaundice Chip resequencing array initially developed at Cincinnati Children’s Hospital and introduced several years ago, provides diagnostic data for several disorders [genes *SERPINA1*(A1ATD), *JAG1* (ALGS), *ATP8B1* (type 1 PFIC), *ABCB11* (type 2 PFIC), and *ABCB4*(type 3 PFIC)][88,89]. The Advances in next generation sequencing (NGS) technology have produced newer cholestasis panels with a comprehensive genetic test menu for a wide range of heritable liver diseases, such as 18 genes ± a specific bile acid defects

panel with 3 genes [90].The included genes (ABCB11, ABCB4, ABCC2, AKR1D1, ATP8B1, BAAT, CLDN1, CYP7B1, EPHX1, HSD3B7, JAG1, NOTCH2, SERPINA1, SLC10A1, SLC25A13, TJP2, VIPAS39, VPS33B) are intended for the screening for ALGS, A1ATD, citrin deficiency, PFIC, ARC, NISCH and bile acid synthesis defects. Currently it is possible to study even larger panels of genes involved in adult and neonatal cholestasis [91,92].

Nevertheless, a high index of suspicion will continue to be an essential aspect of the search for the correct diagnosis. Clinicians should be aware that guidelines may not be adequate, particularly in the genetic/metabolic cholestatic conditions category, in which known genes cannot account for all familial cases. For example, mutations in NR1H4, which encodes the farnesoid X receptor (FXR) the bile acid-activated nuclear hormone receptor that regulates bile acid metabolism, have been reported very recently in 4 individuals from 2 unrelated families with low to normal GGT-neonatal cholestasis resembling PFIC 1-2 [93]. In particular, homozygous loss of FXR function due to NR1H4 mutations causes a low GGT form of severe PFIC 1-2. The clinical features of NR1H4-related cholestasis include neonatal onset with rapid progression to end-stage liver disease, early onset vitamin K-independent coagulopathy, low-to-normal serum GGT, high serum bile salts, elevated serum alpha-fetoprotein and undetectable liver BSEP expression.

Again, a new familiar defect in bile acid synthesis affecting peroxisomal enzymes [94] and a low GGT PFIC 1 like condition due to myosin 5B (MYO5B) mutations in the setting of Microvillus inclusion disease (MVID) have been recently described [95,96]. More will

be discovered in the future which will further shorten the list of idiopathic neonatal cholestasis.

7.2 **New and prospective therapeutic options**

7.2.1 **Adjuvant medical therapy after Kasai portoenterostomy**

The role of adjuvant medical therapy (steroids, prophylactic antibiotics and choleretic agents) in the postoperative management of the Kasai surgical repair procedure in BA is controversial. Here we discuss the main aspects.

7.2.2 **Adjuvant steroid therapy**

Steroids have become popular in the post-operative management of BA although their benefits remain uncertain. A 2011 meta-analysis found no significant effect of steroid over standard therapyin normalizing serum bilirubin levels at 6 months nor in delaying the need for liver transplantation following a HPE [97].

The position of the American Association for the Study of Liver Diseases, American Society of Transplantation and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition is that high-dose corticosteroid therapy initiated within 72 hours of HPE is not recommended because not shown to improve bile drainage at 6 months, nor did it enhance transplant-free survival up to 2 years of age [98], although a small clinical benefit could not be excluded [99].

A more recent systematic review and meta-analysis to determine effect of treatment with steroids on bile drainage post HPE showed that prednisolone 4-5mg/kg/day for 1-2 weeks, followed by tapering doses, accelerated the regression of jaundice, especially in

the subgroup who underwent HPE before70 days of age [100]. Adjuvant steroid treatment following HPE may improve short-term (≤1 year) clearance rate of jaundice, but without significant effects on long-term (≥2 years) clearance rate of jaundice and native liver survival rate [101].

7.2.3 **Adjuvant therapy with choleretic agents**

First preliminary reports suggested that early postoperative administration of 1st generation choleretics (i.e. phenobarbital and cholestyramine) had no effect on residual cholestasis in BA after HPE procedure [102]. UDCA is metabolized to lithocholic acid which promotes DNA strand breakage and cell transformation, and is co-mutagenic. Caution therefore is needed with the use of UDCA, considering that of the narrow difference between the recommended therapeutic (13 mg/kg/day) and toxic dose (approximately 28 mg/kg/day) [103,104].

The new FXR ligands (e.g. obeticholic acid) appear to have surpassed UDCA by combining the choleretic effects with substantial suppression of bile acid synthesis and increased bile acid-independent bile flow [54]. As shown in **figure 1,** these new agents are capable of turning on FXR in both ileum and liver. Suppression of hepatic bile acid synthesis happens via induction of ileal endocrine hormone FGF19-mediated CYP7A1 suppression and via FXR-short heterodimer partner 1 (SHP)-mediated CYP7A1 repression. Altogether this reduces bile acid pool size. Additionally, FXR agonists may limit cellular bile acid accumulation by blocking bile acid uptake at ileal (via apical sodium dependent bile acid transporter, ASBT) and hepatic (via sodium taurocholate co-transporting polypeptide, NTCP) level and by implementing bile acid export (via organic

solute transporter α/β,OSTα/β). As a consequence, bile acids waste into feces and decline in systemic circulation [54].A preliminary pediatric study to assess the dosage and effects of obeticholic acid in BA is now in progress [105]

7.3**Cholestatic pruritus**

Cholestatic pruritus is an extremely tormenting symptom especially in disorders such as ALGS, PFIC, NSC, and occasionally, in infants with BA. Pruritus may degrade the quality of life of affected children to the point of becoming an indication for liver transplantation even when liver functions are still preserved. Several biologically active agents have been proposed as pruritogenic, including histamine, bile salts, endogenous opioids, and progesterone metabolites, but none have proven to be a causal mediator. While the different pathogenetic mechanisms in the development of pruritus are not fully understood, new findings help to clarify the molecular mechanisms and receptors involved in the regulation of the enterohepatic circulation of bile acids, and suggest new targeted therapeutic strategies. These new approaches suggest that overall anti-cholestatic effects may be achieved with agents which target impaired bile flow, combined with others that reduce bile acid accumulation and decrease bile acid pool size (e.g. obeticholic acid).

Sertraline, a serotonin reuptake inhibitor widely used as an antidepressant drug, has been recommended for treatment of pruritus in adults with chronic cholestasis. A promising pilot study of sertraline therapy for refractory pruritus in pediatric patients with cholestasis reported improvement in 75% of cases without significant adverse effects [67]. Lysophosphatidic acid (LPA), a potent neuronal activator, is typically increased in

cholestatic adult patients with pruritus. The major source of LPA is lysophospholipase autotaxin (ATX) and serum ATX activity has been found to correlate with itch intensity. These associative findings suggest that ATX may be a promising target for future investigations [106].

Finally, hereditary cholestatic disorders caused by mutations causing misfoldedbile acid transporters (e.g. ATP8B1, MDR3, and BSEP) might find in the future an appealing answer in chemical chaperones to improve targeting of these proteins [54,106].

8. **Key issues**

- Infants with prolonged jaundice (>14 days) must be evaluated for cholestasis using a thorough clinical examination and a few simple laboratory tests (total and fractionated serum bilirubin levels, hepatobiliary enzymes, serum albumin determinations, and coagulation tests).
- Imaging and diagnostic procedures, including liver biopsy, and when necessary, exploratory laparotomy are available. To establish a precise diagnosis, cholestatic infants must be further investigated using newer specific biochemical, and genetic tests.
- When treatment is available, it must be initiated as soon as possible, and include ursodeoxycholic acid as well.
- Caloric intake should be in excess of their commended dietary intake for healthy infants, using MCT supplements as a rich and readily absorbable source of calories.
- Adequate supplementation with vitamins A, D, E, and K must be provided and closely monitored for potential complications.
- Recent advances in understanding the basic molecular mechanisms of cholestasis and

pruritus are opening the way for newer targeted therapeutic approaches.

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Figures legends

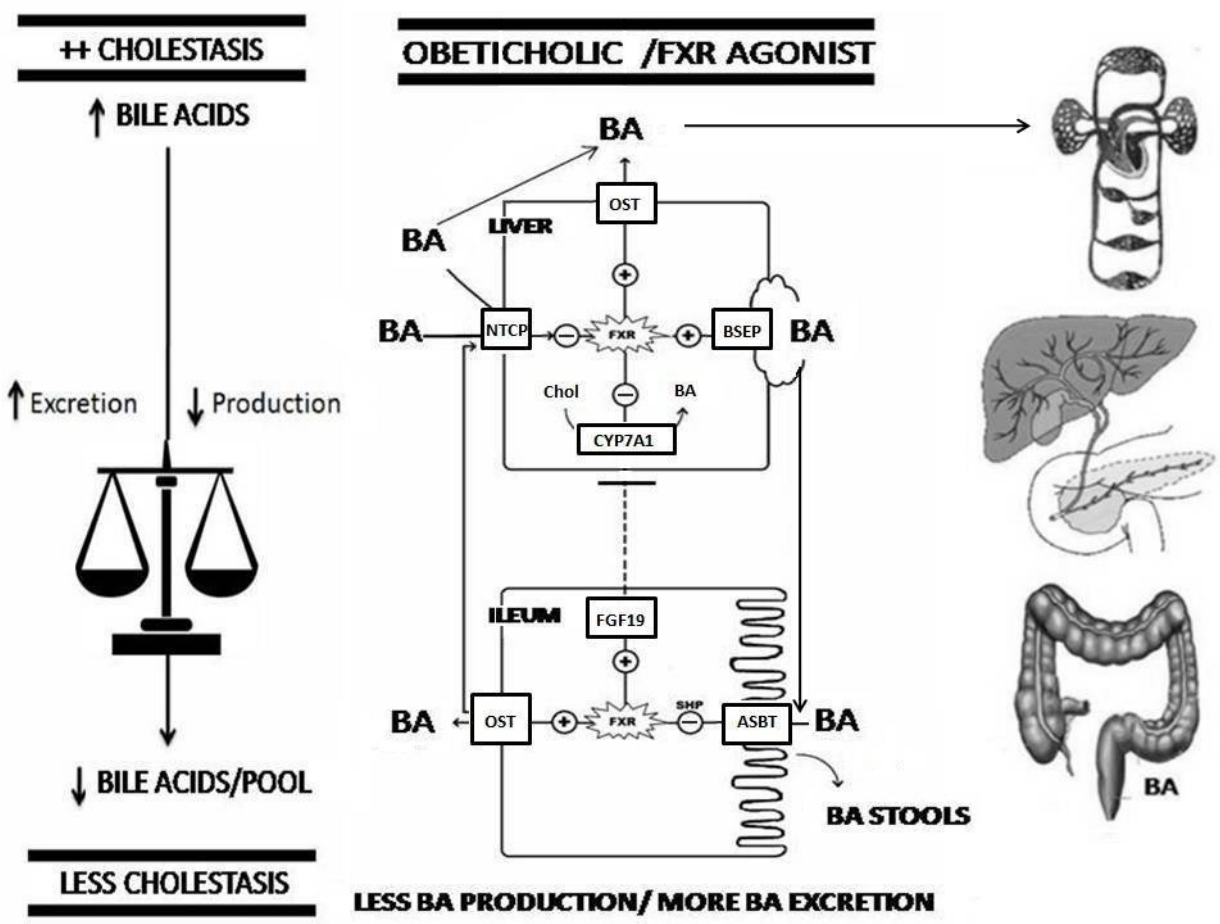

Figure 1.

New choleretic agent Obeticholic acid turns on Farnesoid X receptor (**FXR**) in ileum and in liver. Suppression of hepatic bile acid (**BA**) synthesis happens via induction of ileal Fibroblast growth factor 19 (**FGF19**)-mediated cytochrome P450 7A1(**CYP7A1**) suppression, and via FXR-short heterodimer partner 1 (**SHP**)-mediated CYP7A1 repression. Altogether this leads to a reduction of bile acid pool size.

Additionally, FXR agonists limit cellular bile acid accumulation by blocking bile acid uptake at ileal (via apical sodium dependent bile acid transporter, **ASBT**) and hepatic (via sodium taurocholate co-transporting polypeptide, **NTCP**) level, and by implementing bile

acid export (via organic solute transporter α/β,**OST**α/β). As a consequence, bile acids tend to waste into feces and don't reach systemic circulation.

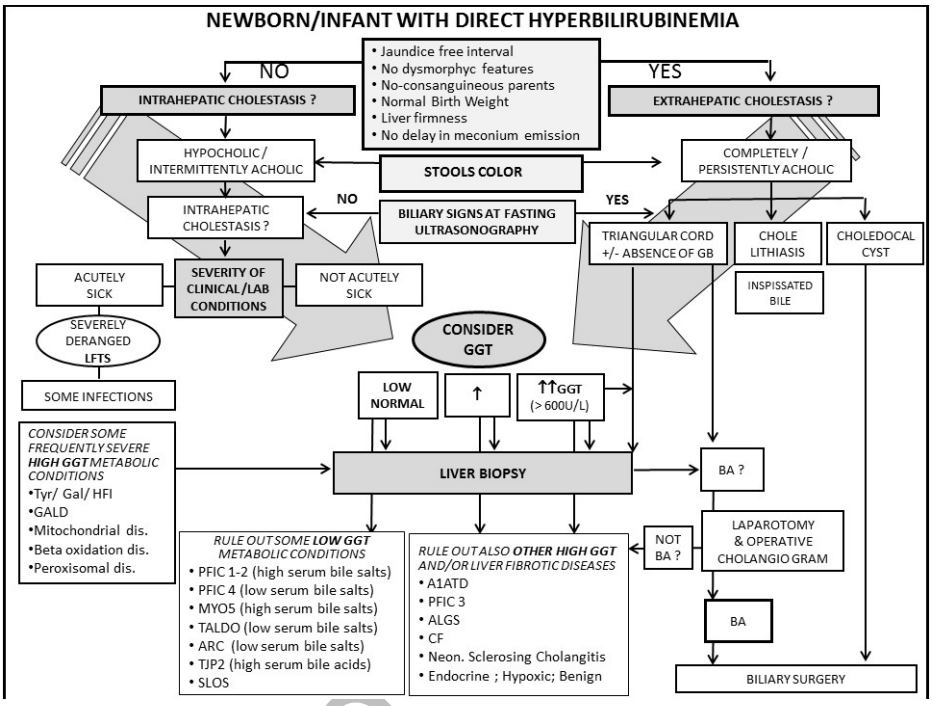

Figure 2.

Flow chart for Formula-fed infant jaundiced. Cholestasis is suspected on the basis of prolonged jaundice (>14 days), acholic/hypocholic stools, dark urine, hepatomegaly, direct bilirubin > 20% total bilirubin

Abbreviations to figure 2:A1ATD = α1-antitrypsin deficiency, **ALGS**= Alagille syndrome, **ARC=** arthrogryposis, renal dysfunction and cholestasis, **BA=** Biliary atresia**, CF=** Cystic Fibrosis, **Gal =** Galactosemia, **GALD =**gestational alloimmune liver disease, **GB=**Gallbladder, **GGT=** Gamma-Glutamyltranspeptidase, **HFI =** hereditary fructose intolerance, **LFTs=** Liver function tests**, PFIC=** Progressive familiar intrahepatic

cholestasis**, SLOS=** Smith-Lemli-Opitz, **TALDO=** Transaldolase Deficiency**, TJP2=** Tight junction protein2, **Tyr=** Tyrosinemia

TABLE 1. Causes of neonatal cholestasis

Infections	**Viral:** Adenovirus, CMV, Coxsackie, EBV, Echovirus, HAV, HBV, HSV, HIV, Parvovirus, Reovirus, Rubella **Bacterial:** Urinary tract infection, Sepsis, Listeriosis, Syphilis, TBC **Parasitic:** Malaria, Toxoplasmosis
Bile duct anomalies	BA, Choledochal cyst, Ductal Plate malformation, Non-syndromic bile duct paucity, NSC
Endocrine disorders	Hypopituitarism, Hypothyroidism
Genetic and metabolic disorders	A1ATD ALGS Arginase deficiency Bile acid synthetic defects (or PFIC 4) (e.g.3-beta-hydroxy-delta-5-c27-steroid oxidoreductase deficiency) Cholesterol synthesis defects Citrin deficiency Cystic fibrosis Fatty acid oxidation defects (SCAD, LCAD) Galactosemia Hereditary fructose intolerance Lipid storage diseases (e.g. Wolman, Gaucher, Farber) Mitochondrial respiratory chain disorders Mutations in TJP2 GALD Niemann-Pick disease type C Ornithine transcarbamylase Deficiency Peroxisomal disorders (including Zellweger syndrome) PFIC 1, 2, 3, 4 (bile acids synthesis defect) and MYO5B mutation Trisomy 13, 18, or 21, Turner syndrome Tyrosinemia Urea cycle defects
Neoplastic disorders	Neonatal leukemia, Histiocytosis X, Neuroblastoma, Hepatoblastoma
Miscellaneous	Toxic: parenteral nutrition, drugs, Choledocholithiasis, gallstones, hemolysis and inspissated bile syndrome

Abbreviations to table1: **A1ATD**= α1-antitrypsin deficiency, **ALGS=** Alagille syndrome, **BA=**Biliary atresia, **CMV=**Cytomegalovirus, **EBV=**Epstein Bar Virus, **GALD =**gestational alloimmune liver disease, **HAV=**Hepatitis A virus, **HBV=**Hepatitis B virus, **HIV=**Human immuno-deficiency virus, **HSV**=Herpes simplex virus, **LCAD=** long-chain acyl-CoA dehydrogenase, **NSC=**Neonatal sclerosing cholangitis, **PFIC =** progressive familial intrahepatic cholestasis, **SCAD=** Short-chain acyl-CoA dehydrogenase, **TBC =** Tuberculosis, **TJP2=** Tight junction protein2

TABLE 2. More common parameters of history and physical examination to evaluate in cholestasis

	Parameters of clinical interest	**Possible clinical finding**
Family history	Consanguinity	Increased risk of autosomal disorders
Personal history	Cholestasis of pregnancy	Possible mother heterozygote for PFIC
	Maternal infection in pregnancy	Toxoplasmosis, Rubella, Cytomegalovirus, Herpes simplex
	Low birth weight or SGA	Intrahepatic cholestasis
	Neonatal infection	Sepsis related cholestasis
	Newborn screeening	Cystic fibrosis, galactosemia or hypothyroidism
	Parenteral nutrition	PNAC
	Delayed meconium emission	Cystic fibrosis, hypothyroidism
	Vomiting, lethargy, poor feeding, hypotonia, hypoglicemia	Metabolic disease, sepsis
Physical examination	Ill appearance	Infection, metabolic disease
	Dysmorphic findings: a. broad nasal bridge, triangular facies, deep set eyes, pointed chin, prominent forehead b. flattened face, broad nasal bridge, high forehead, upslanting palpebral fissures, epichantal folds, microcephaly or macrocephaly, protruding tongue, neck skinfolds, cataracts, glaucoma, nystagmus	a. ALGS (facial features are often non-specific and can make difficult the individualization of clinical features in neonates) b. Zellweger Syndrome
	Cardiac murmur Peripheral pulmonic stenosis Fallot tetralogy	ALGS
	Hearing evaluation	PFIC 1, TJP2
	Hepatomegaly, liver increased consistency, umbilical hernia	BA
	Abdominal mass	Choledochal cyst
	Splenomegaly	Cirrhosis; Metabolic storage disease

	Stool color	Acholic or hypocholic stools
	Fundus oculi or slit lamp anomalies	Infections ALGS Niemann-Pick disease type C
	Neurological manifestation as language development delay and a pyramidal syndrome.	ARC, genetic disease

Abbreviations to table 2: ALGS= Alagille syndrome; **ARC=**Arthrogryposis, renal dysfunction and cholestasis; **BA=**Biliary atresia, **PFIC=** Progressive familiar intrahepatic cholestasis; **PNAC=** Parenteral nutrition–associated cholestasis; **SGA=** Small for gestational age; **TJP2=** Tight junction protein 2