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"Recent Developments in diagnostics and treatment of neonatal cholestasis"

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Abstract

Neonatal cholestasis is characterized by conjugated hyperbilirubinemia in the newborn and young infant and is a sign common to over 100 hepatobiliary and/or metabolic disorders. A timely evaluation for its etiology is critical in order to quickly identify treatable causes such as biliary atresia, many of which benefit from early therapy. An expanding group of molecularly defined disorders involving bile formation, canalicular transporters, tight junction proteins and inborn errors of metabolism are being continuously discovered because of advances in genetic testing and bioinformatics. The advent of next generation sequencing has transformed our ability to test for multiple genes and whole exome or whole genome sequencing within days to weeks, enabling rapid and affordable molecular diagnosis for disorders that cannot be directly diagnosed from standard blood tests or liver biopsy. Thus, our diagnostic algorithms for neonatal cholestasis are undergoing transformation, moving genetic sequencing to earlier in the evaluation pathway once biliary atresia, "red flag" disorders and treatable disorders are excluded. Current therapies focus on promoting bile flow, reducing pruritus, ensuring optimal nutrition, and monitoring for complications, without addressing the underlying cause of cholestasis in most instances. Our improved understanding of bile formation and the enterohepatic circulation of bile acids has led to emerging therapies for cholestasis which require appropriate pediatric clinical trials. Despite these advances, the cause and optimal therapy for biliary atresia remain elusive. The goals of this review are to outline the etiologies, diagnostic pathways and current and emerging management strategies for neonatal cholestasis.

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Declaration of Competing Interest

None

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Introduction

A subset of infants who present with jaundice have elevated serum direct or conjugated bilirubin concentrations, which represents impaired bile formation or flow (cholestasis) and is not physiological (normal). Conditions causing neonatal cholestasis include surgical and non-surgical disorders, some of which require rapid diagnosis and institution of treatment to avoid irreversible injury to other organs and to allow for best possible outcomes from surgical intervention for biliary atresia (BA). In this review, newer genetic causes of neonatal cholestasis will be outlined, current and future diagnostic paradigms will be suggested, and emerging therapeutic approaches will be described.

Definition of Cholestasis in Neonates

Cholestasis in infancy is defined as serum conjugated/direct bilirubin level >1 mg/dL and > 20% of the total bilirubin[1]. Although historically 2 mg/dL had been arbitrarily used as a threshold for conjugated/direct bilirubin, this has recently been reduced to 1 mg/dL to more accurately reflect clinical experience[2–4]. Recent reports suggest that during the first 5 days of life lower direct/conjugated bilirubin levels (>0.3–0.5 mg/dl) and direct/conjugated bilirubin >10% of total bilirubin are abnormal and should raise the suspicion for cholestasis at this age and require further evaluation[2–4]. Jaundice beyond 2 weeks of age in a breast-fed infant or at 2 weeks of age in a formula-fed infant should elicit fractionation of the serum bilirubin to differentiate the much more common breast milk-associated indirect hyperbilirubinemia from cholestasis. If conjugated/direct hyperbilirubinemia is identified, further evaluation for hepatobiliary causes should proceed immediately. The diagnostic pathway for neonatal cholestasis has evolved in recent years, largely because of the expanding identification of new genetic causes of cholestasis, the advent of next generation sequencing to identify genetic variants, the recognition of best outcomes for BA when hepatoportoenterostomy (HPE) is performed before 30–45 days of life[5–7], and emerging new potential therapies.

Causes of Neonatal Cholestasis

The frequency of neonatal cholestasis has been estimated to be 1 in every 2,500 live births[8, 9]. Etiologic categories include both extrahepatic and intrahepatic disorders, including anatomic obstruction of the biliary system (BA, choledochal cyst, cholelithiasis), neonatal infections, genetic and inborn errors of metabolism, endocrine disorders, toxin and drug exposures, hypoxia/ischemia, idiopathic neonatal hepatitis (INH) now referred to as transient neonatal cholestasis (TNC), and other miscellaneous causes[10] (Table 1). Although there are over 100 conditions that can present with cholestasis, establishing a prompt diagnosis is critical for optimal outcomes so focusing on the most common etiologies may be of benefit. BA accounts for 25–45% of cases; Alagille syndrome (arteriohepatic dysplasia; syndromic

paucity of interlobular bile ducts) in 2–14%; genetic and metabolic diseases including α1-antitrypsin (A1AT) deficiency, cystic fibrosis, tyrosinemia, galactosemia, progressive familial intrahepatic cholestasis (PFIC), bile acid metabolism disorders, Niemann-Pick type C and citrin deficiency in 10–20%; hypopituitarism up to 5%; inspissated bile syndrome; parenteral nutrition associated cholestasis; and INH/TNC[11–13] Urinary tract infection, bacteremia, herpes simplex virus, and cytomegalovirus are uncommon but important treatable etiologies. In preterm infants, cholestasis may occur in 10–20% because of immaturity of the enterohepatic circulation, absence of feedings, intestinal inflammation or dysfunction, repeated bacterial or fungal infections, and use of parenteral nutrition[14–17].

A better understanding of genetic disorders of bile formation and biliary physiology has been clarified over the past 10 years primarily because of technological advances in gene sequencing, advanced microscopy and cell biology. Many sites involved in the enterohepatic circulation of bile acids within the hepatocyte, cholangiocyte and enterocyte that regulate bile acid synthesis, transport and metabolism can lead to neonatal cholestasis (Figure 1). For example, many infants previously labeled as INH/TNC have been found to harbor disease-causing variants (either one or two alleles) in recently discovered genes, such as the those coding for canalicular transporters: ATP8B1 coding for FIC1, ABCB11 coding for bile salt export protein (BSEP), and ABCB4 coding for multidrug resistance protein 3 (MDR3), a phospholipid flippase[11],[18–21]. Moreover, the bile acid synthesis and conjugation enzymatic pathways have been fully defined, with mutations in many of the genes coding for these enzymes now identified to cause neonatal cholestasis (AKR1D1, AMACR, CYP7B1, HSD3B7, CYP7A1, CYP27A1, BAAT, SLC27A5). PFIC was originally a clinical description for idiopathic neonatal cholestatic conditions associated with progressive cholestasis and hepatic fibrosis, generally with normal/low serum γ-glutamyl transpeptidase (GGTP) and cholesterol concentrations and familial occurrence. It is now clear that there are over 10 genes (Table 1) that can cause a progressive cholestatic disease beginning in infancy consistent with PFIC, straining the original classification of PFIC 1 (ATP8B1), PFIC 2 (ABCB11) and PFIC 3 (ABCB4). Many of these genes have enlightened our understanding of the role of the cytoskeleton and tight junctions in bile formation (TJP2, MYO5B, UNC45, USP53, KIF12, PLEC, DCDC2), of the critical role of nuclear hormone receptors in the regulation of bile secretion (NR1H4) and of the role of mitochondrial energy production in promoting bile acid transport (DGUOK, MPV17, POLG). In addition to achieving a precise diagnosis and being able to provide families with genetic testing, defining the genetic etiology of each cholestatic infant will potentially allow for application of newer therapies based on specific gene mutations, promoting precision medical treatment to achieve optimal outcomes.

Diagnostic Evaluation

Is this jaundice cholestasis?

The timing of initial differentiation of indirect from direct/conjugated hyperbilirubinemia is the first question that must be addressed in the evaluation of a jaundiced infant. Breast milk-associated jaundice (indirect hyperbilirubinemia) can be present in 10–20% of two-week old breast milk-fed infants[22], thus being >300 times more common than cholestasis at this

age. For this reason, cholestasis frequently goes unnoticed at this age and the breast-fed infant is labeled as having breast milk-associated jaundice. As the indirect hyperbilirubinemia improves over the next few weeks, the jaundice appears to improve despite the possibility of underlying cholestasis. Thus, many cholestatic infants who are breast-fed are discovered to still be jaundiced at their 2 month well child visit to a caregiver. This common sequence is an unfortunate missed opportunity to diagnose BA and other causes of cholestasis within the first 30–45 days of life, when surgery for BA has its best outcomes[5–7]. To prevent this delay in diagnosis of BA, it is recommended that breast-fed infants who remain jaundiced undergo serum bilirubin fractionation at 2–3 weeks of age. If conjugated or direct hyperbilirubinemia is identified, immediate referral is indicated for expedited evaluation by a pediatric gastroenterologist or hepatologist.

Initial Biochemical Testing

The current approach for evaluating an infant with cholestasis (Figure 2) centers on initially excluding BA (and A1AT deficiency in appropriate populations), following “red flags” that indicate the likelihood of a specific etiology (e.g., extrahepatic features of Alagille syndrome), and searching for other treatable conditions (Table 2 and Table 3). If this evaluation is not fruitful, then specific testing for less common and rarer conditions proceeds. The initial evaluation should include serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, GGT, and albumin, although these rarely discriminate among the etiologies[8]. However, a significantly elevated GGT (>150–200 U/L) is suggestive of BA, mechanical bile duct obstruction, paucity of interlobular bile ducts, A1AT deficiency, cystic fibrosis, neonatal sclerosing cholangitis or PFIC type 3. A normal or low GGT (<125 U/L) suggests PFIC type 1, 2, 4–6, bile acid synthesis or metabolism disorders, panhypopituitarism or INH/TNC. Elevated prothrombin time or international normalized ratio suggests either vitamin K malabsorption and deficiency or synthetic liver failure (particularly if unresponsive to parenteral vitamin K administration), metabolic disease or sepsis. Low serum albumin may indicate malnutrition or hepatic synthetic dysfunction. Serum α-fetoprotein levels can be elevated in tyrosinemia type 1 and other conditions [23, 24].

Tiers of Testing for Etiology of Neonatal Cholestasis

Testing for common and treatable etiologies of cholestasis should include a first tier of initial tests, which can be modified by historical and physical findings. A1AT deficiency should be excluded before exploratory surgery for BA by normal serum A1AT levels, the absence of protease inhibitor (PI) ZZ or SZ on electrophoresis, or by rapid genotyping the SERPINA1 gene, whichever can be performed within 24–48 hours. The newborn metabolic screen should be carefully reviewed (or repeated) to exclude hypothyroidism, tyrosinemia type 1, or galactosemia (treatable causes of cholestasis), or specific testing should be performed (thyroid function tests, urine succinylacetone for tyrosinemia, urinary-reducing substances or red blood cell galactose-1-phosphate uridyl transferase drawn before administration of any blood products for galactosemia). If infection is suspected, blood and urine cultures should be obtained in addition to appropriate viral cultures and IgM serologies. In low GGT patients, total serum bile acids (which are paradoxically low or normal in the majority of

cases of bile acid synthesis disorders) can be measured[25]. Receiving oral ursodeoxycholic acid (UDCA) can elevate serum bile acid levels so this must be withheld for 5 days before total serum bile acid testing. A second tier of testing can include: serum amino acids (for citrin deficiency), urine organic acids, acylcarnitine profile (for fatty acid oxidation defects); very long-chain fatty acid levels (for peroxisomal disorders); and a sweat test, serum immunoreactive trypsinogen, or CFTR genotyping (for cystic fibrosis). If clinical, family history or biochemical 'red flags' (Table 3) are suggestive of a specific disorder (Figure 2), evaluation for that disease should proceed early in the evaluation process.

Imaging Evaluation

Early in the diagnostic evaluation, BA, the most common cause of neonatal cholestasis, must be excluded in virtually every cholestatic infant and imaging plays a major role in this determination. Abdominal ultrasonography should be performed to assess for liver size, position and composition, spleen number and size, ascites, and presence of a choledochal cyst, mass, gallstone or obstructing sludge. Several sonographic findings are associated with BA, including absent or abnormal gallbladder, triangular cord sign, hepatic artery–portal vein ratio, increased hepatic subcapsular blood flow, or findings of heterotaxia and laterality defects[8, 26]. Hepatobiliary scintigraphy with a technetium-labeled iminodiacetic acid analogue may be helpful in identifying patency of bile ducts from liver to intestine, excluding BA[8, 27], however the specificity of a non-excreting scan for BA is low (33–80%)[27, 28]. Endoscopic retrograde cholangiopancreatography, magnetic resonance cholangiopancreatography or percutaneous transhepatic cholecystocholangiography have been used in selected centers to define bile duct patency[29–32], however intraoperative cholangiography (IOC) remains the definitive diagnostic test for BA in most centers.

Liver Biopsy

Percutaneous liver biopsy may assist in differentiating BA from other etiologies and histopathology may point to other specific diagnoses. It has been used classically early in the evaluation of cholestasis in order to direct the evaluation to IOC if characteristic BA findings were present. The accuracy of liver biopsy histology for predicting the diagnosis of BA has been in the range of 85–95%[33, 34]. Histological features of BA include portal bile duct plugs, ductal reaction, and portal fibrosis. However, other causes of cholestasis can mimic BA histology, including A1AT deficiency, parenteral nutrition-associated cholestasis, MDR3 deficiency, DCDC2 disease and cystic fibrosis. Liver biopsy can also be helpful in identifying other diseases causing cholestasis (Table 4). Early in the course of BA (before age 4 weeks), not all of the characteristic histologic findings may be present, so liver histology should not be overinterpreted at that age. Recent diagnostic algorithms (Figure 2, Figure 3) have placed less emphasis on liver biopsy and more on genotyping in evaluating the cholestatic infant.

Liver Elastography

Noninvasive measures of liver stiffness fibrosis (such as acoustic radiation force impulse [ARFI], transient elastography, sheer wave elastography, or magnetic resonance

elastography) as surrogate markers for the degree of hepatic fibrosis have achieved widespread use in adult hepatology. Because elastography measures are significantly higher in BA than other neonatal cholestatic conditions[35], it has been proposed that measuring liver stiffness could be used to speed the diagnostic path to IOC, avoid liver biopsy and ultimately shorten the time to HPE. For example, in a study from Taiwan using transient elastography in 48 cholestatic infants, among which 15 had BA, liver stiffness above 7.7 kPa predicted BA (area under the receiver operated characteristic curve (AUC) of 0.85) with an odds ratio of 128 ($P<0.001$)[36]. The utility of these noninvasive procedures for distinguishing BA from other cholestatic disorders is promising but requires further study.

Other Biomarkers

Recent studies have indicated that mRNA expression of matrix metalloproteinase 7 (MMP7) is strongly upregulated in liver biopsies obtained at the time of diagnosis of BA compared to similarly age intrahepatic cholestasis controls[37, 38] and in the newborn mouse viral model of BA[39, 40], and that the protein is highly expressed intrahepatic bile ducts. This led to several groups reporting that elevated serum MMP7 at 1– 2 months of age discriminates those with BA from all others with neonatal cholestasis, with positive predictive values exceeding 90% and negative predictive values exceeding 95%[37, 41, 42]. A potential role of rapidly measuring serum MMP7 by ELISA early in the evaluation of neonatal cholestasis to guide the further work-up for BA has been proposed and remains to be tested prospectively. Similarly, IL-33 appears to be overexpressed in the bile ducts of patients with BA[43] leading to a proposed used of serum IL-33 to differentiate BA from non-BA in a similar manner as MMP7[44].

Next Generation Sequencing (NGS)

New gene sequencing technologies have allowed for rapid testing of large numbers of genes, including an individual's entire genome, within days to weeks, thus challenging our prior use of genotyping late in the traditional paradigm for evaluation of cholestasis[11, 45–48]. NGS also allows for the possibility of discovering new genetic causes of neonatal cholestasis. NGS is performed by several high-throughput platforms using massively parallel processing of spatially separated amplified DNA templates[47]. Targeted gene panels (TGPs) analyzing dozens of genes known to cause cholestatic disorders, whole exome sequencing (WES), and whole genome sequencing (WGS) are now clinically available tools (as costs have decreased substantially) in many centers and countries that can provide an opportunity to identify all known gene variants that have been associated with cholestatic diseases in a single test. Various bioinformatics platforms can make it possible to obtain genetic results in a few days although the turnaround time is generally measured in weeks. Most TGP panels include at least JAGGED1 and NOTCH2 (Alagille syndrome), ATP8B1 (PFIC type 1), ABCB11 (PFIC type 2), ABCB4 (PFIC type 3), SERPINA1 (A1AT deficiency), ABCC2 (Dubin–Johnson syndrome), and SLC25AJ3 (neonatal or infantile intrahepatic cholestasis caused by citrin deficiency), as well as many other rarer conditions[45, 49–52].

There are several published reports of the utility of using NGS panels in the evaluation of neonatal cholestasis. One study used a multi-gene panel that sequenced 61 cholestasis-related genes in 141 patients and made a potential genetic diagnosis in 22%[50]. A second study of gene sequencing in 51 infants with cholestasis without a etiology, a molecular diagnosis was established in 27%[45]. A Japanese study using NGS in 109 patients with neonatal cholestasis \reported a molecular diagnosis in 26%[51]. A North and south American study using a 66 gene panel in 716 infants and older children with cholestasis or liver disease of unknown etiology reported a positive or likely positive molecular diagnosis in 11.7% and a single pathogenic or likely pathogenic variant in another 12.7%[52]. It needs to be emphasized that variants of unknown significance (VOUS) need to be interpreted cautiously and should not be used to establish a molecular diagnosis. Viewed in total, these reports indicate that a molecular diagnosis can be made in a significant number of cholestatic infants using TGPs. The possible use of WES expands these capabilities and opens the window for discovery of new genetic causes of cholestasis.

The advents of more rapid turn-around time, decreasing cost, and automation of bioinformatics for TGP and WES genotyping begs the question of whether multigenic sequencing may become an early diagnostic test in the evaluation of neonatal cholestasis, replacing the current diagnostic algorithm (Figure 3). Thus, molecular diagnostics may become a cost- and time-effective means to determine the etiology in patients for whom BA has been excluded and while other treatable or “red flag” indicated disorders are being evaluated. This shift in the paradigm for evaluation of neonatal cholestasis is being implemented at selected centers around the world and may become the standard in coming years.

Management of Neonatal Cholestasis

As outlined in a recent review article[53], therapy of neonatal cholestasis can be tailored to the three stages of disease (Figure 4): early, chronic and end-stage, each with specific goals for management. Treatable etiologies should be identified and specific therapy initiated expeditiously (Table 2). This is best illustrated by BA in which early timing (at < 30–45 days of life) of HPE dictates best short- and long-term outcomes[5, 6]. Early diagnosis and initiation of therapy is beneficial in hypopituitarism, tyrosinemia type 1, and infections, among other conditions[54]. Targeted pharmacologic, dietary or surgical treatments are available for a few genetic etiologies of cholestasis (Tables 2), including oral cholic acid which replaces the primary bile acid pool for several disorders of bile acid synthesis[55]; and nitisinone which prevents accumulation of toxic intermediates (maleylacetoacetic acid and fumarylacetoacetic acid) in tyrosinemia type 1[56]. Lactose and galactose-restricted diets are required for galactosemia and fructose, sucrose and sorbitol-restricted diets for hereditary fructose intolerance[57].

To promote bile flow and reduce the severity of cholestasis-induced pruritus, UDCA is commonly prescribed at doses of 15–20 mg/kg/day in many of the cholestatic diseases of infancy, despite a paucity of efficacy data in most of these diseases. UDCA is generally well tolerated without significant side effects. If pruritus does not respond to UDCA, antihistamines, rifampicin (20 mg/kg/day), naltrexone (1–2 mg/kg/day), sertraline (1–4

mg/kg/day), bile acid binding resins (e.g, cholestyramine) and other measures are frequently used off label, often in combination, to reduce pruritus and improve the quality of life for the infant and their family. Surgical interruption of the enterohepatic circulation of bile acids (partial external or internal biliary diversion or ileal exclusion) may be effective in a subset of patients with PFIC type 1, PFIC type 2 and Alagille syndrome, with improvement in pruritus, stabilization of cholestasis and improved growth[58].

To promote growth and development and prevent the consequences of malnutrition, maximizing nutrition is an essential component of treatment for all cholestatic infants. Since intraluminal bile acid concentrations above the critical micellar concentration are required for intestinal absorption of dietary fat and fat-soluble vitamins (vitamins A, D, E and K), cholestatic infants are at high risk for steatorrhea and fat-soluble vitamin malabsorption and deficiencies. In the early stage of cholestasis, breast milk or standard infant formulas may be adequately absorbed with milder degrees of cholestasis or after successful bile drainage for BA[59]. However, more severe cholestasis will require infant formulas containing larger amounts of medium-chain triglycerides (MCT) and adequate amounts of essential fatty acids or MCT oil supplements, fat that is absorbed despite poor bile flow[60]. Infants with chronic cholestasis often require at least 125–140% of the recommended caloric requirement based on ideal body weight because of increased oxygen consumption compounding the fat malabsorption[61]. To achieve this goal, supplemental nasogastric tube nocturnal drip feedings or home parenteral nutrition[62] may be necessary, especially in end-stage cholestasis when children are placed on waiting lists for liver transplantation. The importance of achieving adequate nutrition is underscored by the fact that liver transplantation graft and patient survival are directly related to nutritional status [63, 64].

From the early stage of neonatal cholestasis, infants require fat-soluble vitamin supplementation and careful monitoring vitamin status to prevent and treat deficiencies. Deficiency of vitamin K has been fatal in infants with cholestasis as presentation with intracranial hemorrhage from vitamin K deficiency coagulopathy has been reported multiple times. Vitamin D deficiency can lead to severe rickets and bone fractures, vitamin A deficiency to corneal and retinal abnormalities that may lead to blindness, and vitamin E deficiency to irreversible neurologic and muscular morbidities[61]. Thus, monitoring is essential each 2–3 months during the first year of life by obtaining serum 25-hydroxy vitamin D, serum retinol and the retinol: retinol binding protein ratio, prothrombin time and international normalized ratio, and serum alpha tocopherol normalized to serum lipids. For those in the end-stage of cholestasis, it is essential to accelerate the childhood immunization schedule to achieve full immunization before liver transplantation, particularly for the live vaccines which should not be administered during significant immunosuppression[65–68]. Under immunization of pediatric liver transplant recipients remains a significant problem[69]. Through the chronic and end-stages of cholestasis monitoring is advised for signs of portal hypertension, ascites, pruritus and hepatocellular carcinoma. Attention should also be directed to the motor and cognitive development and emotional status of the child and the well-being of the family since developmental delays and impaired quality of life are common[70, 71].

Emerging Potential Therapies for Cholestasis

Although there are currently no U.S. Food and Drug Administration approved drugs for treatment of cholestasis in children, UDCA and 6-ethylchenodeoxcholic acid or obetacholic acid (OCA) are approved for treatment of adults with the cholestatic disease primary biliary cholangitis (PBC)[72–77]. New potential therapeutic opportunities for cholestatic disorders have emerged in recent years based on recent insights into our understanding of bile formation and secretion, bile acid signaling along the enterohepatic circulation and regulatory networks (Figure 5). Pharmacologic agents have been developed to either block or stimulate many of these pathways and have advanced to early or late phase therapeutic trials in adults or children with chronic cholestasis. In this section, we will review these agents with knowledge that they may not all be effective or safe and that carefully conducted clinical trials in children will be needed.

A final common pathway leading to cholestatic liver injury and fibrosis is the accumulation of excess amounts of hydrophobic bile acids in the cholestatic hepatocyte[78]. Thus, efforts to reduce or prevent hepatocyte retention of bile acids would theoretically be beneficial. UDCA, a hydrophilic largely non-toxic bile acid, may improve cholestasis by reducing hepatocyte bile acid concentrations through stimulating canalicular secretion through MRP2 and BSEP and basolateral export through MRP3 and MRP4, and by dilution of the pool of toxic bile acids. Other proposed mechanisms include cytoprotective, anti-inflammatory and antifibrotic properties of UDCA[79–81]. Despite its common use in pediatric cholestatic disorders there is a paucity of clear evidence of clinical effectiveness in this age group, although UCDA is generally well tolerated. It has been shown that excessively large doses of UDCA (>28 mg/kg/day) in adults with primary sclerosing cholangitis were associated with worse clinical outcomes[82].

Nor-UDCA is a side chain shortened derivative of UCDA that is resistant to amidation and enhances shunting of secreted bile acids from the bile duct lumen back to the hepatocyte[83]. This process induces a bicarbonate rich hypercholeresis that may counteract intrinsic bile acid toxicity to biliary epithelia[84] and could be of potential benefit in a number of cholestatic disorders, particularly cystic fibrosis liver disease. Nor-UDCA also has anti-inflammatory, anti-lipotoxic, antiproliferative and anti-fibrotic properties[83, 85–87] and is currently undergoing trials in several adult cholestatic liver diseases.

The farnesoid X receptor (FXR) agonists are a new class of drugs developed for the treatment of cholestasis. FXR is a nuclear hormone receptor within hepatocytes (and enterocytes) which is activated by bile acids (most strongly by chenodeoxycholic acid and cholic acid), which then transactivates expression of BSEP and MRP2 in the hepatocyte and FGF19 in the ileal enterocyte. FXR also indirectly down regulates bile acid synthesis by activating expression of the small heterodimer partner (SHP) in the hepatocyte which then inhibits expression of the rate limiting enzyme for bile acid synthesis, cholesterol 7-alpha hydroxylase (CYP7A1), as well as CYP8B1, and also downregulates NTCP to prevent uptake of circulating conjugated bile acids by the hepatocyte[88]. The combined effect is to reduce hepatocyte bile acid concentrations which may protect the hepatocyte from potential bile acid mediated toxicity. Thus it has been proposed that FXR agonists could activate these

pathways to stimulate bile flow and reduce hepatocyte bile acid concentrations and cholestatic hepatocyte injury[89, 90]. Activation of FXR may alter intestinal bile acid concentrations and composition, change the intestinal microbiome, and modify the gut–liver axis that is believed to potentiate cholestatic liver injury[91–93]. FXR agonists have yet to undergo clinical trial testing in neonatal cholestatic conditions or children with other liver disorders.

Agonists for other nuclear receptor (for example, PXR, CAR, and PPARα) and the membrane bound G-protein coupled bile acid receptor TGR5 are under development as these pathways have properties that regulate bile acid homeostasis, and may also have potential hepatic anti-inflammatory properties[94]. PPARα agonists (e.g., bezafibrate) can increase MDR3 expression and its redistribution to the canalicular membrane which would stimulate biliary phospholipid secretion that may provide biliary epithelial protection against bile acid toxicity. PPARα agonists may also suppress CYP7A1 and CYP27A1 and thus reduce bile acid synthesis, induce CYP3A4 which may aid in bile acid detoxification, and may have anti-inflammatory, anti-fibrotic and antipruritic actions[93]. TGR5 is not expressed in hepatocytes, but is in cholangiocytes, gallbladder epithelium, endothelial cells and Kupffer cell. It may be involved in biliary HCO3- secretion and has anti-inflammatory properties and hepatoprotective effects from bile acid retention[48, 95, 96]. This, together with TGR5 polymorphisms in PSC[97],makes it an attractive therapeutic target.

Another attractive pharmacological target for treatment of cholestasis (based on the effectiveness of surgical biliary diversion in PFIC and Alagille syndrome) is blocking ileal bile acid uptake by highly selective, non-absorbed apical sodium–bile acid transporter (ASBT) inhibitors (for example, maralixibat)[58, 98]. These drugs bind to ASBT, interrupting the enterohepatic circulation of bile acids by increasing fecal excretion and reducing portal venous bile acid levels, thereby reducing hepatic uptake of bile acids which, in turn, reduces hepatic FXR activation and increases bile acid synthesis in the liver, with alterations in the composition of bile[90, 99, 100]. In addition, blocking ileal bile acid uptake reduces ligand binding and activation of ileal enterocyte FXR, thereby reducing intestinal FGF19 secretion into the portal circulation[101]. Since FGF19, through binding to FGFR4/βKL on the hepatocyte basolateral membrane, normally suppresses CYP7A1, the effect of ASBT blockade is to release this hepatocyte suppression of bile acid synthesis. Thus, ASBT inhibitors both reduces the bile acid pool size and increases bile acid synthesis and secretion by the hepatocyte. In maralixibat and A4250 trials in Alagille syndrome and PFIC, preliminary reports demonstrate reduction of pruritus, serum bile acid levels or both in a subset of patients [98, 102, 103]. Similarly, inhibitors of the hepatocyte basolateral bile acid uptake protein, NTCP, such as myrcludex B, could also reduce the bile acid burden in the liver[104], although NTCP is already downregulated to some extent in models of cholestasis. Bile acid sequestrants that are already in clinical use to prevent hypercholesterolaemia and pruritus (for example, cholestyramine or colesevelam) might also be beneficial in enhancing fecal bile acid excretion, although they have not been shown to alter the course of the liver disease itself and palatability is an issue in small children[105]. A major difference between ASBT inhibitors and sequestrants is that ASBT inhibitors and not sequestrants allow free bile acids to enter the colon, where they can still signal through FXR and may induce secretion of GLP-1, which can have hepatoprotective effects[48].

A number of the genetic causes of cholestasis result in perturbations of the synthesis or structure of bile transport proteins or their intracellular trafficking to the canalicular membrane. As in other genetic diseases, small-molecule chemical chaperones that influence the folding or stability of the malformed proteins to enhance their trafficking to the canalicular membrane may be an effective therapeutic strategy[106]. Early testing of potential chaperones, such as 4-phenylbutyrate, has been performed for selective cases of PFIC types 1 and 2 with missense mutations that result in cholestasis[107–110]. It is hoped that more chaperones can be developed for specific genetic variants that allow for precision medical treatments based on genotype.

There is a great deal of interest in developing novel therapies to reduce inflammation and fibrosis in pediatric as well as adult cholestatic diseases[111]. As an anti-inflammatory, high-dose corticosteroid therapy in BA did not demonstrate a short-term or long-term benefit after HPE in the START trial conducted by the NIH-funded Childhood Liver Disease Research Network[112]. Similarly, in an attempt to block innate immune injury in BA, a phase 1/2A clinical trial of intravenous immunoglobulin was of no benefit[113]. Some believe that a subset of patients with BA, who perhaps will be identified in the future through cellular and molecular immunotyping, could benefit from anti-inflammatory agents. This approach to precision pediatric hepatology awaits confirmation. Several anti-inflammatory or anti-fibrotic agents currently under investigation in adult liver diseases (e.g., the CCR2 or CCR5 antagonist, cenicriviroc) may prove to be beneficial and would be attractive agents for trials in pediatric cholestasis. Gene-editing and gene transfer strategies as potential cures for genetic causes of cholestasis may revolutionize how these diseases are treated in the future. These approaches are under investigation in a number of animal models; safety in children with underlying cholestasis will first need to be established if these therapies reach the human clinical trial stage.

Conclusions

The evaluation and management of neonatal cholestatic disorders is in the midst of a transformation driven largely by advances in genetics, technology and new and emerging pharmacotherapeutics. With the identification of molecular causes for many of these disorders, new categorization schemes are likely to be developed to replace soon outmoded terminology, such as PFIC (how many PFIC types will we have?). Incorporating genomics into the cholestasis evaluation paradigm has already begun, and only cost, availability and turn-around time of whole genome sequencing is keeping it from replacing many of the biochemical tests and the use of liver biopsy in the current diagnostic paradigm. For many diseases, through molecular definition of each patient, it is hoped that precision medicine approaches can be developed in the future that will allow each patient to receive the best therapy tailored to their own personal pathophysiology. Defining the etiology and pathogenesis of BA still remains a challenge as is the development of newer effective therapies. The next decade will see many clinical trials of newer agents (Figure 5) in infants and children with cholestasis, hopefully providing families with new hope for a better quality of life for their affected children.

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Abbreviations

A1AT	α-1-antitrypsin deficiency
BA	Biliary atresia
BSEP	Bile salt export protein
FXR	Farnesoid X receptor
GGT	γ-glutamyl transpeptidase
HPE	Hepatoportoenterostomy
INH	Idiopathic neonatal hepatitis
IOC	Intraoperative cholangiography
MCT	Medium chain triglycerides
MDR3	Multidrug resistance protein 3
MMP 7	Matrix metalloproteinase 7
NGS	Next generation sequencing
OCA	Obetacholic acid
PBC	Primary biliary cholangitis
PFIC	Progressive familial intrahepatic cholestasis
TGP	Targeted gene panel
TNC	Transient neonatal cholestasis
UDCA	Ursodeoxycholic acid
WES	Whole exome sequencing
WGS	Whole genome sequencing

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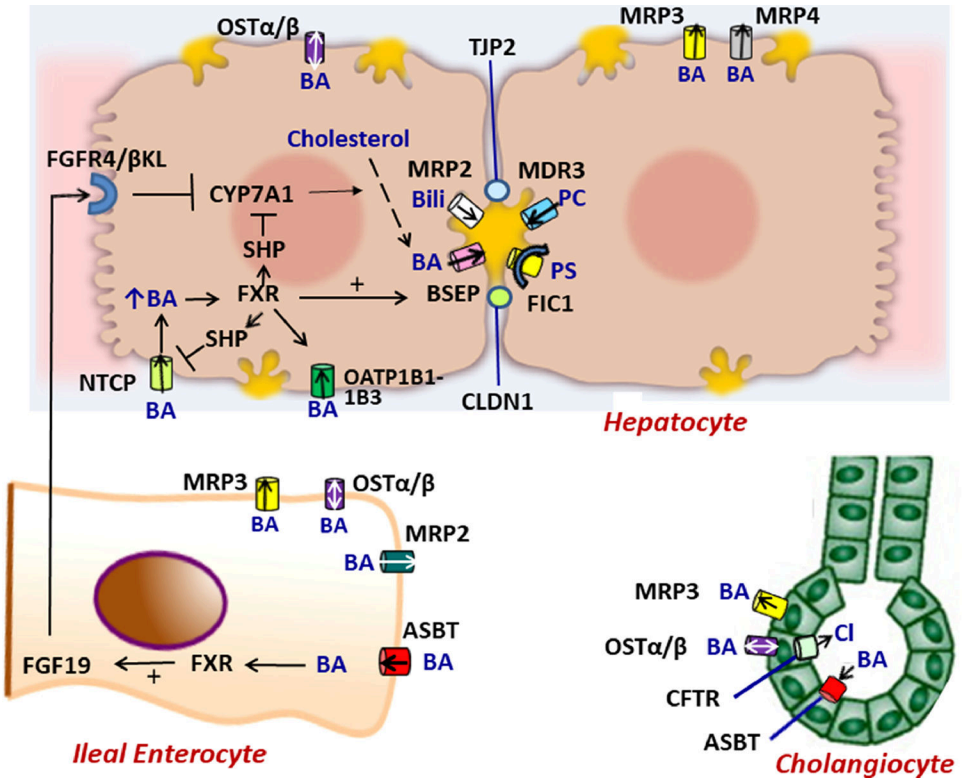

Figure 1.

Molecular regulation of the enterohepatic circulation of bile acids.In hepatocytes, bile acids are taken up by NTCP, OSTα/β or OATP1B1–1B3 on the basolateral membrane or are synthesized from cholesterol by CYP7A1. The ATP-dependent BSEP transports bile acids into bile across the canalicular membrane. FXR, a nuclear hormone receptor, is a master switch that upregulates mRNA expression of BSEP and downregulates NTCP and CYP7A1 (through SHP). MRP2 (conjugated bilirubin transporter), MDR3 (phospholipid transporter) and FIC1 (phosphotidylserine flippase) participate in bile formation across the canalicular membrane. MRP3 and MRP4 transport bile acids from the hepatocyte to the sinusoids. Tight junction proteins (TJP2 and CLDN1) maintain the boundary between canalicular bile and the hepatocyte, preventing the toxicitiy of secreted bile acids. In ileal enterocytes, ASBT promotes absorption of luminal bile acids across the enterocyte brush border which are transported by OSTα/β or MRP3 into portal blood to return to the liver where they interact with FXR to suppress further bile acid synthesis. Within the enterocyte, absorbed bile acids may also activate FXR which upregulates synthesis and secretion of FGF19 into the portal circulation. When it reaches the liver FGF19 binds to the FGFR4/ βKL receptor on the hepatocyte basolateral membrane and triggers suppression of CYP7A1. In cholangiocytes, ASBT in the apical membrane may transport bile acids from bile back into the cell and subsequently into the portal circulation by OSTα/β or MRP3 in the basolateral membrane. CTFR actively secretes chloride into bile. Perturbations of bile formation can result from genetic variants in many of these proteins (FIC1, BSEP, MDR3, MRP2, CFTR, TJP2, CLDN1) and from adaptive changes in transporter expression and function in response to inflammatory, obstructive, or drug-induced insults. . BA = bile acid, PC = phosphatidylcholine, Bili = conjugated bilirubin, Cl = chloride, PS= phosphatidylserine.

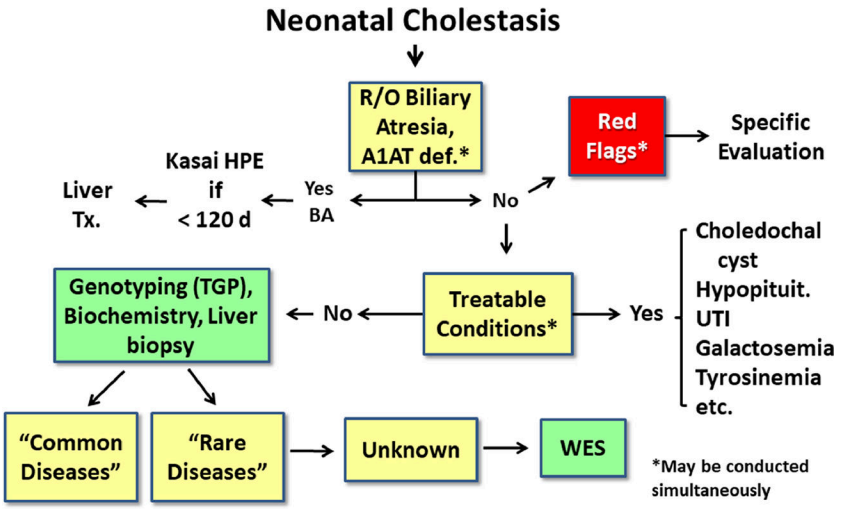

Figure 2.

Current paradigm for evaluation of neonatal cholestasis.In the current sequential evaluation of neonatal cholestasis, alpha-1 antitrypsin deficiency (A1AT), biliary atresia, and treatable causes of cholestasis (such as choledochal cyst or urinary tract infection) are excluded in a timely fashion, which may require liver biopsy and intraoperative cholangiogram to determine if biliary atresia is present. If a "red flag" points to a specific diagnosis (Table 2), evaluation for that disease should proceed promptly.If no diagnosis if found, the next tier of testing may include multiple blood and urine tests to evaluate for infectious, genetic and metabolic causes. If no diagnosis is confirmed, targeted gene panels or specific gene testing is performed and whole exome sequencing (WES) is reserved for those cases without a diagnosis after this exhaustive evaluation. R/O= rule out, HPE= hepatoportoenterostomy, UTI= urinary tract infection, PFIC= progressive familial intrahepatic cholestasis, TGP= targeted gene panel, WES= whole exome sequencing.

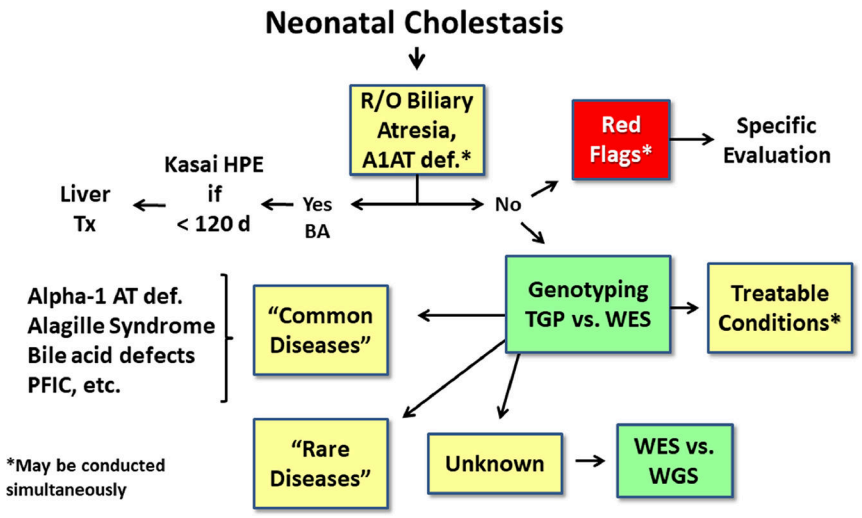

Figure 3.

Future evaluation of neonatal cholestasis. In the near future, it is anticipated that following exclusion of biliary atresia and A1AT deficiency, investigation of "red flags" pointing to specific etiologies, and testing for treatable conditions, rapid turn-around genotyping via whole exome sequencing (WES) or whole genome sequencing (WGS) will be utilized to evaluate for common and rare genetic causes. R/O= rule out, HPE= hepatoportoenterostomy, UTI= urinary tract infection, PFIC= progressive familial intrahepatic cholestasis, TGP= targeted gene panel, WES= whole exome sequencing, WGS= whole genome sequencing.

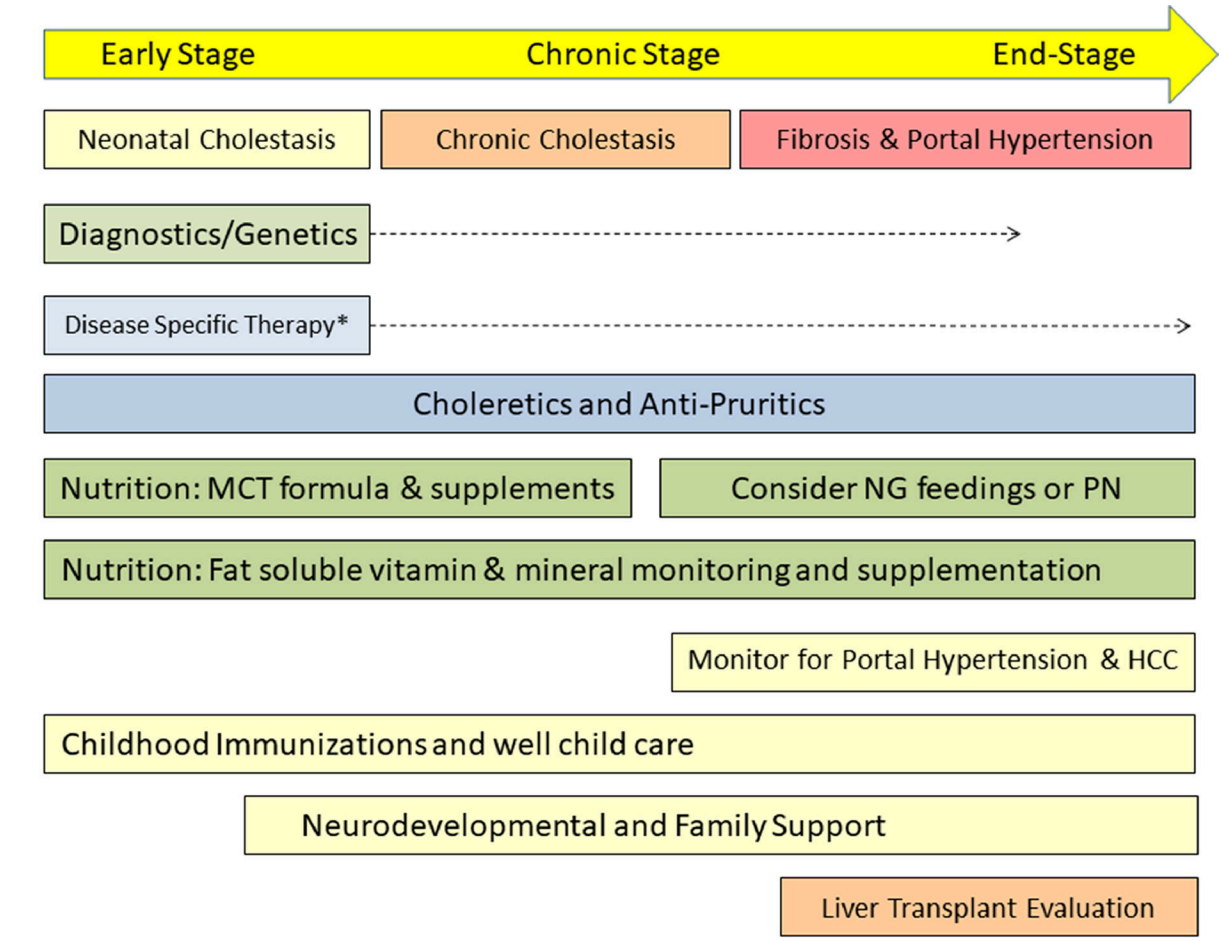

Figure 4.

Holistic Management of Neonatal Cholestasis. Different stages of neonatal cholestasis are defined by the rate of progression from early (neonatal) to chronic (> 6 months of cholestasis) to end-stage liver disease. The latter is characterized by progressive portal fibrosis and synthetic liver failure or complications of portal hypertension. Diagnostic testing may be continued as new etiologies are discovered over time. Disease-specific therapy (if available) should be instituted. Medical therapies can be utilized to improve or treat pruritus, cholangitis and portal hypertension. In the future, new choleretic, anti-fibrotic, anti-inflammatory and bile acid-modifying agents might become available. Medium-chain triglyceride containing infant formula and fat-soluble vitamin supplementation are essential for most infants who remain cholestatic. Monitoring and therapy are initiated for complications of portal hypertension; screening for hepatocellular carcinoma is initiated as indicated. Immunization schedules are accelerated if liver transplantation is planned. Developmental services and family support are provided as needed. MCT= medium chain triglycerides, NG = nasogastric, PN = parenteral nutrition, HCC = hepatocellular carcinoma, HPE = hepatoportoenterostomy, NTBC = 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione

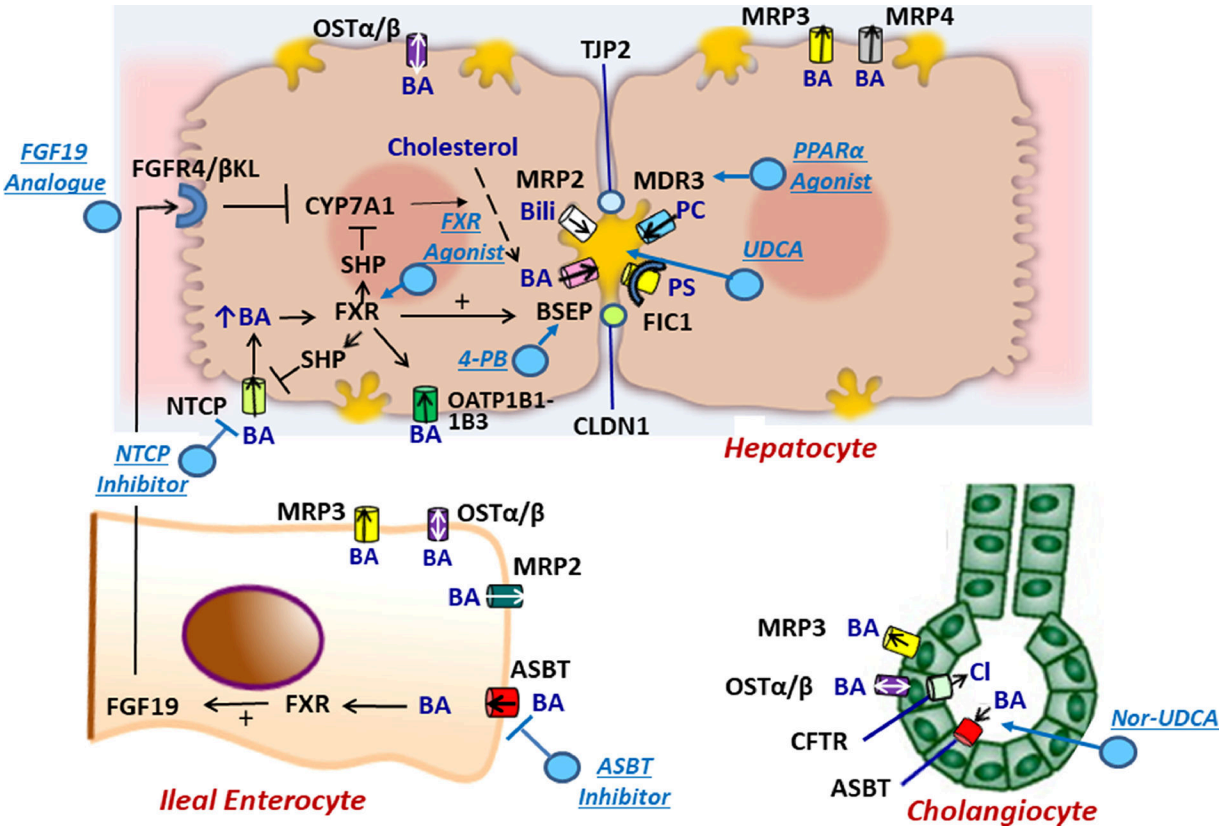

Figure 5.

Emerging therapeutic targets for the treatment of cholestasis. Inhibitors of NTCP may reduce the bile acid uptake by hepatocytes and subsequent toxicity. FXR agonists may upregulate hepatocyte efflux of bile constituents through BSEP, MRP2, MRP3, MRP4, and OSTα/β. UCDA, molecular chaperones such as 4-phenylbutyrate or other agents may reduce the toxic bile acid burden of the hepatocyte and improve bile flow and fat absorption. PPARα agonists may induce canalicular MDR3 expression and phospholipid secretion protecting cholangiocytes against bile acid toxicity. Alternatively, another strategy that would reduce hepatocyte bile acid levels is the inhibition of bile acid synthesis through suppressing CYP7A1 by FGF19 agonists, FXR agonists, or short interfering RNAs. In the ileal enterocyte, inhibiting ASBT will increase fecal excretion of bile acids, lower the bile acid pool size, change bile acid composition and alter enterocyte FXR signaling. In the cholangiocyte, nor-UDCA may protect the cholangiocyte from bile acid-induced injury by altering bile pH. 4-PB = 4-phenylbutyrate, BA = bile acid, PC = phosphatidylcholine, Bili = conjugated bilirubin, Cl = chloride, PS= phosphotidylserine, UDCA = ursodeoxycholic acid.

Table 1.

Causes of Neonatal Cholestasis

Anatomic Obstruction
Biliary atresia, choledochal cyst, cholelithiasis, biliary sludge, inspissated bile, spontaneous perforation of common bile duct, tumor
Infections
Viral, bacterial, spirochete, parasites
Toxins
Drugs, endotoxin, total parenteral nutrition associated cholestasis, herbal products, ? biliatresone
Endocrine
Hypothyroidism, panhypopituitarism
Immune
Gestational alloimmune liver disease
Genetic and Inborn Errors of Metabolism*
Alpha-1-antitrypsin deficiency (SERPINA1)
Alagille syndrome (JAGGED1, NOTCH2)
Arthrogryposis/Renal/Cholestasis (VPS33B, VIPAR)
Congenital hepatic fibrosis (PKHD1)
Citrin Deficiency (Adult citrullinemia type 2) (SLC25A13)
Cystic fibrosis (CFTR)
Bile acid synthesis defects (AKR1D1, AMACR, CYP7B1, HSD3B7, CYP7A1, CYP27A1)
Bile acid conjugation defects (BAAT, SLC27A5)
Fatty acid oxidation defects (SCAD, LCAD)
Galactosemia (GALT)
Glycogen storage disease type IV (GBE1)
Hereditary fructose intolerance (ALDOB)
Mitochondrial respiratory chain disorders (DGUOK, MPV17, POLG)
Neonatal ichthyosis sclerosing cholangitis syndrome (CLDN1)
Neonatal sclerosing cholangitis (DCDC2)
Niemann Pick type C disease (NPC1, NPC2)
Peroxisomal disorders (PEX1, PEX6, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, PEX2, PEX26, PEX3, PEX5, PEX7)
Progressive Familial Intrahepatic Cholestasis
Bile transport defects (ATP8B1, ABCB11, ABCB4, NR1H4, OSTα/β, WDR83OS, ABCC12)
Cytoskeleton defects (TJP2, MYO5B, UNC45, USP53, KIF12, PLEC)
Other defects (LSR, PPM1F)
Smith Lemni Opitz syndrome (DHCR7)
Lipid Storage Diseases (SCP2)
Tyrosinemia type 1 (FAH)
Urea cycle defects
Other

Idiopathic neonatal hepatitis (transient neonatal cholestasis)
Ischemia, Hypoxia, Hepatic Congestion
Hemophagocytic lymphohistiocytosis (HLH)
Malignancy

* Causative genes in parentheses

Adapted from Feldman AG, Sokol RJ, Nat Rev Gastroenterol Hepatol, 2019: Box 1

Table 2.

Treatable causes of neonatal cholestasis

Disorder	**Treatment**
Infection (viral, bacterial, spirochete, parasite)	Antimicrobials
Galactosemia	Galactose-free diet
Tyrosinemia type 1	NTBC (2-(2- nitro-4-trifluoromethylbenzol)-1,3-cyclohexanedione), low tyrosine or phenylalanine diet
Hereditary fructose intolerance	Fructose or sucrose free diet
Hypothyroidism	Thyroid hormone replacement
Cystic fibrosis	Pancreatic enzymes
Hypopituitarism	Thyroid, growth hormone, cortisol replacement
Bile acid synthesis defects	Cholic acid or ursodeoxycholic acid supplementation
Biliary atresia	Hepatoportoenterostomy (Kasai procedure)
Choledochal cyst	Mucosectomy and Choledochoenterostomy
Spontaneous perforation of the common bile duct	Surgical drainage
Inspissated bile, common bile duct stone	Biliary tract irrigation
Parenteral nutrition associated cholestasis (intestinal failure associated cholestasis)	Intravenous lipid emulsion modification, advance enteral feedings

Adapted from Feldman AG, Sokol RJ, Nat Rev Gastroenterol Hepatol, 2019: Table 153

Table 3:

"Red flag" findings for Neonatal Cholestasis

Red Flag	Disease to Consider
Maternal History	
Prenatal Ultrasound abnormality	Choledochal cyst, cystic biliary atresia, gallstone
Intrahepatic cholestasis of pregnancy	PFIC, mitochondrial disease
Acute fatty liver of pregnancy	LCHAD
Maternal infection during pregnancy	Congenital infection
Physical Finding	
Acholic stool	Biliary atresia, choledochal cyst, gallstone, biliary sludge
Palpable mass in the right upper quadrant	Choledochal cyst
Heart murmur	Alagille syndrome or biliary atresia
Butterfly vertebrae	Alagille syndrome
Ascites	Spontaneous perforation of the bile duct
Dysmorphic facies	Alagille syndrome, Zellweger syndrome, chromosomal abnormality
Microcephaly	
Posterior embryotoxon	Congenital infection
Chorioretinitis	Alagille syndrome
Cataracts	Congenital infection
Vision abnormalities	Congenital infection, galactosemia
Splenomegaly	Panhypopituitarism, septo-optic-dysplasia
Multiple joint contractures	Nieman Pick type C
Purpura, thrombocytopenia	Arthrogryposis
Hypotonia	Congenital infection, Congenital lupus
Neurologic abnormalities (irritability, lethargy, poor feeding, hypotonia, or seizures	Mitochondrial disorder, peroxisomal disorder
	Sepsis, intracranial hemorrhage, metabolic and mitochondrial disorders, panhypopituitarism
Family History	
Early emphysema	Alpha-1-antitrypsin deficiency
Liver disease in siblings or stillbirths	Genetic disease or inborn error of metabolism
Consanguinity	Autosomal recessive genetic liver disease

Adapted from Feldman AG, Sokol RJ, Nat Rev Gastroenterol Hepatol, 2019: Table 253

Table 4.

Histological Features of Specific Causes of Neonatal Cholestasis

Disorder	**Histology**
Biliary obstruction	Portal tract bile duct plugs, ductular reaction, portal tract fibrosis
A1AT deficiency	Periodic acid Schiff-positive, diastase-resistant hepatocyte globules
Alagille syndrome	Bile duct paucity
Neonatal sclerosing cholangitis	Necro-inflammatory bile duct lesions
Inborn Errors of Metabolism	Hepatic steatosis and pseudo-acinar formation of hepatocytes
PFIC types 1, 2	Electron microscopy - abnormal canalicular bile
Storage diseases	Electron microscopy - lysosomal storage material
Transient Neonatal Cholestasis	Multinucleated giant cells, extramedullary hematopoiesis, and hepatocellular cholestasis without portal tract involvement
Viral infections	CMV inclusions and HSV, CMV on immunohistochemistry