The School of
Allied Medical Sciences

Diagnosing Neonatal Cholestasis

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- Definition of neonatal cholestasis.
- Incidence of Neonatal Cholestasis
- Etiology of Neonatal Cholestasis
- Clinical symptoms of Neonatal Cholestasis
- Bile acids
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Definition of Neonatal cholestasis

Neonatal physiological jaundice: A common and mostly benign symptom.

It typically resolves 2 weeks after birth.

Neonatal cholestasis (NC): Indicated by a conjugated hyperbilirubinemia.

Never benign and indicates the presence of a severe underlying condition.

Incidence of Neonatal Cholestasis

NC is a rare condition with an estimated incidence rate of 1:2500 to 1:5000 worldwide.

The etiological spectrum of NC is diverse and 25% to 50% are now known to be associated with mutations in specific genes.

In Iran, the incidence of NC is estimated to be around 1 in 2500 live births. Studies conducted in Iran have reported different causes of infant cholestasis, with biliary atresia, idiopathic neonatal hepatitis, and bile duct abnormalities being among the most common.

Etiology of Neonatal Cholestasis

Prognosis for neonatal cholestasis depends on its underlying cause.

Timely diagnosis and intervention are crucial for improving outcomes.

Etiology of Neonatal Cholestasis

Extrahepatic (Surgical) causes of cholestasis

- Extra hepatic Biliary atresia
- Idiopathic Neonatal Hepatitis (INH)
- Choledocal cyst
- Cholelithiasis

Intrahepatic (Medical or non-surgical) causes of cholestasis

- Hepatocytes damage (Viral, Metabolic, Idiopathic)
- Defects of the biliary canalicular transport
- Bile acid synthesis and conjugation disorders
- Biliary development defects
- Lysosomal storage disease
- Mitochondrial disorders

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Disease	Laboratory hallmarks and biomarkers	Gene
INTRAHEPATIC CHOLESTASIS		
Cystic fibrosis *	Elevated sweat chloride	*CFTR*
α−1-antitrypsin deficiency	Low serum A1AT	*SERPINA1*
Defects of the biliary canalicular transport		
PFIC1	High sBA; low GGT; elevated sweat chloride	*ATP8B1*
PFIC2	High sBA; low GGT; high AFP	*ABCB11*
PFIC3	High sBA; high GGT	*ABCB4*
FXR deficiency (PFIC5)	High sBA; low GGT; high AFP	*NR1H4*
MYO5B cholestasis	High sBA; low GGT	*MYO5B*
Tight junction defects		
TJP2 deficiency (PFIC4)	High sBA; low GGT	*TJP2*
USP53 deficiency	High sBA; low GGT	*USP53*
NISCH syndrome	High sBA; high GGT	*CLDN1*
Bile acid synthesis and conjugation disorders		
3-β-HSD-oxidoreductase deficiency	Low sBA, GGT; bile acid mass spectrometry	*HSD3B7*
D4–3-oxosteroid 5 β-reductase deficiency	High GGT; low sBA; biliary acid mass spectrometry	*AKR1D1*
Cerebrotendinous xanthomatosis	Low sBA; high cholesterol and cholestanol	*CYP27A1*
BACL deficiency	Low GGT, sBA; biliary acid mass spectrometry	*SLC27A5*
BAAT deficiency	Low GGT, sBA; biliary acid mass spectrometry	*BAAT*
2-methylacil-CoA racemase deficiency	Low GGT, sBA; biliary acid mass spectrometry	*AMACR*
Oxisterol-7α-hydroxylase deficiency	Low GGT, sBA; biliary acid mass spectrometry	*CYP7B1*
Biliary development defects		
Alagille syndrome	High GGT, sBA, cholesterol, triglycerides	*JAG1, NOTCH 2*
Neonatal sclerosing cholangitis	High GGT, sBA	*DCDC2; CLDN1*
ARC syndrome	High sBA; low or high GGT; metabolic acidosis, hypophosphatemia	*VPS33B, VIPAS39*
Caroli disease	High GGT, sBA	*PKHD1*
Ciliopathies	High GGT, sBA	*different genes*

Disease	Laboratory hallmarks and biomarkers	Gene
Lysosomal storage disease		
Niemann-Pick disease type C *	Markedly elevated oxysterols and lysosphingolipids in plasma; accumulation of intracytoplasmic unesterified cholesterol in skin fibroblasts (filipin staining); elevated chitotriosidase; sea blue histiocytes in bone marrow	*NPC1; NPC2*
Acid sphyngomyelinase deficiency (Niermann-Pick disease type A and B) *	Markedly elevated oxysterols and lysosphingolipids in plasma; reduced acid sphingomyelinase activity (dried blood spot, leukocytes, fibroblasts);	*SMPD1*
Lysosomal acid lipase deficiency (Wolman disease) *	Reduced acid lipase activity (dried blood spot, leukocytes, fibroblasts); abnormal lipid profile; vacuolated lymphocytes; macrophage activation	*LIPA*
Gaucher disease (neurologic) *	Reduced glucocerebrosidase activity (dried blood spot, leukocytes, fibroblasts); foamy cells in bone marrow; high angiotensin-converting enzyme, tartrate-resistant acid phosphatase, chitotriosidase; thrombocytopenia	*GBA*
Mitochondrial disorders		
Mitochondrial DNA depletion syndrome	Hypoglycemia; lactic acidosis; high plasma alpha-fetoprotein; hyperferritinemia iron overload; coagulopathy; abnormal urine organic acid	*POLG, DGUOK, MPV17*
SUCLG1, C10ORF2, elongation factor G1,TRMU related and BCS1L deficiency	Hypoglycemia; lactic acidosis; abnormal urine organic acid	*SUCLG1, C10ORF2, EGF1, TRMU, BCS*
Mitochondrial Fatty Acid Oxidation defects		
LCHAD/MTP deficiency*	Hypoketotic hypoglycemia; high levels of CPK; abnormal blood acylcarnitine and urine organic acids profiles	*HADHA, HADHB*
Peroxisomal disorders		
Zellweger spectrum disorders	Elevated very long-chain fatty (VLCFA), phytanic and pristanic acids in plasma; reduced plasmalogenes in erythrocytes and fibroblasts; reduced catalase activity in fibroblasts	*PEX genes*

Disease	Laboratory hallmarks and biomarkers	Gene
Aminoacidopaties		
Tyrosinemia type 1[a]	Elevated succinylacetone (dry blood spot, plasma, urine); elevated tyrosine and methinine (dry blood spot, plasma); elevatedAFP; coagulopathy; hypophosphatemia; hypoglycemia; hyperaminoaciduria; elevated delta-aminolevulinic acid urine	*FAH*
Adenosine kinase deficiency [a]	Hypoglycemia (hyperinsulinemic); elevation (intermittent) of methionine in plasma; coagulopathy; elevated adenosine in dry blood spot and urine (transient)	*ADK*
S-adenosylhomocysteine hydrolase deficiency[a]	Elevated methionine, S-adenosyl-homocysteine, S-adenosyl-methionine and homocysteine in plasma; high levels of CPK	*AHCY*
Inborn error of polyols and pentose metabolism		
Transaldolase deficiency	Coagulopathy; anemia and thrombocytopenia; hypothiroidism; renal tubulopathy; abnormal profile of urinary polyols	*TALDO1*
Carbohydrate metabolism defects		
Classic galactosemia[a]	Elevated galactose (dry blood spot, erythrocytes); reduced GALT activity in erythrocytes; coagulopathy; hypoglycemia; renal tubulopathy; neonatal *E. Coli* sepsis; positive urinary reducing substances	*GALT*
Glycogen storage disease type IV	Fasting hypoglycemia; high levels of CPK; PAS positive inclusions at liver and muscle histology; reduced enzyme activity (liver tissue, muscle, leukocytes, fibroblasts)	*GBE1*
Congenital disorders of glycosylation	Low coagulation factors (VII, IX, X, XI, AT-III, protein C and S); abnormal serum transferrin isoforms	*different genes*

Disease	Laboratory hallmarks and biomarkers	Gene
Urea cycle defects		
Urea cycle defects*	Hyperammonemia; abnormal aminoacid profile in plasma; elevated orotic acid in urine	*OTC, ASS, ASL, ARG*
Citrin deficiency (NICCD)	Elevated citrulline (dry blood spot; plasma); elevated galactose and AFP; hypoglycemia; hyperammonemia	*SLC25A13*
Cholesterol metabolism disorders		
Smith-Lemli-Opitz syndrome	Elevated 7-dehydrocholesterol and 8-dehydrocholesterol in plasma	*DHCR7*
Mevalonic aciduria	Anemia and thrombocytopenia; hyper-Ig D; abnormal organic urine acids	*MVK*
Cellular trafficking abnormalities		
NBAS deficiency	Hypoglycemia; lactic acidosis; coagulopathy, abnormal urine organic acid	*NBAS*
CALFAN syndrome	Low GGT	*SCYL1*
Metal metabolism disorder		
MEDNIK syndrome	Low serum copper and ceruloplasmin; high urinary copper; mild elevation of plasma VLCFA	*AP1S1*
Syndromic cholestasis (most relevant)		
Down and Edwards Syndrome	Abnormal kariotype	*Trisomy 21, 18*
Kabuki syndrome	Low or high GGT	*KMT2D, KDM6A, MLL2*
Noonan syndrome	High GGT	*PTPN11, SOS1, RAF1 and KRAS*
Aagenaes syndrome	High GGT	*LSC1, CCBE1*
ENDOCRINOLOGIC DISEASES		
Thryroid disorders	TSH and FT4 values	*different genes. in congenital hypotiroidism (e.g., FOXE1, NKX2–1/5, PAX8, SLC26A4, TSHR)*
Panhypopituitarism	TSH and FT4, ACTH, cortisol, GH, IGF1, PRL, LH, FSH, stimulating test, brain MRI	*different genes in genetic forms (e.g., HESX1, PROP1, POUF1, LHX3, LHX4, GLI2, SOX3)*
Adrenal insufficiency	ACTH, cortisol stimulating test	*monogenic forms (e.g., POR, MC2R, MRAP, StAR, AYP11A1, NNT, TRXR2) syndromic forms (eg.CDKN1C, MCM4, SAMD9, SGPL1)*

Disease	Laboratory hallmarks and biomarkers	Gene
INFECTIOUS DISORDERS		
Cytomegalovirus, Herpes virus type 1–2–6; toxoplasma; rubella; parvovirus B19; enterovirus (including coxsackievirus, echovirus), adenovirus, syphilis, HIV, listeria monocitogenes, congenital tubercuolosis	Low or high GGT, serology in the proband and mother, specific direct nucleic acid testing via PCR	NA
HEMATOLOGIC AND IMMUNE-MEDIATED DISORDERS		
Hemophagocitic lymphoistiocytosis	HLH clinical and testing criteria (cytopenia, hypertriglyceridemia or hypofibrinogenemia, hemophagocytosis in biopsy samples, low or absent NK activity, high serum ferritin, elevated CD25 levels	*different genes (e.g., PRF1, UNC13D, STX11, STXBP2, RAB 27, XLP)*
Neonatal hemocromatosis (GALD and non-GALD)	Hypoglycemia, coagulopathy, high ferritin, high alpha-fetoprotein, low trasferrin and high iron saturations	*non-GALD (e.g., DGUOK, SRD5B1, BCS1L)*
Congenital lupus	ANA, positive Coombs-test	NA
Post-hemolytic cholestasis	Hemolytic disease of the newborn due to Rh or ABO alloimmunization	NA
TOXIC AND SECONDARY CHOLESTASIS		
Parenteral nutrition associated cholestasis (PNALD); drugs; intestinal obstruction; cardiovascular disorders, neoplastic disorders; perinatal asphyxia	Low or High GGT	NA

Ranucci G, Della Corte C, Alberti D, Bondioni MP, Boroni G, Calvo PL, Cananzi M, Candusso M, Clemente MG, D'Antiga L, Degrassi I. Diagnostic approach to neonatal and infantile cholestasis: a position paper by the SIGENP liver disease working group. Digestive and Liver Disease. 2022 Jan 1;54(1):40-53.

There are numerous causes, which are **identified** by

- ✓ Laboratory testing
- ✓ Hepatobiliary scan
- ✓ Liver biopsy
- ✓ Surgery

Neonatal cholestasis (NC) is a diagnostic dilemma frequently countered in a neonatal care unit**, due to its diverse etiology.**

Clinical symptoms of Neonatal Cholestasis

Infants by neonatal cholestasis have these signs:

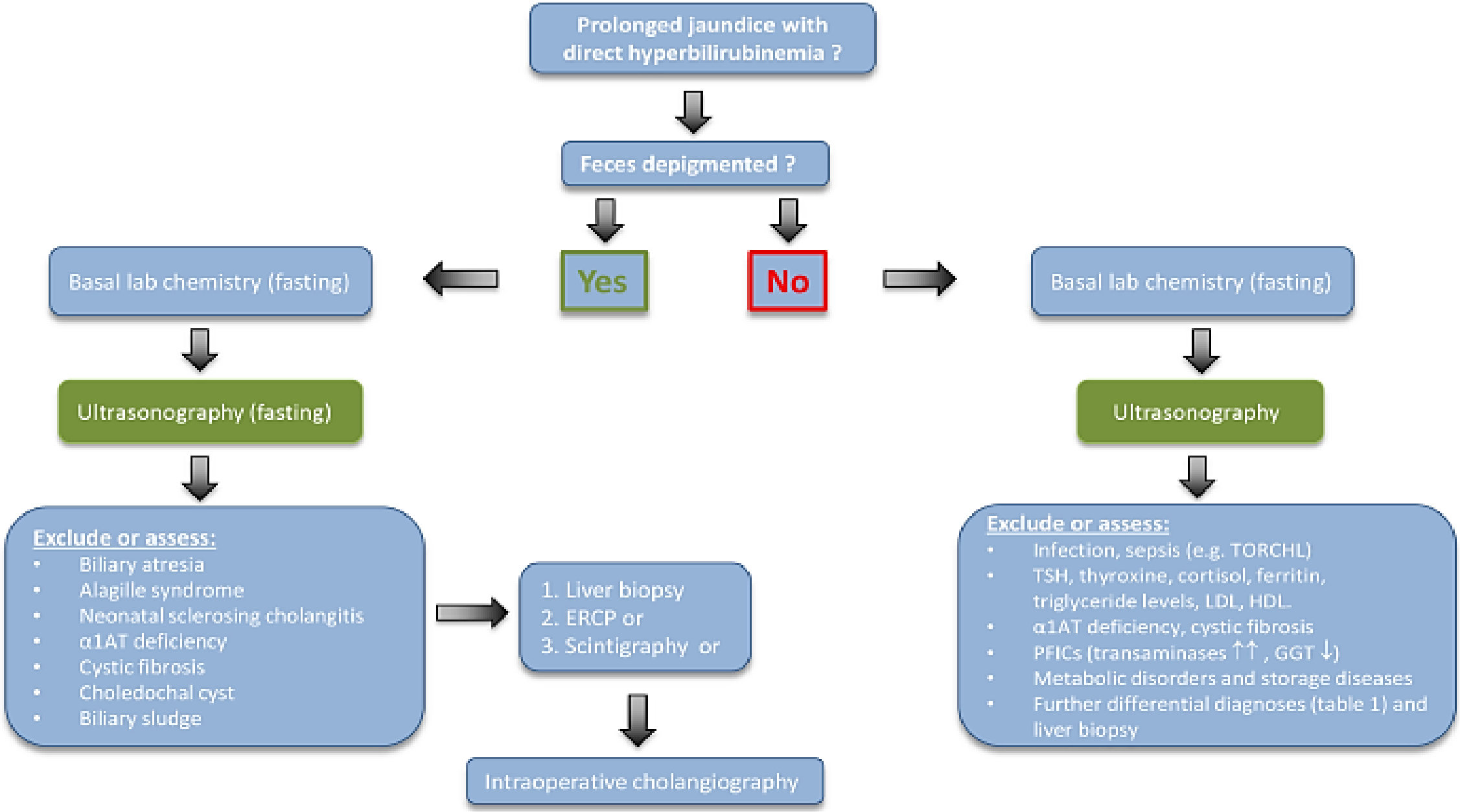
- ✓ Jaundiced
- ✓ Dark urine (containing conjugated bilirubin)
- ✓ Acholic stools
- ✓ Hepatomegaly

The clinical symptoms of patients with different types of neonatal cholestasis do not differ much and cannot be the basis for differentiating different types of cholestasis.

Therefore, the difference between the types of disease should be directed to paraclinical studies.

Treatment depends on cause.

Diagnostic algorithm for neonatal cholestasis

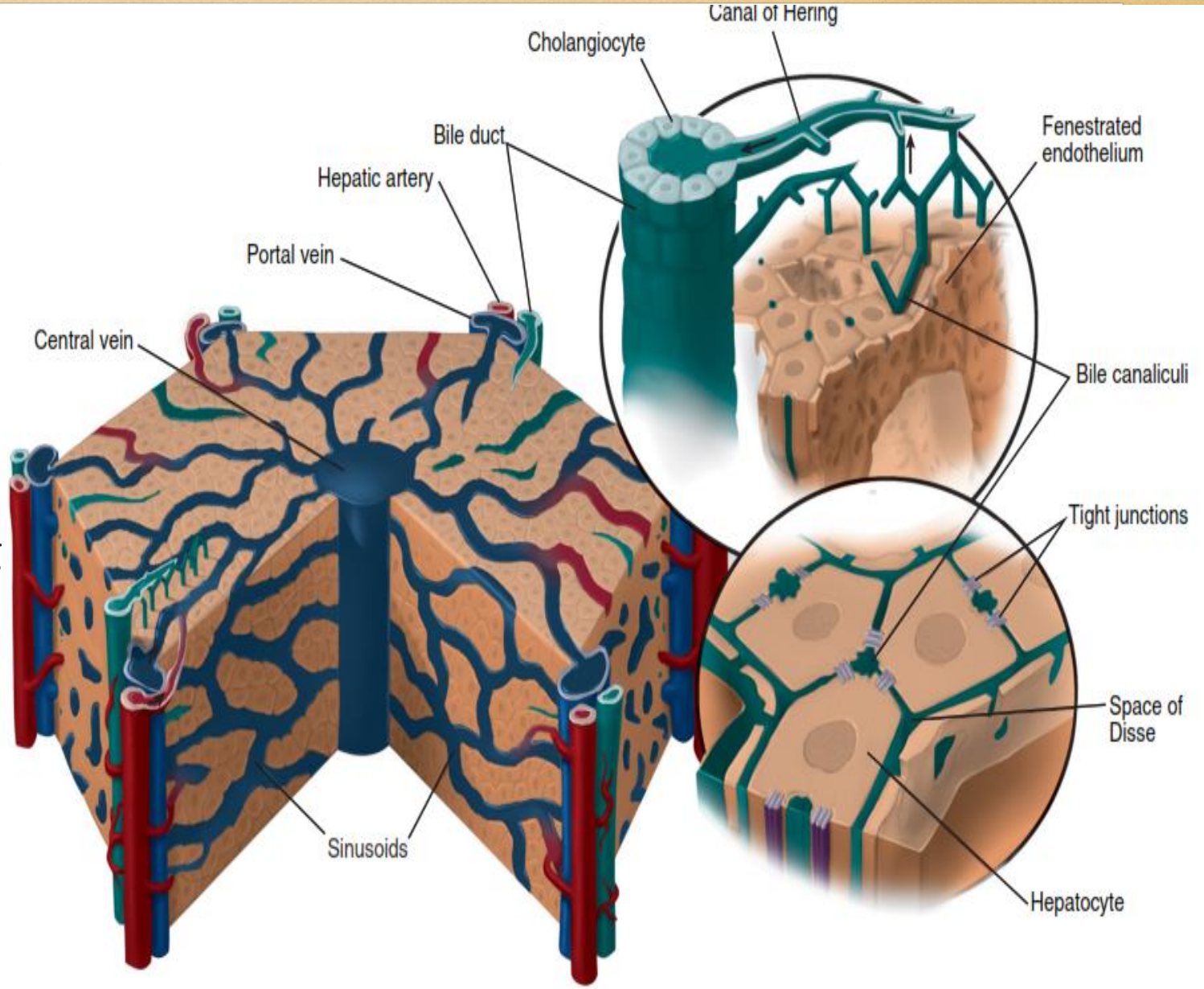


A defect of the intrahepatic production or the transmembrane transport of bile, or a mechanical obstruction preventing bile flow leads to an accumulation of bile components in the liver, in the blood and extrahepatic tissues.

Bile acids

Bile is formed in the liver and is a blend of bile acids, bilirubin, and fats.

It is secreted into canaliculus; from that point, it flows into bile ducts and is at last discharged into the intestine after transient stockpiling inside the gallbladder.



Bile acids

In the liver, **primary bile acids** are formed by catabolism of cholesterol.

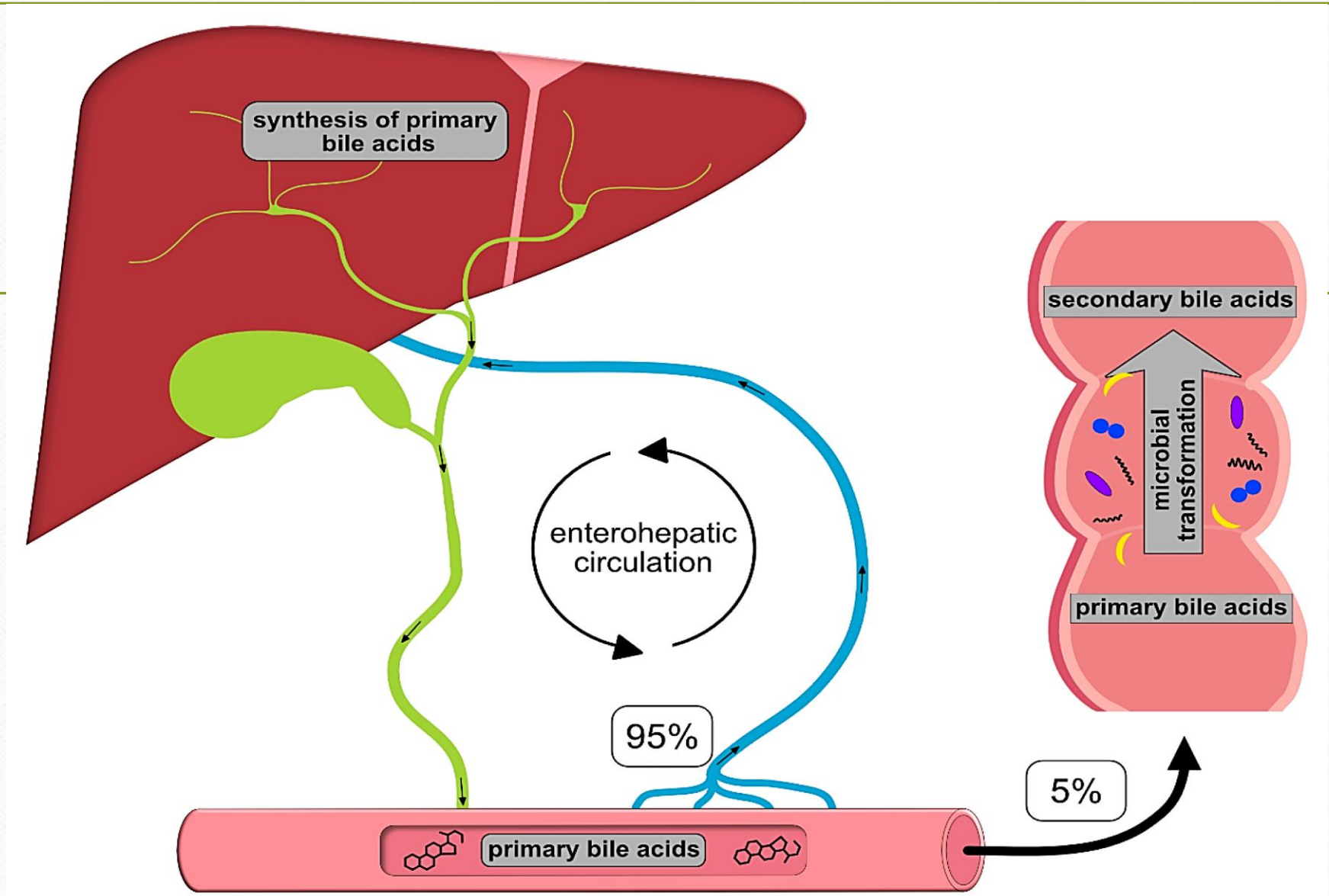
Cholic acid (CA) + Chenodeoxycholic acid (CDCA)

Bile acids are conjugated to glycine and taurine to form the hydrophilic **bile salts**.

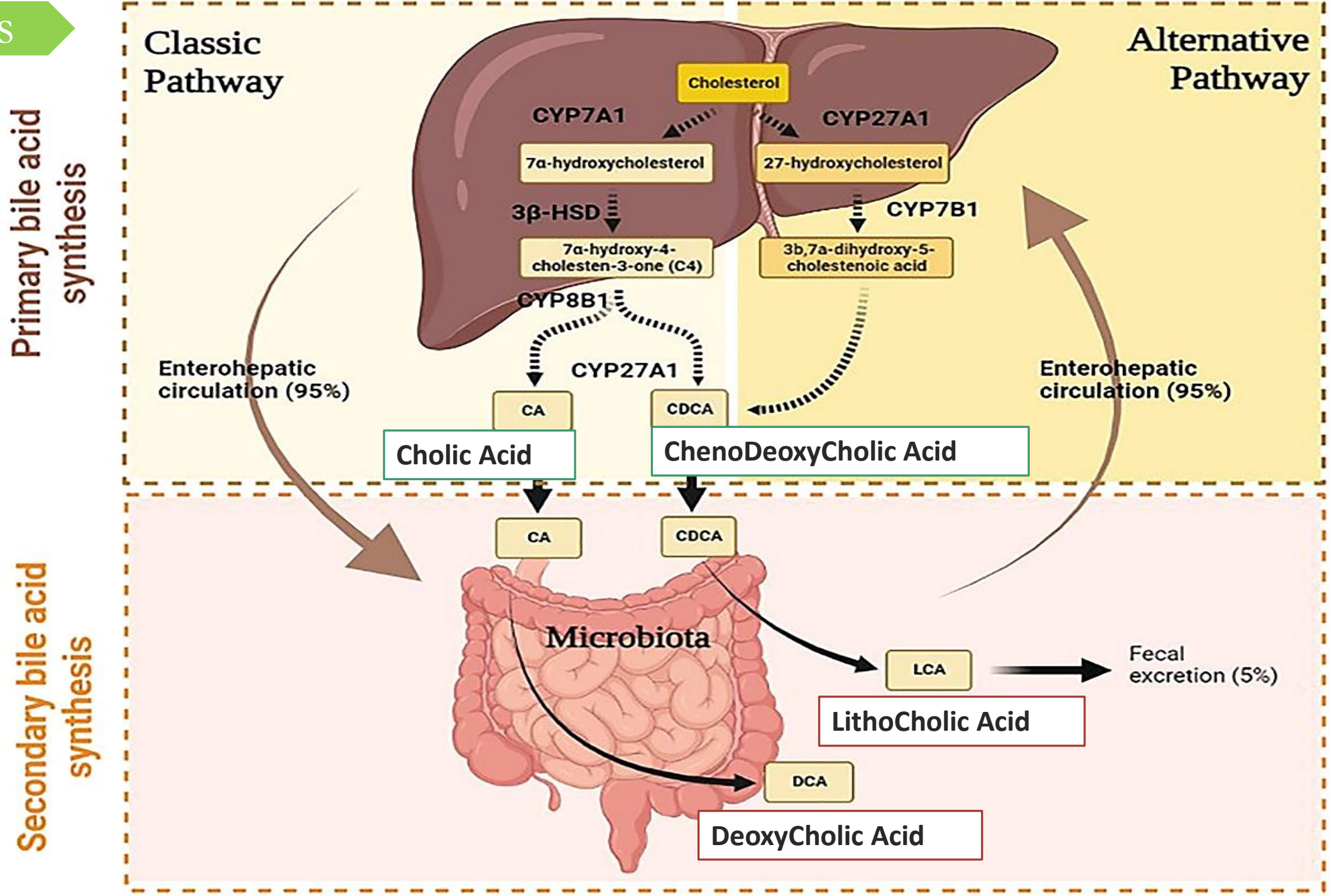
In the human gut, a small proportion (~5%) of the bile salt pool undergoes microbiota-mediated deconjugation of glycine and taurine to reform the hydrophobic, cytotoxic, and potentially antimicrobial primary bile acids CDCA and CA, and these compounds may be metabolized further to the **secondary bile acids**, and by microbiota in the large bowel.

Deoxycholic acid (DCA) + Lithocholic acid (LCA)

Bile acids


synthesis of primary bile acids
enterohepatic circulation
95%
5%
primary bile acids
secondary bile acids
microbial transformation
primary bile acids

Bile acids


Classic Pathway
Alternative Pathway
Primary bile acid synthesis
Cholesterol
CYP7A1
CYP27A1
7α-hydroxycholesterol
27-hydroxycholesterol
3β-HSD
CYP7B1
7α-hydroxy-4-cholesten-3-one (C4)
3b,7a-dihydroxy-5-cholestenoic acid
CYP8B1
CYP27A1
Enterohepatic circulation (95%)
Enterohepatic circulation (95%)
CA
CDCA
Cholic Acid
ChenoDeoxyCholic Acid
Secondary bile acid synthesis
CA
CDCA
Microbiota
LCA
Fecal excretion (5%)
LithoCholic Acid
DCA
DeoxyCholic Acid

Bile acids in neonatal cholestasis

Bile acids play a crucial role in diagnosing neonatal cholestasis.

Bile acids serve as biomarkers for cholestasis, aiding in its diagnosis and assessment.

In cholestasis, the primary failure is of bilirubin excretion, resulting in excess conjugated bilirubin in the bloodstream and decreased bile salts in the gastrointestinal (GI) tract. As a result of inadequate bile in the GI tract, there is malabsorption.

Ursodeoxycholic acid (UDCA) is prescribed to promote bile flow and alleviate cholestasis-induced pruritus in neonates.

Development of scoring systems based on bile acid profiles enhances diagnostic accuracy, particularly in estimating the risk of specific complications like biliary atresia in infants with cholestasis

Bile acids in Neonatal cholestasis

Inherited disorders of bile acid synthesis cause ~2% of persistent cholestasis in infants.

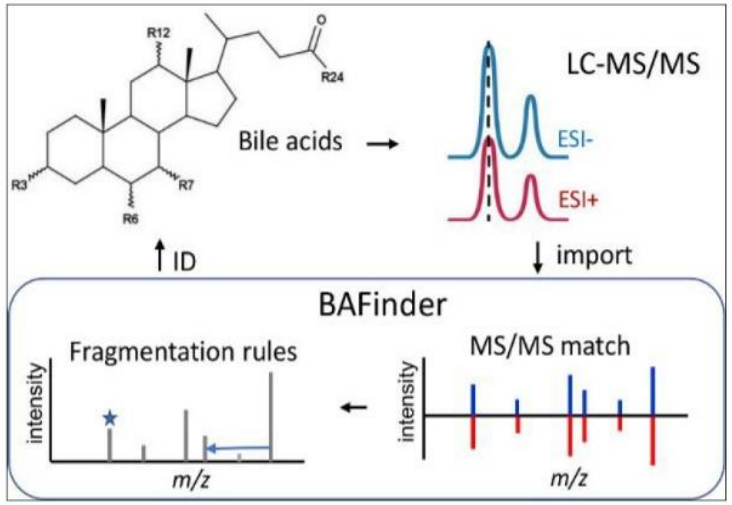
In contrast to other causes of NC, the serum bile acid levels are normal.

Cholestasis is thought to result from an imbalance and inadequate production of primary bile acids. This results in an accumulation of aberrant hepatotoxic bile acids and intermediary metabolites leading to an impaired bile flow

Bile acids measurement

Bile acids/salts : Hydrophobic and ionizable

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) **techniques** are considered the **gold standard** for analysis of BA profile

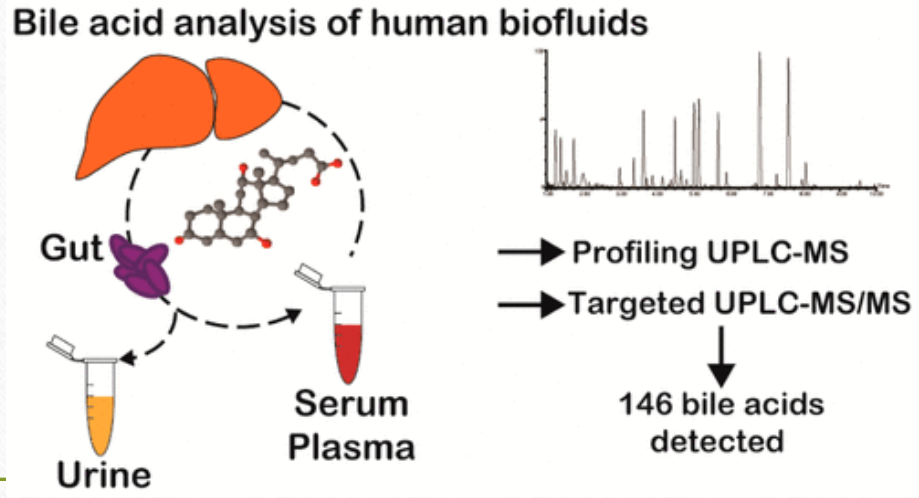


Bile acids measurement

Various methods may be used to measure blood levels of bile salts.

They can be measured separately using sophisticated analytical methods such as:

- High-performance liquid chromatography (HPLC)
- Gas chromatography coupled with mass spectrometry (GC-MS)
- Ultra performance liquid chromatography–mass spectrometry (UPLC-MS)/MS
- Enzymatic Spectrophotometric Methods (ELISA KIT)
- Tandem GC-MS



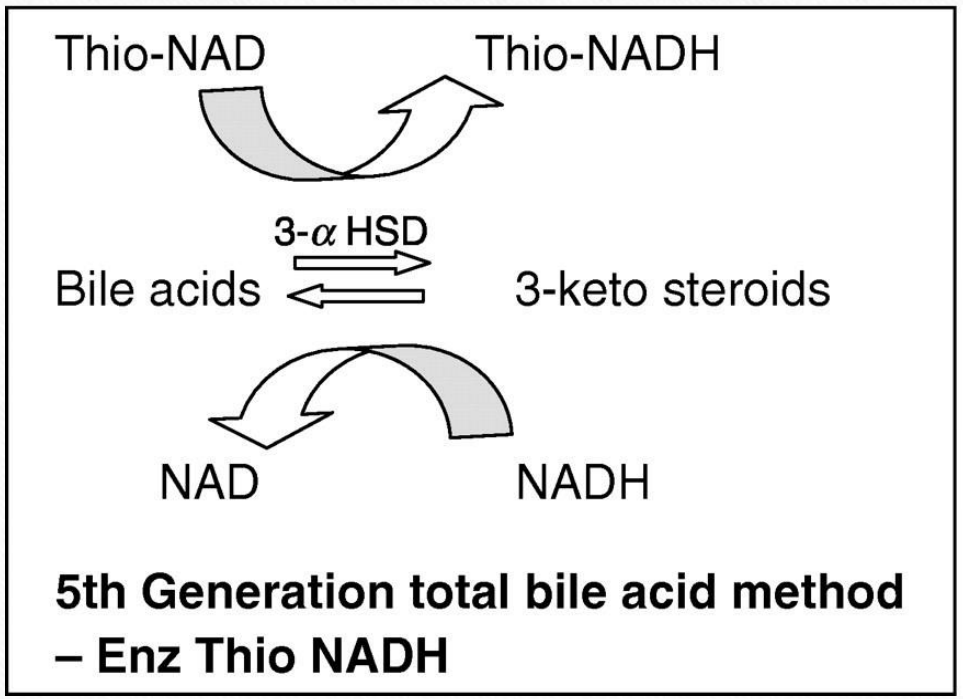
Bile acids measurement

Total bile acids can easily be quantified with conventional enzymatic spectrophotometric methods, based on the use of dehydrogenases acting on hydroxyl groups in BA structures, and thus allowing quantification of total BA, using the 3-alphahydroxysteroid dehydrogenase (3α-HSD) enzyme to convert bile acids to 3-ketosteroids and NADH.

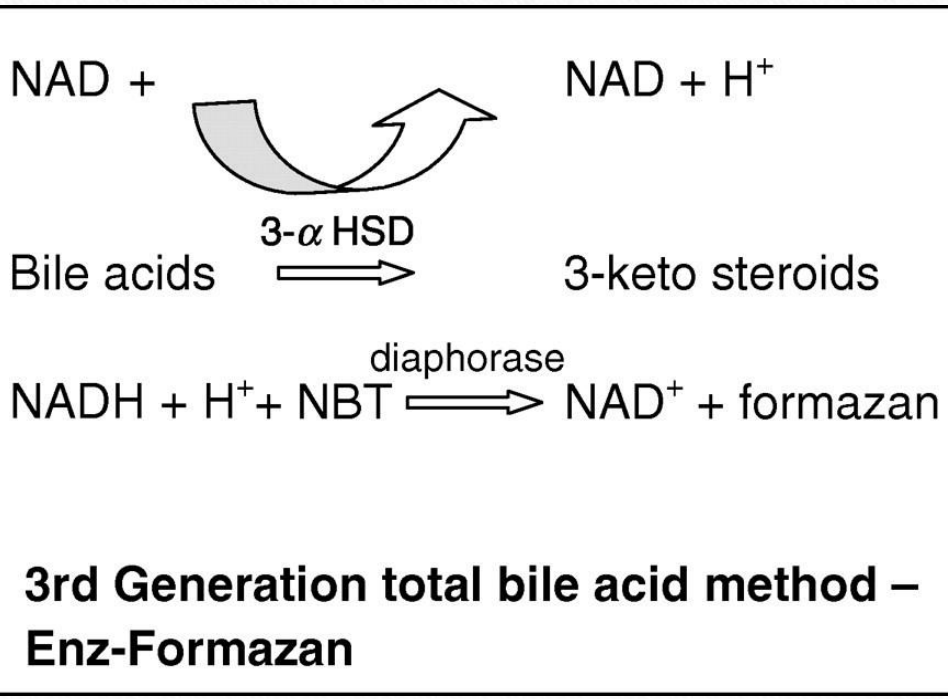
Bile acids measurement

Monitor the rate of formation of Thio-NADH at 405 nm

Measure the formation of formazan at 540 nm after addition of nitrotetrazolium blue and Diaphorase



5th Generation total bile acid method – Enz Thio NADH



3rd Generation total bile acid method – Enz-Formazan

Bile acids measurement in IRAN

آزمایشگاه مرجع معاونت بهداشت

آزمایشگاه خصوصی در تهران

- ✓هزینه
- ✓زمان
- ✓تعداد نمونه

Suggestion

- ✓راه اندازی آزمایشگاه/ شبکه/ سانتر جهت اندازه گیری پنل اسیدهای صفراوی
- ✓ جمع آوری تمام نمونه ها از مراکز مختلف مانند مرکز طبی، مرکز گوارش، بیماری های متابولیک
- ✓انجام طرح های تحقیقاتی مشترک میان بالین و دانشکده/مراکز تحقیقاتی
- ✓طراحی الگوریتم جهت تسهیل فرآیند تشخیص کلستاز نوزادی و جلوگیری از هدر رفت زمان

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NIC is defined in presence of:

Conjugated bilirubin more than 1mg/dL + total bilirubin of <5.0 mg/dL

OR

Conjugated bilirubin >20% of the total, if total bilirubin is >5.0 mg/dL

A parenteral report of depigmented feces suggests an extrahepatic obstructive process.

Neonatal Delirium

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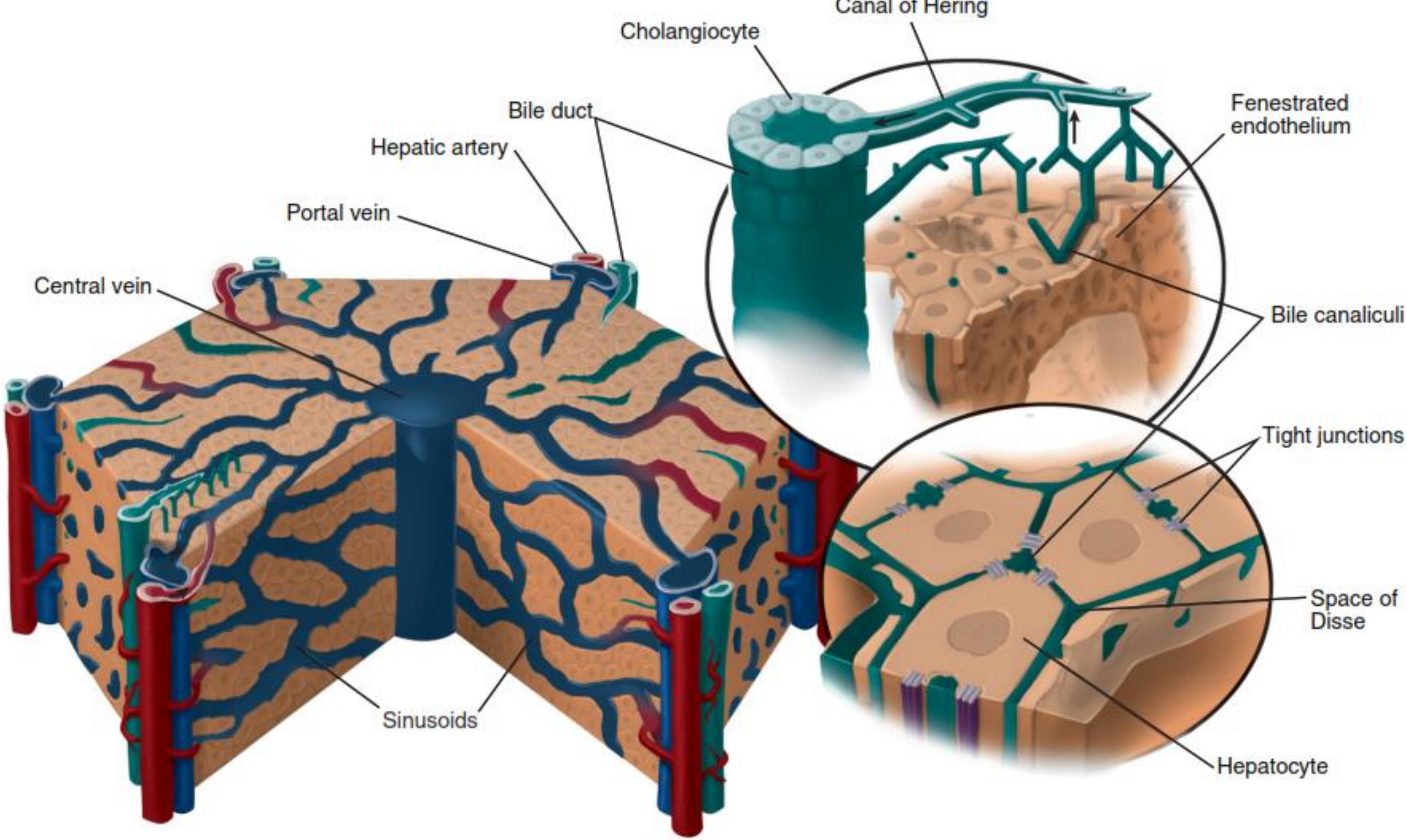
CHOLESTASIS

Neonatal jaundice associated with a rise in conjugated bilirubin is indicative of a defect or insufficiency in bile secretion, biliary flow, or both and is always pathologic

Conjugated hyperbilirubinemia, however, is likely pathologic and is defined by a conjugated bilirubin level greater than 1 mg/dL (if total bilirubin is less than 5 mg/dL) or greater than 20% of the total bilirubin (if total bilirubin is greater than 5 mg/dL)


Canal of Hering
Cholangiocyte
Bile duct
Hepatic artery
Portal vein
Central vein
Fenestrated endothelium
Bile canaliculi
Tight junctions
Space of Disse
Hepatocyte
Sinusoids

BILE ACID SYNTHESIS

- There are two pathway for bile production. Neutral and acidic pathway

Neutral or classic bile acid pathway

The rate-limiting cytochrome P450 enzyme, cholesterol 7α-hydroxycholesterol (CYP7A1), initiates the conversion of cholesterol to the primary bile acid, cholic acid (CA).

Acidic or alternative pathway

cholesterol 27-hydroxylase (CYP27A1), a mitochondrial P450 enzyme, catalyzes the first reaction that leads to the final production of chenodeoxycholic acid (CDCA). the acidic pathway has been found to be more important in those with liver disease and in neonates

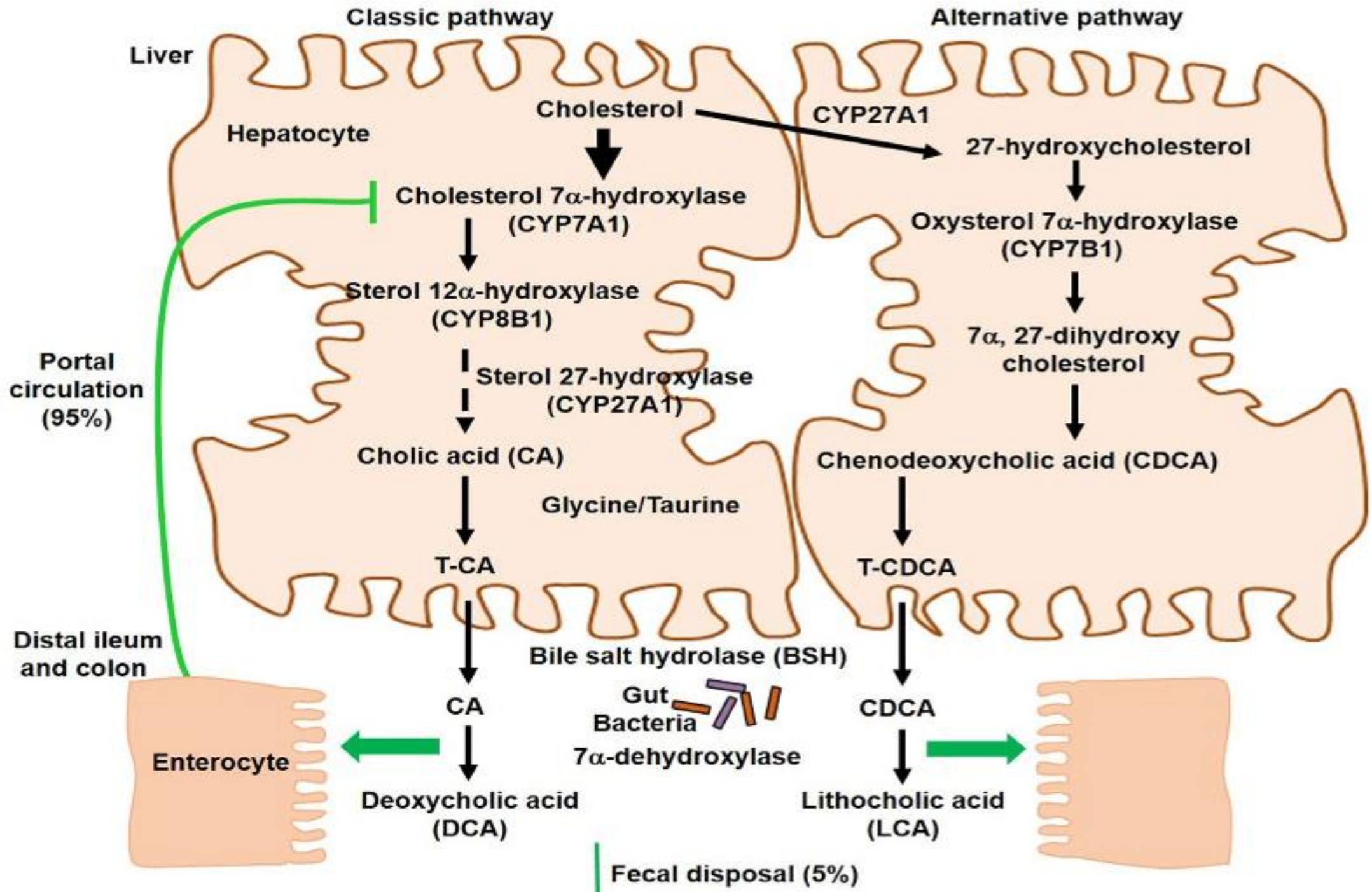

Classic pathway
Alternative pathway
Liver
Hepatocyte
Cholesterol
CYP27A1
27-hydroxycholesterol
Cholesterol 7α-hydroxylase
(CYP7A1)
Oxysterol 7α-hydroxylase
(CYP7B1)
Sterol 12α-hydroxylase
(CYP8B1)
7α, 27-dihydroxy
cholesterol
Portal
circulation
(95%)
Sterol 27-hydroxylase
(CYP27A1)
Cholic acid (CA)
Chenodeoxycholic acid (CDCA)
Glycine/Taurine
T-CA
T-CDCA
Distal ileum
and colon
Bile salt hydrolase (BSH)
CA
Gut
Bacteria
CDCA
Enterocyte
7α-dehydroxylase
Deoxycholic acid
(DCA)
Lithocholic acid
(LCA)
Fecal disposal (5%)

Table 89.1 Bile Acid Synthetic Disorders.

Diagnosis	Genetics	Clinical Symptoms
CYP7A1 deficiency[a]	Chromosome 8q11-12	Hyperlipidemia, increased hepatic cholesterol, gallstones, premature cardiovascular disease
CYP7B1 deficiency	Chromosome 8q21.3	Severe neonatal cholestasis and cirrhosis, elevated transaminases but normal γ-GT
3β-Hydroxysteroid-Δ^5-C_{27}-steroid dehydrogenase deficiency[a]	Chromosome 16p11.2-12	Progressive neonatal cholestasis, hepatomegaly, fat malabsorption, fat-soluble vitamin deficiencies, rickets
Δ^4-3-Oxosteroid 5β-reductase deficiency (AKR1D1 or SRD5B1)[a]	Chromosome 7q32-33	Severe cholestasis, elevated transaminases but normal γ-GT, fat-soluble vitamin deficiencies
Cerebrotendinous xanthomatosis; sterol 27-hydroxylase (CYP27A1) deficiency[a]	Chromosome 2q33-qter	Elevated plasma concentrations of cholesterol and cholestanol, neurologic symptoms, including dementia, psychiatric disturbances, pyramidal or cerebellar signs, and seizures; diarrhea; tendon xanthomas; cataracts
2-Methyl coenzyme A racemase deficiency	Chromosome 5p11-13	Neuropathy (in adults), coagulopathy (in infants), vitamin D and E deficiency, mild liver impairment but no neurologic disease
Peroxisomal disorders		
1. D-bifunctional protein deficiency (HSD-17B4)	Chromosome 5q2	Neonatal seizures, hypotonia, hepatomegaly; usually fatal before 2 years of age
2. Trihydroxycholestanoic acid CoA oxidase deficiency	Chromosome 3p14.3	Ataxia with onset around 3 years of age, liver dysfunction
Amidation defects		
1. Bile acid CoA ligase	Chromosome 19q13.43	Cholestasis, elevated transaminases
2. Bile acid CoA amino acid N-acyl transferase	Chromosome 9q31.1	Neonatal cholestasis, fat-soluble vitamin deficiencies, growth failure

Cholestatic causes

Cholestatic disorders can be separated into:

extrahepatic or biliary causes

(structural abnormalities such as biliary atresia)

intrahepatic or hepatocellular causes

(impairment in bile transport, genetic or metabolic disorders, infection).

Genetic/metabolic disorders of Cholestasis

Alagille syndrome	*JAG1* and *NOTCH2*
Alpha-1 antitrypsin deficiency	*SERPINA1*
Arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome	*VPS33B* and *VIPAS39* (*VIPAR*)
Bile acid synthesis defects	
- 3β-hydroxy-C_{27}-steroid oxidoreductase deficiency (3β-HSD; most common)	*HSD3B7*
- Δ^4-3-oxosteroid 5β-reductase deficiency (5β-reductase deficiency)	*AKR1D1*
- Defective amidation (bile acid-coenzyme A (CoA) ligase deficiency and bile acid-CoA:amino acid N-acyl transferase deficiency)	*SLC27A5* and *BAAT*
- 27-hydroxylase deficiency or cerebrotendinous xanthomatosis (CTX)	*CYP27A1*
- Oxysterol 7α-hydroxylase deficiency	*CYP8B1*
- 2-methylacyl- CoA racemase deficiency	*AMACR*
- Side-chain oxidation defects (acyl-CoA oxidase and 3α,7β,12α-trihydroxy-5β-cholestanoic acid-CoA oxidase deficiency)	*ACOX2*
CoA oxidase deficiency	
- Adenosine trisphosphate (ATP) binding cassette subfamily D member 3 deficiency	*ABCD3*
Biliary atresia splenic malformation (BASM) syndrome	*PKD1L1*
Caroli disease and congenital hepatic fibrosis	*PKHD1*
Chromosomal defects	Trisomy 17, 18, 21; Turner syndrome
Crigler Najjar and Gilbert syndromes	*UGT1A1*
Cystic fibrosis	*CFTR*
Dubin-Johnson syndrome	*ABCC2*
Fatty acid oxidation defects:	
- Short-chain acyl-CoA dehydrogenase deficiency (SCAD)	*ACAD5*
- Long chain acyl-CoA dehydrogenase deficiency (LCAD)	*ACADL*

Fructosemia (most commonly fructose-1-phosphate aldolase)	*ALDOB*
Galactosemia (classic/type 1)	*GALT*
Glycogen storage diseases (GSD)	*G6PC and SLC37A4*
- GSD type Ia (glucose-6-phosphatase) and Ib (glucose-6-phosphatase transporter)	*GAA*
- GSD type II (lysosomal acid alpha-glucosidase)	*AGL*
- GSD type III (debrancher enzyme)	*GBE1*
- GSD type IV (glycogen debrancher)	*PYGL*
- GSD type VI (hepatic phosphorylase)	*PHKA2 (X-linked); PHKB, PHKG2 (recessive)*
- GSD type IX (phosphorylase kinase)	
Gaucher disease (beta-glucocerebrosidase)	*GBA*
HMG CoA lyase deficiency (3-hydroxy-3-methylglutaryl-CoA lyase deficiency)	*HMGCL*
Lysosomal acid lipase deficiency (Wolman disease)	*LIPA*
Methylmalonic acidemia (MMA)	
- Isolated MMA	*MMUT, MMAA, MMAB, MMADHC, MCEE*
- MMA with homocystinuria	*MMACHC, MMADHC, LMBRD1, ABCD4, HCFC1*
Mitochondrial respiratory chain disorders (mitochondrial DNA depletion syndromes)	*DGUOK, MPV17, POLG*
Neonatal ichthyosis-sclerosing cholangitis (NISCH) syndrome	*CLDN1*
Neonatal sclerosing cholangitis	*DCDC2*
Niemann-Pick disease	
- Types A and B	*SMPD1*
- Type C	*NPC1* and *NPC2*
Peroxisomal disorders (Zellweger spectrum)	*PEX1-3, PEX5-7, PEX10-14, PEX16, PEX19, PEX26*

Progressive familial intrahepatic cholestasis (PFIC)	
- PFIC type1 (FIC1 deficiency)	*ATP8B1*
- PFIC type 2 (BSEP deficiency)	*ABCB11*
- PFIC type 3 (MDR3 deficiency)	*ABCB4*
- PFIC type 4 (TJP2 deficiency)	*TJP2*
- PFIC type 5 (FXR deficiency)	*NR1H4*
- MYO5B deficiency	*MYO5B*
Tyrosinemia (fumarylacetoacetate)	*FAH*
Urea cycle defects	
- Citrin deficiency	*SLC25A13*
- Citrullinemia (type I)	*ASS1*
- Ornithine trans-carbamylase deficiency	*OTC*
Wilson disease	*ATP7B*

Infectious causes of neonatal cholestasis

CMV	33.5
Sepsis	24.7
Congenital syphilis (*Treponema pallidum*)	10.8
E. coli UTI	9.8
Rubella	6.2
Toxoplasmosis (*Toxoplasma gondii*)	3.6
Hepatitis B (HBV)	1.6
Herpes simplex (HSV)	1.0
Other	8.8

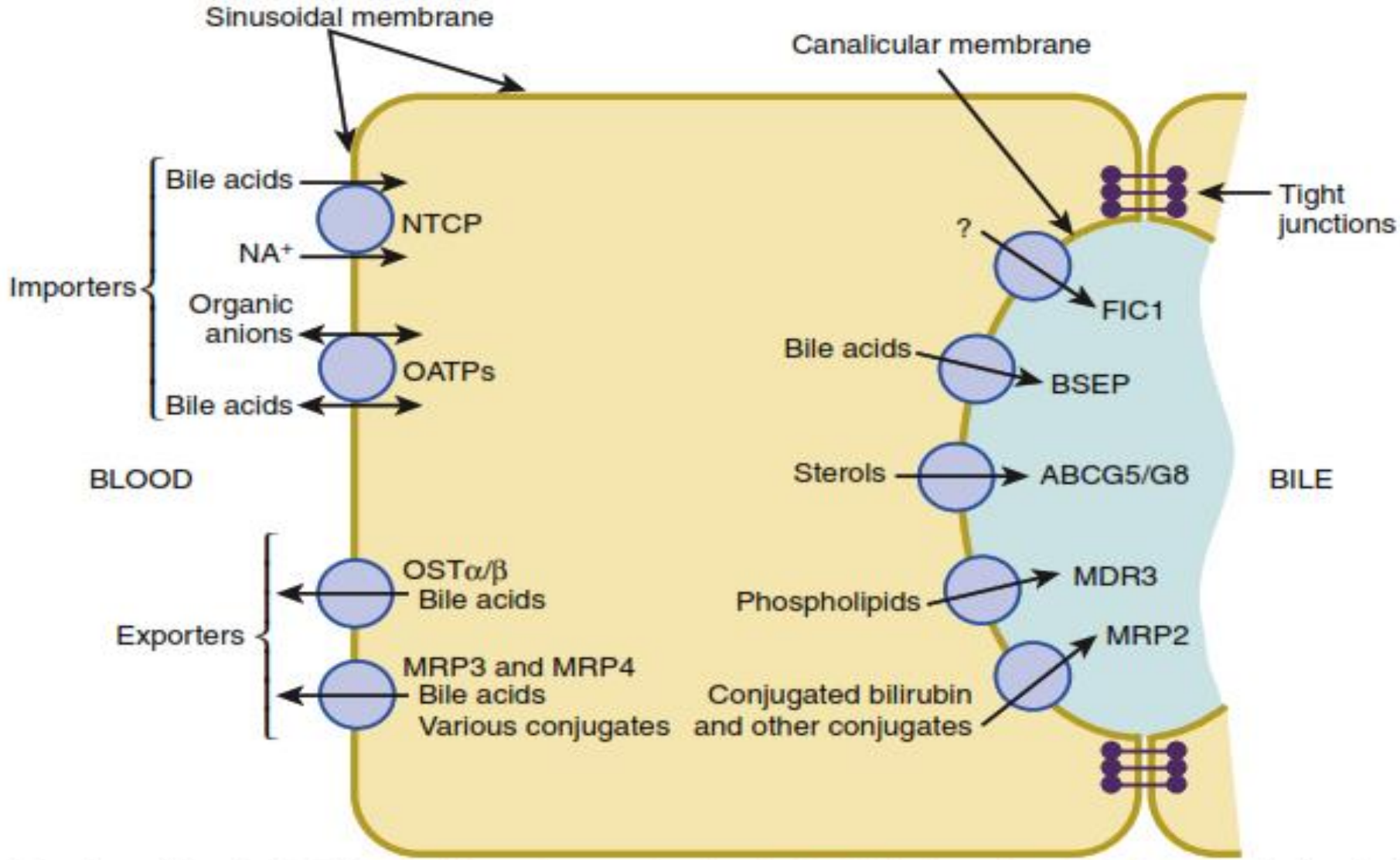


Fig. 89.7 Composite schematic of select hepatobiliary transporters involved in bile formation. The hepatocyte is oriented with sinusoidal (BLOOD) transporters on the left, canalicular (BILE) on the right. *Arrows* connote general directions that the listed solutes may be transported. *Arrows* denote distinct membranes and the tight junctions that help define these membrane domains. Note that the resident canalicular transporters are responsible for active secretion of nearly all components of bile. *BSEP*, Bile salt export pump; *MDR*, multidrug resistance protein; *MRP*, multidrug resistance-associated protein; *NTCP*, Na^+/taurocholate cotransporting polypeptide; *OA*, organic anion; *OATP*, organic anion-transporting polypeptide; *OC*, organic cation; *OST*, organic solute transporters.

EXTRA-HEPATIC CAUSES		
Biliary atresia	High platelets count; high GGT, sBA.	NA
Choledocal cyst	-	NA
Cholelithiasis	-	NA
Inspissated bile/mucous plug	-	NA
Congenital perforation of common bile duct	-	NA
INTRAHEPATIC CHOLESTASIS		
Cystic fibrosis *	Elevated sweat chloride	*CFTR*
$\alpha-1$-antitrypsin deficiency	Low serum A1AT	*SERPINA1*
Defects of the biliary canalicular transport		
PFIC1	High sBA; low GGT; elevated sweat chloride	*ATP8B1*
PFIC2	High sBA; low GGT; high AFP	*ABCB11*
PFIC3	High sBA; high GGT	*ABCB4*
FXR deficiency (PFIC5)	High sBA; low GGT; high AFP	*NR1H4*
MYO5B cholestasis	High sBA; low GGT	*MYO5B*
Tight junction defects		
TJP2 deficiency (PFIC4)	High sBA; low GGT	*TJP2*
USP53 deficiency	High sBA; low GGT	*USP53*
NISCH syndrome	High sBA; high GGT	*CLDN1*
Bile acid synthesis and conjugation disorders		
3-β-HSD-oxidoreductase deficiency	Low sBA, GGT; bile acid mass spectrometry	*HSD3B7*
D4–3-oxosteroid 5 β-reductase deficiency	High GGT; low sBA; biliary acid mass spectrometry	*AKR1D1*
Cerebrotendinous xanthomatosis	Low sBA; high cholesterol and cholestanol	*CYP27A1*
BACL deficiency	Low GGT, sBA; biliary acid mass spectrometry	*SLC27A5*
BAAT deficiency	Low GGT, sBA; biliary acid mass spectrometry	*BAAT*
2-methylacil-CoA racemase deficiency	Low GGT, sBA; biliary acid mass spectrometry	*AMACR*
Oxisterol-7α-hydroxylase deficiency	Low GGT, sBA; biliary acid mass spectrometry	*CYP7B1*
Biliary development defects		
Alagille syndrome	High GGT, sBA, cholesterol, triglycerides	*JAG1, NOTCH 2*
Neonatal sclerosing cholangitis	High GGT, sBA	*DCDC2; CLDN1*
ARC syndrome	High sBA; low or high GGT; metabolic	*VPS33B, VIPAS39*

Caroli disease	High GGT, sBA	*PKHD1*
Ciliopathies	High GGT, sBA	*different genes*
Lysosomal storage disease		
Niemann-Pick disease type C *	Markedly elevated oxysterols and lysosphingolipids in plasma; accumulation of intracytoplasmic unesterified cholesterol in skin fibroblasts (filipin staining); elevated chitotriosidase; sea blue histiocytes in bone marrow	*NPC1; NPC2*
Acid sphyngomyelinase deficiency (Niermann-Pick disease type A and B) *	Markedly elevated oxysterols and lysosphingolipids in plasma; reduced acid sphingomyelinase activity (dried blood spot, leukocytes, fibroblasts);	*SMPD1*
Lysosomal acid lipase deficiency (Wolman disease) *	Reduced acid lipase activity (dried blood spot, leukocytes, fibroblasts); abnormal lipid profile; vacuolated lymphocytes; macrophage activation	*LIPA*
Gaucher disease (neurologic) *	Reduced glucocerebrosidase activity (dried blood spot, leukocytes, fibroblasts); foamy cells in bone marrow; high angiotensin-converting enzyme, tartrate-resistant acid phosphatase, chitotriosidase; thrombocytopenia	*GBA*
Mitochondrial disorders		
Mitochondrial DNA depletion syndrome	Hypoglycemia; lactic acidosis; high plasma alpha-fetoprotein; hyperferritinemia iron overload; coagulopathy; abnormal urine organic acid	*POLG, DGUOK, MPV17*
SUCLG1, C10ORF2, elongation factor G1,TRMU related and BCS1L deficiency	Hypoglycemia; lactic acidosis; abnormal urine organic acid	*SUCLG1, C10ORF2, EGF1, TRMU, BCS1L*

Mitochondrial Fatty Acid Oxidation defects		
LCHAD/MTP deficiency*	Hypoketotic hypoglycemia; high levels of CPK; abnormal blood acylcarnitine and urine organic acids profiles	*HADHA, HADHB*
Peroxisomal disorders		
Zellweger spectrum disorders	Elevated very long-chain fatty (VLCFA), phytanic and pristanic acids in plasma;	*PEX genes*
Aminoacidopaties		
Tyrosinemia type 1*	Elevated succinylacetone (dry blood spot, plasma, urine); elevated tyrosine and methinine (dry blood spot, plasma); elevatedAFP; coagulopathy; hypophosphatemia; hypoglycemia; hyperaminoaciduria; elevated delta-aminolevulinic acid urine	*FAH*
Adenosine kinase deficiency *	Hypoglycemia (hyperinsulinemic); elevation (intermittent) of methionine in plasma; coagulopathy; elevated adenosine in dry blood spot and urine (transient)	*ADK*
S-adenosylhomocysteine hydrolase deficiency*	Elevated methionine, S-adenosyl-homocysteine, S-adenosyl-methionine and homocysteine in plasma; high levels of CPK	*AHCY*
Inborn error of polyols and pentose metabolism		
Transaldolase deficiency	Coagulopathy; anemia and thrombocytopenia; hypothiroidism; renal tubulopathy; abnormal profile of urinary polyols	*TALDO1*
Carbohydrate metabolism defects		
Classic galactosemia*	Elevated galactose (dry blood spot, erythrocytes); reduced GALT activity in erythrocytes; coagulopathy; hypoglycemia; renal tubulopathy; neonatal *E. Coli* sepsis; positive urinary reducing substances	*GALT*
Glycogen storage disease type IV	Fasting hypoglycemia; high levels of CPK; PAS positive inclusions at liver and muscle histology; reduced enzyme activity (liver tissue, muscle, leukocytes, fibroblasts)	*GBE1*

Urea cycle defects		
Urea cycle defects*	Hyperammonemia; abnormal aminoacid profile in plasma; elevated orotic acid in urine	*OTC, ASS, ASL, ARG*
Citrin deficiency (NICCD)	Elevated citrulline (dry blood spot; plasma); elevated galactose and AFP; hypoglycemia; hyperammonemia	*SLC25A13*
Cholesterol metabolism disorders		
Smith-Lemli-Opitz syndrome	Elevated 7-dehydrocholesterol and 8-dehydrocholesterol in plasma	*DHCR7*
Mevalonic aciduria	Anemia and thrombocytopenia; hyper-Ig D; abnormal organic urine acids	*MVK*
Cellular trafficking abnormalities		
NBAS deficiency	Hypoglycemia; lactic acidosis; coagulopathy, abnormal urine organic acid	*NBAS*
CALFAN syndrome	Low GGT	*SCYL1*
Metal metabolism disorder		
MEDNIK syndrome	Low serum copper and ceruloplasmin; high urinary copper; mild elevation of plasma VLCFA	*AP1S1*
Syndromic cholestasis (most relevant)		
Down and Edwards Syndrome	Abnormal kariotype	*Trisomy 21, 18*
Kabuki syndrome	Low or high GGT	*KMT2D, KDM6A, MLL2*
Noonan syndrome	High GGT	*PTPN11, SOS1, RAF1 and KRAS*
Aagenaes syndrome	High GGT	*LSC1, CCBE1*

ENDOCRINOLOGIC DISEASES		
Thryroid disorders	TSH and FT4 values	*different genes. in congenital hypotiroidism (e.g., FOXE1, NKX2–1/5, PAX8, SLC26A4, TSHR)*
Panhypopituitarism	TSH and FT4, ACTH, cortisol, GH, IGF1, PRL, LH, FSH, stimulating test, brain MRI	*different genes in genetic forms (e.g., HESX1, PROP1, POUF1, LHX3, LHX4, GLI2, SOX3)*
Adrenal insufficiency	ACTH, cortisol stimulating test	*monogenic forms (e.g., POR, MC2R, MRAP, StAR, AYP11A1, NNT, TRXR2) syndromic forms (eg.CDKN1C, MCM4, SAMD9, SGPL1)*
INFECTIOUS DISORDERS		
Cytomegalovirus, Herpes virus type 1–2–6; toxoplasma; rubella; parvovirus B19; enterovirus (including coxsackievirus, echovirus), adenovirus, syphilis, HIV, listeria monocitogenes, congenital tubercuolosis	Low or high GGT, serology in the proband and mother, specific direct nucleic acid testing via PCR	NA
HEMATOLOGIC AND IMMUNE-MEDIATED DISORDERS		

(*continued on next page*)

Hemophagocitic lymphoistiocytosis	HLH clinical and testing criteria (cytopenia, hypertriglyceridemia or hypofibrinogenemia, hemophagocytosis in biopsy samples, low or absent NK activity, high serum ferritin, elevated CD25 levels	*different genes (e.g., PRF1, UNC13D, STX11, STXBP2, RAB 27, XLP)*
Neonatal hemocromatosis (GALD and non-GALD)	Hypoglycemia, coagulopathy, high ferritin, high alpha-fetoprotein, low trasferrin and high iron saturations	*non-GALD (e.g., DGUOK, SRD5B1, BCS1L)*
Congenital lupus	ANA, positive Coombs-test	NA
Post-hemolytic cholestasis	Hemolytic disease of the newborn due to Rh or ABO alloimmunization	NA
TOXIC AND SECONDARY CHOLESTASIS		
Parenteral nutrition associated cholestasis (PNALD); drugs; intestinal obstruction; cardiovascular disorders, neoplastic disorders; perinatal asphyxia	Low or High GGT	NA

METABOLIC DISORDERS, STORAGE DISEASES, AND OTHERS		
Cystic fibrosis	Newborn screening (not in Germany), trypsinogen content in stool, genetic analysis	*CFTR* gene; Sokol and Durie (57)
A1AT deficiency	A1AT levels ↓ PI analysis (type ZZ, SZ, MZ)	*SERPINA1* gene analysis for prenatal diagnosis; Perlmutter (59), Fregonese and Stolk (58)
Inborn errors of bile acid synthesis	Urinary bile acid analysis, molecular–genetic analysis	Clayton et al. (66); *BAAT and SLC27A5* gene, Setchell et al. (67)
Gaucher disease	AP ↑, β-glucocerebrosidase ↓, chitotriosidase ↑, BM biopsy: "crinkled paper" cytoplasm and glycolipid-laden macrophages, foam cells (Gaucher cells)	Rosenbloom et al. (69)
Niemann–Pick type C	Filipin positive reaction (detection of cholesterol in fibroblasts), genetic testing, chitotriosidase ↑	*NPC1, NPC2* gene; Patterson et al.(74)
Wolman disease, LAL deficiency	Lysosomal lipase acid ↓↓ in PBMC	*LIPA* gene; Zhang and Porto (75)
Mitochondrial disorders	Fasting and postprandial lactate, plasma lactate/pyruvate ratio >20, functional assays, genetics	*SCO1, SUCLG1, BCS1L, POLG1, C10ORF2, DGUOK*, and *MPV17* gene mutations; Fellman and Kotarsky (76), Wong et al. (77)
Neonatal Intrahepatic cholestasis caused by citrin deficiency (NICCD)	Citrulline ↑, α-fetoprotein ↑, and ferritin↑	*SLC25A13* gene; Lu et al. (78), Kimura et al. (79), Song et al. (80)
Peroxismal disorders (Zellweger's spectrum and others)	Zellweger's: Typical craniofacial dysmorphism, mental retardation, hepatomegaly, glomerulocystic kidney disease, cataracts, pigmentary retinopathy VLCFA ↑, pattern of plasmalogenes, phytanic acid, pristanic acid	Moser et al. (81, 82)
Tyrosinemia	Newborn screening, urinary excretion of succinylacetone ↑, 4-hydroxy-phenylketones, and δ-aminolevulinic acid↑ Cave: HCC (AFP↑) (83)	*FAH* gene, de Laet et al. (84)
Classic galactosemia	Newborn screening, galactose-1-phosphate uridyl transferase activity in red blood cells ↓↓	Mayatepek et al. (85)
Congenital disorders of glycosylation (CDG)	Dysmorphic facies, convergent strabism, inverted mammils, mental retardation, seizures, dystrophy, hepatomegaly, hepatic fibrosis/steatosis, cyclic vomiting and diarrhea, coagulopathy, protein losing enteropathy with hypoalbuminemia (CDG1b) Lab chemistry: Triglycerides ↑, ATIII ↓, factor XI ↓, protein C and S ↓, Transferrin IEF	Jaeken (86), Freeze (87, 88), Linssen et al. (89)

Box 8:1 Evaluation of the Infant with Cholestasis

Initial investigations to establish the presence of cholestasis, define the severity of the liver disease, and detect readily treatable disorders:

- History (including pregnancy, early neonatal course);
- Presence of extrahepatic anomalies or extrahepatic disease;
- Stool color;
- Fractionated serum bilirubin analysis;
- Serum tests for liver injury and liver function: alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, serum glucose, serum albumin, prothrombin time, complete blood count;
- If concerns for infection: blood and urine cultures.

Investigations to establish a specific diagnosis:

- Abdominal ultrasound;
- Alpha-1 antitrypsin level and phenotype;
- Infectious work-up as indicated from history and physical examination (blood cultures, viral cultures, serologies);
- Metabolic testing: serum amino acids, urine organic acids, acylcarnitine, newborn screen;
- Thyroid hormone, thyroid stimulating hormone (if low GGT and concern for hypopituitarism or hypothyroidism);
- Urine and serum analysis for bile acid and bile acid precursors (especially if low GGT);
- Urine reducing substances and/or red blood cell galactose-1-phosphate uridyltransferase for galactosemia;
- Echocardiogram, eye exam for posterior embyrotoxon, spine films if concerned for Alagille syndrome;
- Liver biopsy for histology, immunohistochemistry, electron microscopy and snap freeze for enzymatic testing if indicated;
- Exploratory laparotomy and intraoperative cholangiogram;
- Genetic testing (targeted gene panels, whole exome sequencing, whole genome sequencing based on clinical suspicion and clinical availability of testing).

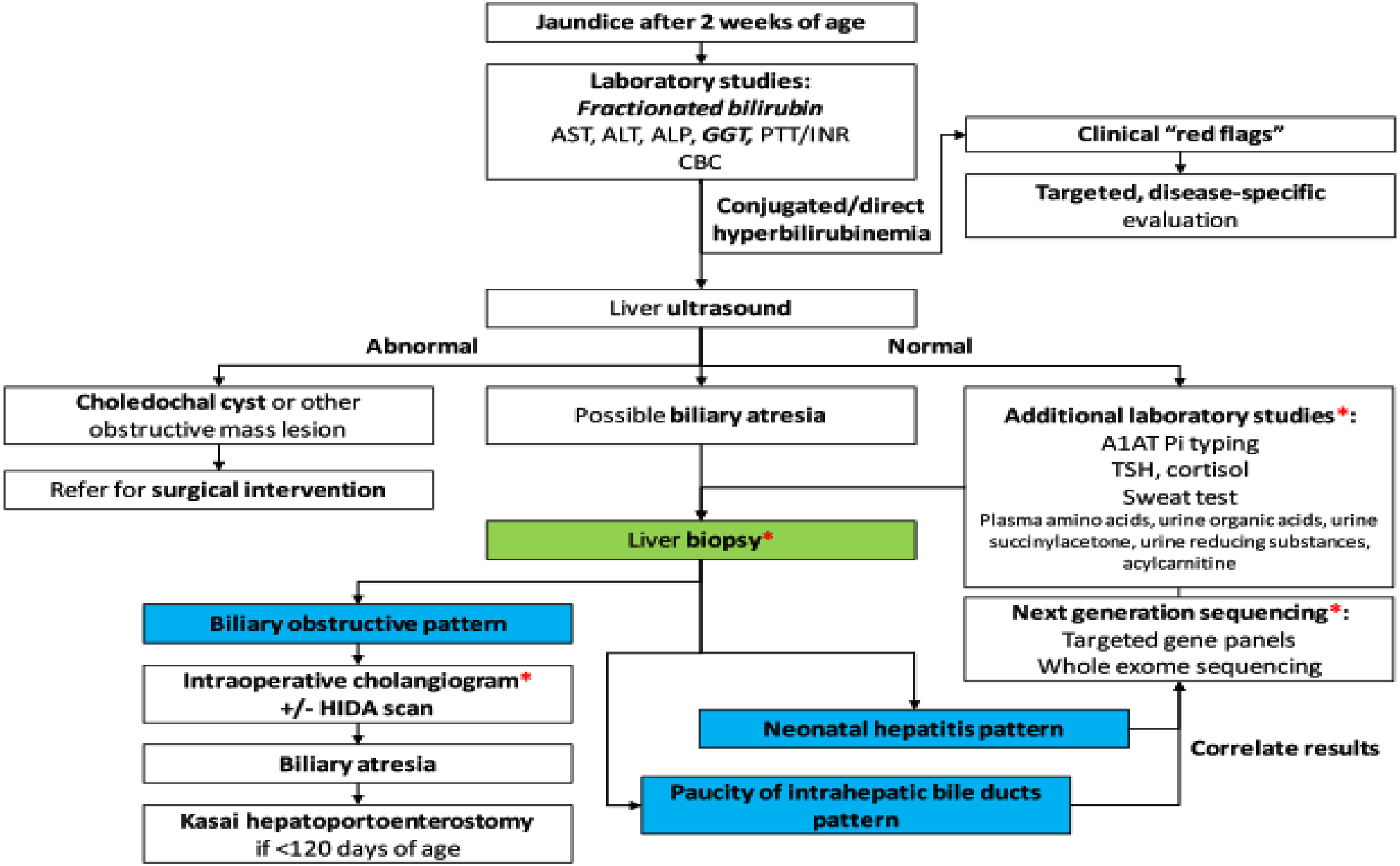
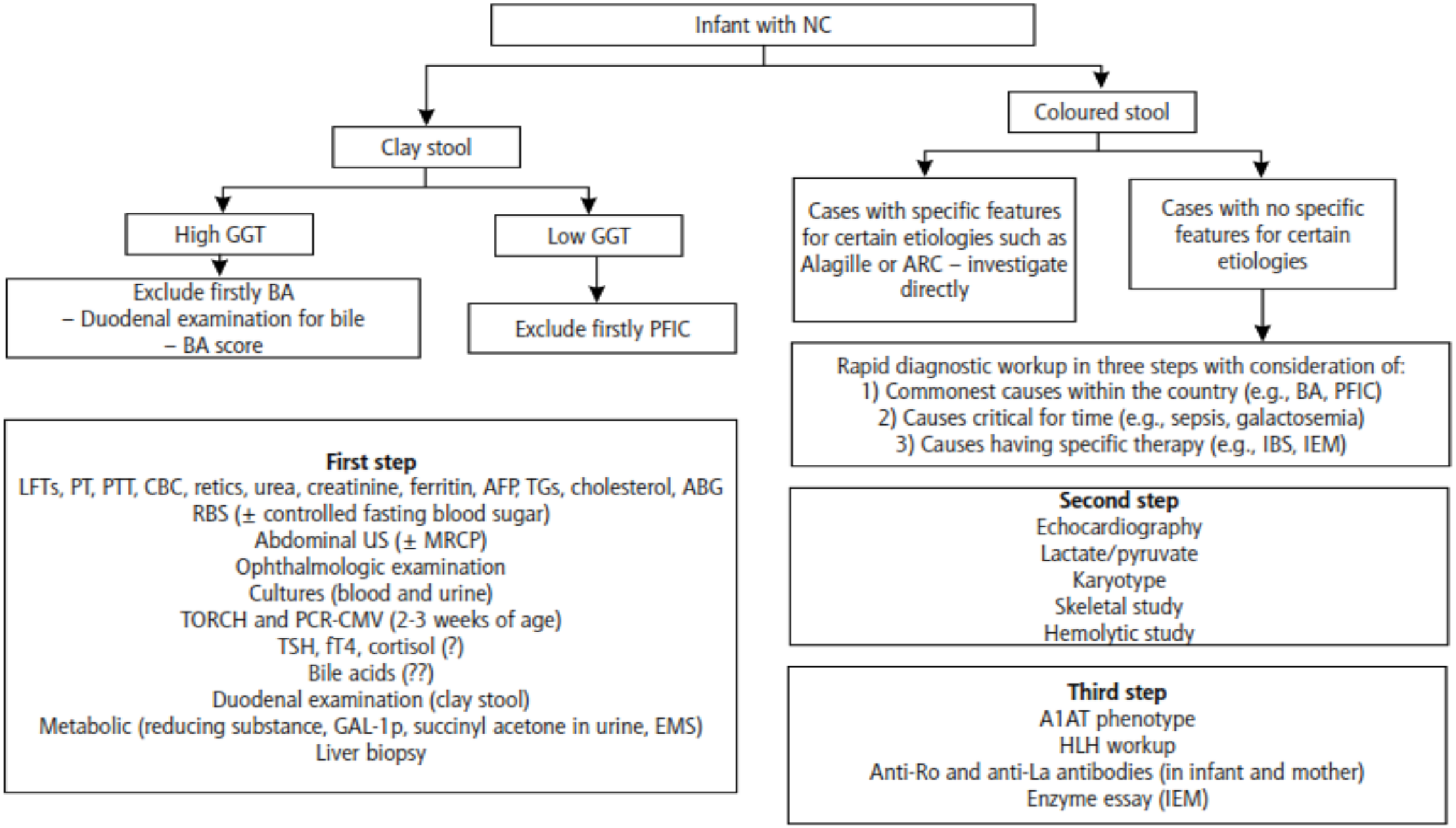
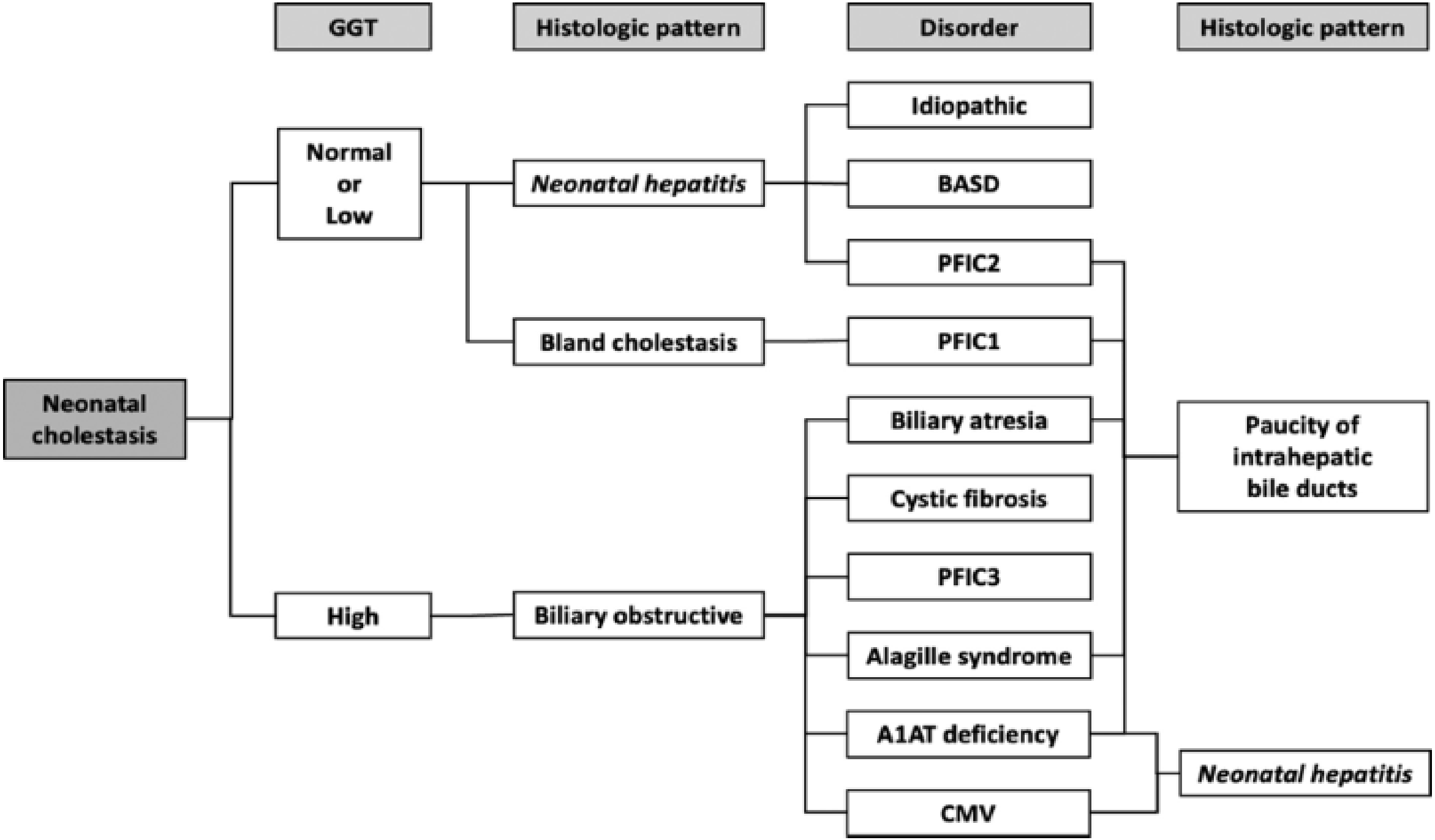


Fig. 2. Liver biopsy in the clinical work-up of neonatal cholestasis. AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; GGT, Gamma-glutamyl transferase; PTT, Prothrombin time; INR, International normalized ratio; CBC, Complete blood count; A1AT, Alpha-1 antitrypsin; Pi, Protease inhibitor; TSH, Thyroid stimulating hormone; HIDA, hepatobiliary iminodiacetic acid scan; *, Tests that may occur concurrently.


Infant with NC
Clay stool
Coloured stool
High GGT
Low GGT
Cases with specific features for certain etiologies such as Alagille or ARC – investigate directly
Cases with no specific features for certain etiologies
Exclude firstly BA
– Duodenal examination for bile
– BA score
Exclude firstly PFIC
Rapid diagnostic workup in three steps with consideration of:
1) Commonest causes within the country (e.g., BA, PFIC)
2) Causes critical for time (e.g., sepsis, galactosemia)
3) Causes having specific therapy (e.g., IBS, IEM)
First step
LFTs, PT, PTT, CBC, retics, urea, creatinine, ferritin, AFP, TGs, cholesterol, ABG
RBS (± controlled fasting blood sugar)
Abdominal US (± MRCP)
Ophthalmologic examination
Cultures (blood and urine)
TORCH and PCR-CMV (2-3 weeks of age)
TSH, fT4, cortisol (?)
Bile acids (??)
Duodenal examination (clay stool)
Metabolic (reducing substance, GAL-1p, succinyl acetone in urine, EMS)
Liver biopsy
Second step
Echocardiography
Lactate/pyruvate
Karyotype
Skeletal study
Hemolytic study
Third step
A1AT phenotype
HLH workup
Anti-Ro and anti-La antibodies (in infant and mother)
Enzyme essay (IEM)


GGT
Histologic pattern
Disorder
Histologic pattern
Neonatal cholestasis
Normal or Low
High
Neonatal hepatitis
Bland cholestasis
Biliary obstructive
Idiopathic
BASD
PFIC2
PFIC1
Biliary atresia
Cystic fibrosis
PFIC3
Alagille syndrome
A1AT deficiency
CMV
Paucity of intrahepatic bile ducts
Neonatal hepatitis

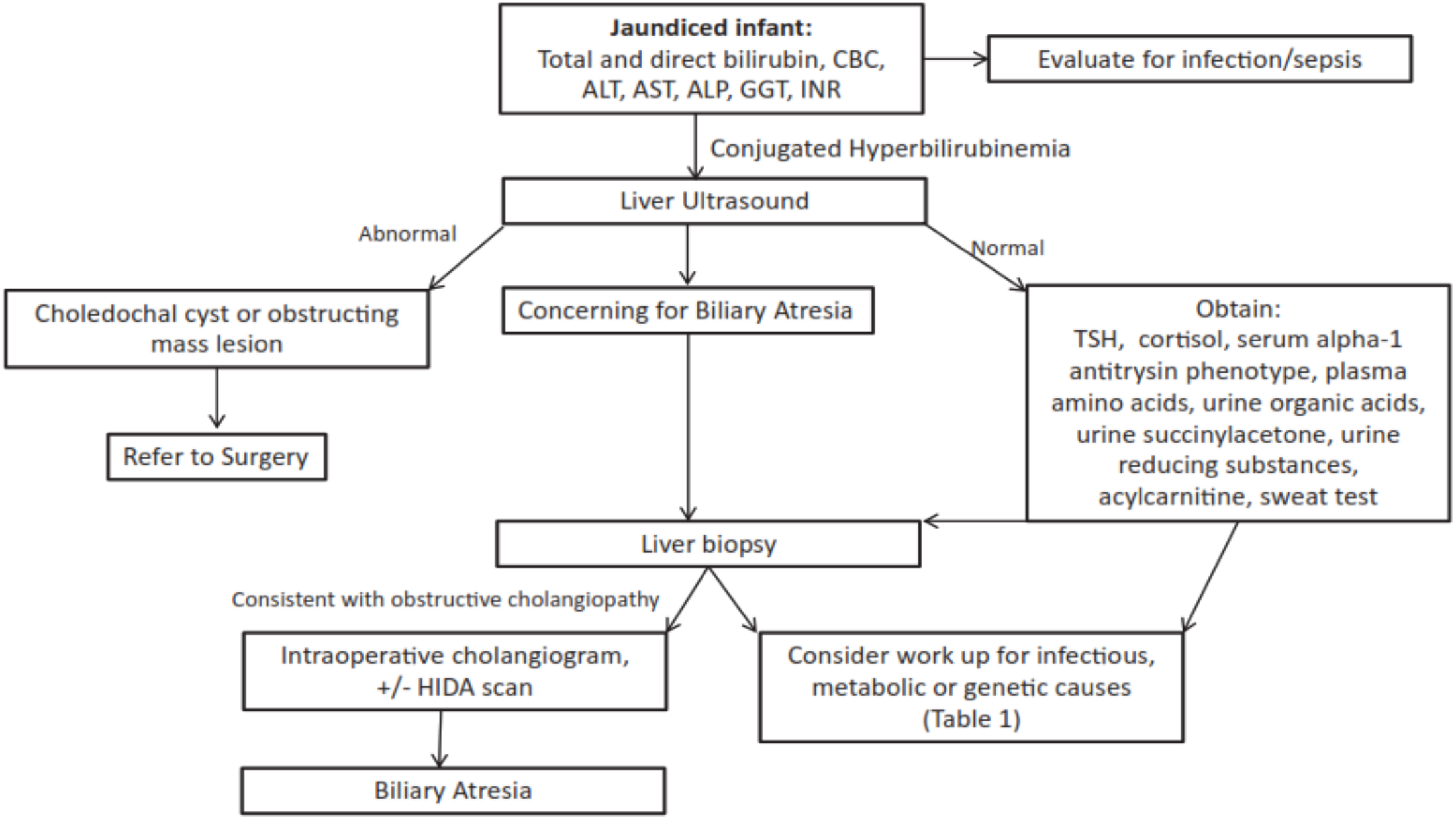


Fig. 1. Algorithmic approach to evaluation of neonatal cholestasis. ALP, alkaline phosphatase; CBC, complete blood count; TSH, thyroid stimulating hormone.