Laboratory Assessment of Hepatic Injury and Function

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The approach to the child with liver disease should be based on an accurate clinical history and a thorough physical examination. Investigating the liver relies on a multidisciplinary approach involving clinical chemistry, hematology, immunology, imaging studies, endoscopy, histopathology, and microbiology. Mutational analyses for many genetic liver diseases are now available. This chapter will outline the basic laboratory assessment of the liver and main disease categories and summarize specialized laboratory investigations which identify the underlying diagnosis.

Baseline Investigations: Biochemical Liver Function Tests

The main functions of the liver include synthesis (albumin, coagulation factors, bile acids), metabolism (carbohydrate, lipid, protein), degradation/detoxification, and excretion (Table 3.1). Biochemical liver function tests (Table 3.2) reflect the severity of hepatic dysfunction but rarely provide diagnostic information on individual diseases.

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Bilirubin: Conjugated bilirubin is nearly always elevated in liver disease [1]. The presence of bilirubin is always abnormal if detected in a fresh urine specimen.

Aminotransferases are intracellular enzymes, which are present in liver, heart, and skeletal muscles. Increases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) indicate hepatic necrosis irrespective of etiology (Table 3.2). ALT is more liver specific than AST but has a longer plasma half-life (approximately 24 h). A rise in AST is an early indication of liver damage and is a useful marker of rejection post-liver transplant. Elevated aminotransferases are often the first indication of the development of nonalcoholic fatty liver disease (NAFLD) in an obese child. Elevated aspartate and/or alanine aminotransferases are also found in muscular dystrophy and this diagnosis should be considered if there are no other signs of liver disease. These enzymes, however, may be normal in compensated cirrhosis.

Alkaline phosphatase is found in the liver, kidney, bone, placenta, and intestine. In pediatric liver disease, a raised alkaline phosphatase indicates biliary epithelial damage, malignant infiltration, cirrhosis, rejection, or osteopenia secondary to vitamin D deficiency. In a growing child, however, the potential contribution from bone makes alkaline phosphatase measurement less specific for liver pathology.

Gamma-glutamyl transpeptidase (*GGT*) is present in biliary epithelia and hepatocytes and also in the cell membrane of many other human

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Table 3.1 Functions of the liver

Function	Effect of dysfunction	Assessment
Metabolism/storage		
Carbohydrate/glycogen	Loss of glucose homeostasis	Hypoglycemia in fasting/stress
Lipid	Lipid accumulation in hepatocytes	High/low cholesterol
	↓Oxidation of fatty acids	↑Lactate, ↑ratio FFA:BOH
		↑Acylcarnitine, organic aciduria
Protein	↑Catabolism	Low BCAA, urea
		↑Ammonia, ↑tyrosine, phenylalanine, methionine
Synthesis		
Albumin	Loss of muscle mass	Low albumin
	protein energy malnutrition	
Factors II, VII, IX, X	Coagulopathy	Prolonged PT/PTT
Degradation		
Drugs	Prolonged drug effect, e.g., sedation	Clinical
Estrogens	Telangiectasia, gynecomastia	Clinical
Toxic products	Encephalopathy	Abnormal EEG/clinical signs
Bile synthesis and excretion	Cholestasis	↑Conjugated bilirubin
	Fat malabsorption	↑GGT ↑ALP ↑cholesterol
	Fat-soluble vitamin deficiency	Anthropometry
	Pruritus, malnutrition	

ALP alkaline phosphatase, *BOH* β-hydroxybutyrate, *BCAA* branched-chain amino acids, *EEG* electroencephalogram, *FFA* free fatty acids, *GGT* gamma-glutamyl transpeptidase, *PT* prothrombin time, *PTT* partial thromboplastin time

Table 3.2 Baseline and initial investigations for the evaluation of liver function

Reference range of test	Liver-associated abnormality
Conjugated bilirubin <20 μmol/l	Elevated: hepatocyte dysfunction or biliary obstruction
Aminotransferases Aspartate (AST) <50 U/l Alanine (ALT) <40 U/l	Elevated: hepatocyte inflammation/damage
Alkaline phosphatase (ALP) <600 U/l (age dependent)	Elevated: biliary inflammation/obstruction, nonspecific
Gamma-glutamyl transpeptidase (GGT) <30 U/l (age dependent)	Elevated: biliary inflammation/obstruction, nonspecific
Albumin 35–50 g/l	Reduced: chronic liver disease
Prothrombin time (PT) 12–15 s	Prolonged: (i) hepatocellular dysfunction, (ii) vitamin K deficiency
Partial thromboplastin time (PTT) 33–37 s	
Ammonia <50 mmol/l	Elevated: abnormal protein catabolism/urea cycle defect/other inherited metabolic disease
Glucose >4 mmol/l	Reduced in: acute or chronic liver failure/metabolic disease/ hypopituitarism

organs, including kidney, pancreas, spleen, brain, breast, and small intestine. An elevated GGT is not specific for liver disease. In addition, the reference range is age related, with higher levels in neonates (up to 385 IU/l). It is elevated in many forms of liver damage. However, GGT does not increase in the serum of patients with bone disease or children with active bone growth, thus helpful in confirming the liver origin of a raised alkaline phosphatase. It may be normal in certain forms of intrahepatic cholestasis (progressive familial intrahepatic cholestasis 1 and 2; PFIC 1 & 2) [2].

Table 3.3 Laboratory assessment in chronic liver disease

Second-line investigations
Bacterial culture of blood, urine, +/– cerebrospinal fluid
Serology for hepatitis A, B, C, E
α1-Antitrypsin level and phenotype
Abdominal ultrasound
Metabolic investigations
Immunoreactive trypsin
Plasma lactate, BOH, FFA, ammonia
Acylcarnitine
Serum iron and ferritin
Plasma amino acids
Cholesterol, triglyceride
α-Fetoprotein
Parathyroid hormone, wrist X-ray for bone age/rickets
Urine: reducing sugars, organic acids, amino acids, succinylacetone, bile salts

BOH β-hydroxybutyrate, *FFA* free fatty acids

Table 3.4 Age-specific investigations in chronic liver disease

Neonate	TORCHES screen
	Galactose 1-phosphate uridyltransferase
	Free T4, TSH, AM cortisol
	Targeted DNA mutational analysis
	Sweat test (>4 weeks)
Older child (>2 years)	Cu, ceruloplasmin, urinary Cu
	C3, C4, ANA, SMA, LKM, immunoglobulins
	EBV
If indicated	Liver biopsy for: histology, electron microscopy, enzyme analysis, immunohistochemistry, culture, copper concentration
	Skin biopsy, ophthalmology, cardiology, bone marrow aspirate
	Endoscopy, ERCP

ANA antinuclear antibodies, *C3*, *C4* complement components 3 and 4, *Cu* copper, *DNA* deoxyribonucleic acid, *EBV* Epstein–Barr virus, *ERCP* endoscopic retrograde cholangiopancreatography, *LKM* liver–kidney microsomal antibodies, *SMA* smooth muscle antibodies, *T4* thyroxine, *TORCHES* toxoplasmosis, rubella, cytomegalovirus, herpes simplex, syphilis, *TSH* thyroid-stimulating hormone

The most useful tests of liver "function" are *plasma albumin* concentration and *coagulation time*. In the absence of excessive urinary or gastrointestinal loss or prolonged starvation, a low serum albumin, which has a half-life of 20 days, indicates chronicity of liver disease. Abnormal coagulation, especially prothrombin time (PT) after vitamin K deficiency is ruled out, indicates significant hepatic dysfunction, either acute or chronic. *Fasting hypoglycemia* in the absence of other causes (e.g., hypopituitarism or hyperinsulinism) indicates poor hepatic function and is a guide to prognosis in acute liver failure. If these baseline investigations suggest hepatic dysfunction, then more specific investigations for metabolic disease are appropriate to consider [3–5] (Table 3.3).

Second-Line Investigations

Hepatic dysfunction may be secondary to sepsis, particularly urinary sepsis, inborn errors of metabolism, or endocrine disorders. It is usual to exclude sepsis by performing bacterial culture of the urine and/or blood and cerebrospinal fluid cultures if appropriate (Tables 3.3 and 3.4).

In neonates, hypopituitarism may be difficult to exclude as thyroid function tests may be equivocal or in the low normal range. It is useful to perform a 09.00 h cortisol level at the same time as measuring free thyroxine and thyroid-stimulating hormone (TSH) [6].

If the infant is unwell, or has evidence of acute liver failure, galactosemia and tyrosinemia should be excluded (see below). Urea cycle defects should also be considered particularly if the serum ammonia is raised.

Alpha-1-antitrypsin deficiency is the most common inherited metabolic liver disease and should always be excluded, regardless of age. As α-1-antitrypsin is an acute-phase protein, it is necessary to measure both concentration and phenotype in order to differentiate between normal and an acute-phase response in the setting of homozygous or heterozygous deficiency.

Although cystic fibrosis is a rare cause of liver disease in the neonatal period, it should be considered in the differential diagnosis of neonatal liver disease and excluded by performing an immunoreactive trypsin test, a sweat test, and mutational analysis if either is positive.

Wilson disease rarely presents before the age of 3 years but may mimic any form of liver disease and should always be excluded in older children [7]. An autoimmune screen and immunoglobulin levels should detect 75 % of children with autoimmune hepatitis.

Serum cholesterol is usually elevated in children with severe cholestasis (e.g., Alagille syndrome, biliary atresia). In contrast, low or normal cholesterol is characteristic of bile acid transport disorders or terminal liver disease.

Plasma ammonia and amino acids (particularly phenylalanine, tyrosine, and methionine) may be raised in either acute or chronic liver failure and are nonspecific indications of hepatic dysfunction. Primitive hepatic cells synthesize α-fetoprotein. The levels are highest in the newborn (>1,000 mg/l) and fall in the first few months of life. It may be a useful screening test in the diagnosis of tyrosinemia type I and hepatoblastoma or for detection of hepatocellular carcinoma in chronic carriers of hepatitis B and C. The α-fetoprotein level can be as high as 100,000 mg/l in hepatoblastoma [8].

Neonatal Liver Disease

Most infants with liver disease present in the neonatal period with persistent jaundice. Although physiologic jaundice is common in neonates, infants who develop severe or persistent jaundice should be investigated to exclude hemolysis, sepsis, or underlying liver disease. Neonatal jaundice that persists beyond 14 days in term infants and 21 days in preterm infants should always be investigated, even in breast-fed babies [1]. It is also necessary to establish whether the jaundice is due to an increase in conjugated or unconjugated hyperbilirubinemia.

Unconjugated hyperbilirubinemia: Common causes include ABO and rhesus incompatibilities, breast-milk jaundice, sepsis, Gilbert syndrome, and rarely, Crigler–Najjar type I or II (Table 3.5).

Table 3.5 Common and uncommon causes of unconjugated hyperbilirubinemia in infancy

Conditions	Frequency	Features/comments
Physiologic jaundice	Very common	Usually benign, ~ 8–20 % of infants with physiologic jaundice may have serum bilirubin >200 μmol/l
Hemolytic jaundice	Common	Causes include ABO and Rh incompatibilities, glucose-6-phosphate dehydrogenase deficiency, red cell membrane defects
Breast-milk jaundice	Common	May overlap with physiologic jaundice and may last till 1–2 months
Sepsis	Common	Sick infants; blood, urine, cerebrospinal fluid cultures, chest X-ray
Hypothyroidism	Common	Thyroid function tests show high thyroid-stimulating hormone and low T4
Gilbert syndrome	Common	Important cause of unconjugated hyperbilirubinemia of unknown cause, benign, polymorphism of the 5′ end of the promoter of the UGT1A1 gene homozygous insertion of the TA pair – genotype UGT1A1*28/*28
Crigler–Najjar syndrome type 1	Rare	Autosomal recessive, mutations in the UGT1A1 gene resulting in either truncated nonfunctional enzyme or nonrecognition of the substrate Bilirubin; rapid rise in conjugated bilirubin early in life may lead to kernicterus
Crigler–Najjar syndrome type 2	Rare	Autosomal recessive, mutations have been reported in exon 1A1 of the UGT1A1 gene, clinically less severe than type 1 disease and responsive to phenobarbital therapy

Table 3.6 Laboratory assessment of the cholestatic infant

Blood	
Liver function tests	Baseline liver investigation (see Tables 3.2 and 3.3)
Electrolytes	Sodium, potassium, urea, creatinine, calcium, phosphate, bicarbonate, glucose
Hematology	Full blood count with differential and reticulocyte count; clotting screen including fibrinogen; blood group and Coombs test
Serology	TORCHES, adenovirus antibody, consider herpes simplex PCR, hepatitis B serology
Metabolic	Fasting bloods for cortisol, glucose, lactate, β-hydroxybutyrate, free fatty acids and amino acids; cholesterol and triglycerides (see also Table 3.11)
Specific metabolic tests	Galactosemia and tyrosinemia screen; thyroid function tests, parathyroid hormone; chitotriosidase; immunoreactive trypsin; α-1-antitrypsin level and phenotype
Vitamin levels	Vitamins A, D, and E
Urine	
General	Urine pH, glucose, ketones, and protein. Protein-to-creatinine ratio if protein is present, culture
Clinical chemistry	Amino acids; reducing substances; bile acids (if possible); tubular reabsorption of phosphate
Virology	CMV culture
Microbiology	Urine microscopy and culture

Note: see also Tables 3.2, 3.3, and 3.11

Conjugated hyperbilirubinemia: A rise in conjugated bilirubin always signifies an underlying liver condition and warrants further assessment (Table 3.6) [1]. It is important to exclude surgical disorders such as biliary atresia in infants with neonatal cholestasis as early surgery is associated with a better outcome (Table 3.7) [9, 10]. Similarly, bacterial infections and metabolic conditions have improved outcomes with early identification and treatment and hence warrant rapid investigation. Although not usually posing a diagnostic dilemma, the successful management of preterm infants as young as 25 weeks' gestation has increased the number of children treated with parenteral nutrition (PN) and a consequential rise in referrals of these infants with persistent jaundice [11]. Other conditions to be considered that can present as neonatal cholestasis are listed in Table 3.8 [1, 12].

Table 3.7 Assessments of infants (2 weeks–6 weeks of age) suspected of biliary atresia

Assessment	Remarks/results
General	Generally well and thriving
Stool color	Progresses to persistently pale (Fig. 3.1)
Liver	Enlarged and usually firm in consistency
Spleen	May be enlarged
Ascites	Rare
Clinical biochemistry	Conjugated hyperbilirubinemia; raised ALT, AST, ALP, and GGT
Abdominal ultrasound	Absent or contracted gall bladder common; presence of triangular cord (a fibrous cone of tissue at the bifurcation of the portal vein); exclude choledochal cyst
Hepatobiliary scintigraphy	Absence of biliary excretion of radioisotope
Histopathology	Preservation of overall hepatic architecture, prominent bile ductular proliferation, canalicular and cellular bile stasis, portal fibrosis

Liver Disease in Older Children

Liver disease in children older than 6 months may be acute or chronic. As in infancy, inherited disorders need to be excluded (Table 3.9), but jaundice may not be a prominent feature. Acute or chronic liver disease may be due to infection, autoimmune disease, drug-induced hepatitis, and metabolic diseases (Table 3.9).

Acute Liver Disease

Underlying causes and clinical presentation depends on the age, but the following clinical features are common: a prodrome of malaise, lethargy, and anorexia, and nausea, vomiting, or diarrhea. There may be weight loss, abdominal discomfort, tender hepatomegaly, splenomegaly, ascites (rarely, except for acute Budd–Chiari), rash, or joint pains. It is noteworthy that jaundice is not always present.

Fig. 3.1 A typical appearance of pale stool

Table 3.8 Differential diagnosis of infantile conjugated hyperbilirubinemia when biliary atresia has been excluded

Categories	Differential diagnosis	Investigations	Results
Infective	Congenital infection Bacteremia, urinary tract infection	Serology for toxoplasma, rubella, CMV buffy coat, herpes simplex, syphilis, PCR urine microscopy, urine and blood culture	Positive testing
Endocrine	Hypothyroidism	TFTs	Raised TSH, low T4
	Hypopituitarism	TFTs, cortisol, glucose	Low TSH, cortisol, hypoglycemia
Metabolic (see also Table 3.11)	Galactosemia	Urine reducing substances Plasma Gal-1-PUT	Positive reducing substances if on a galactose-containing diet Absent or reduced Gal-1-PUT detected
	Tyrosinemia	Urine succinyl acetone, DNA	High succinylacetone Mutations in *FAH*
	Storage disease, e.g., Niemann Pick C	Liver biopsy, bone marrow biopsy Filipin staining DNA	Storage cells on bone marrow and liver biopsy (can be difficult to see in young children), positive Filipin staining of fibroblasts Mutation in *NPC1 and 2*
	Bile salt synthesis disorders	Urinary bile salts (not accurate if on ursodeoxycholic acid), DNA	Abnormal peaks on urine mass spectroscopy Mutation in *AKR1D1*
	Peroxisomal disorders	Plasma very long-chain fatty acids DNA	High levels of very long-chain fatty acids Mutation in *PEX* genes
Genetic	α-1-Antitrypsin deficiency	α-1-Antitrypsin level and phenotype	Low α-1-antitrypsin level and PiZZ or ZS phenotype
	Arthrogryposis, renal dysfunction, cholestasis (ARC) syndrome	GGT DNA	Low GGT cholestasis Mutation in *VPS33B* or *VIPAR*
	Citrin deficiency	Plasma and urine amino acids DNA	Increased plasma and urine citrulline and arginine Mutation in *SLC25A13*
Toxic	Intestinal failure-associated liver disease	Liver biopsy	Cholestasis with hepatocellular necrosis, abundant lipofuscin, fatty infiltration, mild giant cell transformation, portal tract infiltration, bile duct reaction, ±portal fibrosis

Note: *GGT* gamma-glutamyl transpeptidase, *TFTs* thyroid function tests, *TSH* thyroid-stimulating hormone

Table 3.9 Causes of acute liver disease and failure in older children

Disease	Investigations	Comments
Infections		
Viruses:		
Hepatitis A	Anti-HAV IgM	
Hepatitis B	HBsAg, anti-HBc Ab	
Hepatitis C	Anti-hepatitis C Ab, HCV PCR	
Herpes viruses	Antibody	
Epstein–Barr virus	Antibody	
Bacterial	Leptospiral antibody	If clinically indicated
Drugs		
Acetaminophen overdose	Acetaminophen level	Level to be compared to the nomogram by Rumack and Matthews
Metabolic		
Wilson disease	Serum copper, ceruloplasmin 24-h urine copper	With penicillamine challenge when indicated
Others	Serum amino acid	See also Table 3.11
	Urine organic acid	
	Urine reducing sugars	
Autoimmune hepatitis	Autoantibodies	
	Antinuclear antibodies	Type 1 AIH anti-ANA and anti-SM are positive
	Anti-smooth muscle	
	Anti-liver–kidney microsomal antibody	Type 2 AIH
	Immunoglobulins	Raised IgG in both forms
	Liver biopsy	Interface hepatitis, bridging fibrosis, dense mononuclear and plasma cells infiltrate, hepatic regeneration with rosette formation

The differential diagnosis of acute hepatitis in older children includes (Table 3.9) [13, 14]: viral hepatitis A, B, C, and E, sero-negative hepatitis, autoimmune hepatitis, drug-induced hepatotoxicity, and metabolic liver disease especially Wilson disease.

Important causes of neonatal liver failure include viral infections, metabolic liver disease, and ischemic causes (Table 3.10) [15].

Chronic Liver Disease

- Chronic liver disease is frequently asymptomatic but detected through other analyses, such as incidental detection of abnormal liver enzymes or hepatomegaly
- Family screening for hepatitis B/C or metabolic disorders (Wilson disease)
- Transfusion recipient following diagnosis of donor infection
- Coexistent disease, e.g., inflammatory bowel disease and celiac disease
- Recipient of a known toxic agent, e.g., methotrexate

When symptomatic children may present with:

- Intermittent fatigue, anorexia, and weight loss
- Abdominal discomfort
- Variable or fluctuating jaundice with pruritus and pale stools
- Hematemesis or melena from variceal bleeding – especially with portal hypertension

Liver Biopsy and Histopathology

The diagnosis of most chronic liver diseases requires histological confirmation [16]. An aspiration technique, using a Menghini needle (or

Table 3.10 Causes of neonatal acute liver failure

Diseases	Investigations
Infections	
Herpes viruses	Tissue culture
	Direct immunofluorescence of swabs or tissue
	Molecular technique
	(Note: serology is of no value in perinatal infection due to presence of maternal IgG to herpes simplex virus)
Adenovirus	Immunoassay or PCR to detect virus in stool, blood, or liver tissue
Echovirus	Tissue culture
	Direct immunofluorescence of swabs or tissue
Hepatitis B	Hepatitis B surface antigen, anti-HBc antibody
Metabolic (*see also* Table 3.11)	
Galactosemia	Galatose-1-phosphate uridyltransferase
Tyrosinemia	Serum tyrosine, methionine, α-fetoprotein, urine succinylacetone
Gestational alloimmune liver disease (neonatal Hemochromatosis)	Serum ferritin, extrahepatic siderosis (magnetic resonance imaging, or tissue biopsy showing hemosiderosis; oral or buccal mucosa); liver biopsy: immunostaining of hepatocytes for the C5b-9 complex
Mitochondrial	See Table 3.11
Other metabolic conditions	See Table 3.11
Ischemic	
Congenital heart disease	Chest x-ray, ECG, and echocardiogram; cardiac enzymes (if myocarditis is suspected)

disposable variant), has a complication risk of 1:1,000 liver biopsies and may be performed under sedation with local anesthesia. In fibrotic or cirrhotic livers, the use of a Tru-Cut needle, with a cutting-edge beveled end, may be necessary. Transjugular liver biopsies, in which the liver is biopsied through a special catheter passed from the internal jugular vein into the hepatic veins, are now possible for children as small as 6 kg and are a safer way to perform a biopsy if coagulation times remain abnormal despite support (prothrombin time [PT] >5 s prolonged over control value) or for those with large ascites [17]. The complications of this potentially dangerous procedure (see below) are much reduced if performed in expert hands, in specialized units, under controlled conditions [18]. It is essential to be aware of the absolute and relative contraindications of liver biopsy. Biopsy specimens should be obtained for routine histopathology and can be analyzed for microbiology, electron microscopy, immunohistochemistry, and copper (if appropriate) and snap frozen in liquid nitrogen for enzymatic or metabolic investigations. As the interpretation of the histology may be difficult and requires considerable specialist expertise, and tissue preparation for other analyses requires special handling, coordination with the liver pathologist before tissue acquisition is advisable.

In experienced facilities and with careful patient selection, it is possible to carry out a liver biopsy as a day procedure [19, 20]

Complications of Percutaneous Liver Biopsy

Although uncommon, the main complication of percutaneous liver biopsies is bleeding. Subclinical bleeding (as evident on ultrasound imaging) is common and intrahepatic and subcapsular hematomas with no hemodynamic compromise are seen in up to 23 % of patients [21]. Significant nonfatal bleeding (as seen with evidence of active bleeding, shock, or a hemoglobin drop of 2.0 g/l) occurs more frequently in children than adults. In adults significant hemorrhage occurs in 0.3–0.5 % of cases, while bleeding requiring transfusion is seen in up to 2.8 % of children [22]. Evidence of persistent bleeding following liver biopsy despite medical support and blood transfusion warrants urgent hepatic angiography and embolization or surgery.

Other complications include:

- Pneumothorax or hemopneumothorax
- Infection (particularly if the biopsy is combined with another procedure, e.g., dental extraction)
- Perforation of the gall bladder or bile ducts leading to biliary peritonitis

Adequate monitoring of vital signs post biopsy is essential to detect complications such as hemorrhage or infection [22].

Metabolic Investigations (Table 3.11)

Many inborn errors of metabolism present with hepatomegaly and/or liver disease. It is essential to screen for these diseases as part of the investigation of liver disease in neonates and in older children

Bone Marrow Aspiration

Bone marrow aspiration may be useful in infants with undiagnosed neonatal hepatitis and hepatomegaly and splenomegaly, in order to exclude Niemann–Pick type C, or at any age if a storage disorder is suspected and genetic testing unavailable.

Skin Biopsy with Fibroblast Culture

This procedure can be useful in diagnosing inborn errors of metabolism (e.g., Niemann–Pick type A, B, or C or tyrosinemia type I) when genetic testing is not available (Table 3.11).

Genetic Tests (Chromosome and DNA)

With the rapid development of molecular techniques for diagnosis and detection of genetic diseases, samples for DNA analysis and/or chromosomes from both child and parent are essential and now possible for many genetic conditions affecting the liver.

Neurophysiology

Electroencephalography (EEG) is mostly used in the assessment of hepatic encephalopathy. It will identify abnormal rhythms secondary to encephalopathy due to either acute or chronic liver failure or drug toxicity such as posttransplant immunosuppression, but findings are frequently nonspecific. EEG may also be of value in verifying brain death as a flat EEG in the absence of sedation is an indication for withdrawal of therapy.

Ophthalmology (Table 3.12)

A number of inherited conditions have associated ophthalmic lesions (e.g., posterior embryotoxin in Alagille syndrome, Kayser–Fleischer rings in Wilson disease), and thus, ophthalmological examination should be part of the assessment process when these conditions are suspected. Additionally, children with Alagille syndrome have a higher-than-normal incidence of benign intracranial hypertension, and thus, annual fundoscopy for papilledema is essential [23].

Endoscopic Retrograde Cholangiopancreatography (ERCP)

This procedure is invaluable for the assessment of extrahepatic biliary disease in older children (e.g., choledochal cysts, primary sclerosing cholangitis) or for the assessment of chronic pancreatitis. It involves an endoscopic technique where a fiberoptic duodenoscope is passed into the first part of the duodenum, the ampulla of Vater is identified, the pancreatic and biliary ducts are cannulated, and radiological contrast is injected. The technique has an 80 % success rate in skilled hands. Although this technique should be of value in the differential diagnosis of neonatal cholestasis, technical difficulties in the cannulation of bile ducts in small infants may provide equivocal information. Recently, in some

Table 3.11 Specific investigations for metabolic liver diseases

Clinical presentation	Disorders	Enzymes defect/ mutations	Investigations	Results
Liver failure	Galactosemia	Galactose-1-phosphate uridyltransferase (*GALT*) gene, numerous mutations identified	(Serum, erythrocyte, dried blood spots) Galactose-1-phosphate Total galactose (Gal) GALT activity DNA mutation analysis	Increased Increased Reduced/absent enzyme activity More than 130 *GALT* gene (located at 9p13) mutations identified
	Tyrosinemia type 1	Fumarylacetoacetase deficiency	Plasma amino acids (AA) Urine organic acids (OA) (succinylacetone) Urine porphyrins Serum α-fetoprotein Mutational analysis	Increase in tyrosine, methionine Increased succinylacetone (diagnostic), 4-OH-phenyl derivatives Increase δ-aminolevulinic acid Increased serum Mutation in *FAH* gene (located at 15q25.1)
	Hereditary fructose intolerance	Common mutation A149P in aldolase B gene/ Aldolase B	Plasma lactate Urine DNA mutation analysis Enzyme analysis on liver biopsy	Increased Increased urine glucose, albumin, amino acids, reducing substance, positive effect of withdrawing fructose Mutation in *ALBOD* gene (located at 9q31.1) Reduced/absent
	Mitochondrial respiratory chain defects	Respiratory chain defects Mitochondrial DNA (mtDNA) defects	Plasma and CSF lactate Urine OA Plasma and CSF AA Glucose challenge CSF protein mtDNA analysis (blood) Muscle biopsy for DNA, histology, histochemistry, and enzyme analysis	Increased Increase in lactate, ketones Increase in alanine, threonine Markedly increased serum lactate (>20 %) Increased Mutation, depletion, heteroplasmy Ragged-red fibers
	Long-chain fatty acid oxidation defects (usually with associated hypoglycemia)	Long-chain acyl-CoA dehydrogenase (LCAD) deficiency	Urine OA Plasma/blood spot acylcarnitines DNA mutation analysis	Increase in C6—C14 decarboxylic acids Increase in C14:1, ratio C14:1/C12:1 mtDNA mutation
	α-1 antitrypsin (αAT) deficiency		Serum α1-antitrypsin and phenotype	ZZ genotype, decrease in αAT
Encephalopathy or "Reye-like" illness	Fatty acid oxidation defect	Acyl-CoA dehydrogenases deficiency/ abnormality	Plasma/serum Acylcarnitine profile (dried blood spot) Urine OA Fibroblast enzymes Liver biopsy DNA mutation analysis for MCAD and LCHAD deficiency	Markedly increase in plasma NH_3, lactate, liver enzymes, creatine kinase, myoglobin Markedly reduced glucose, ketones, free fatty acids Reduced total serum carnitine Increase in acylcarnitine/total carnitine ratio Increase in specific metabolites Increase in specific decarboxylic acids; specific acylglycines Reduced or absent enzyme activities, Fatty degeneration Mutation K329E in *ACADM* gene; mutation E510Q, *HADHA* gene
	Organic acidemias	Enzyme disorder involving complex metabolism of branched-chain amino acid	Plasma/serum Blood count Urine OA and plasma AA Acylcarnitine profile (dried blood spot) Carnitine	Increase in lactate, NH_3 Ketosis/ketoacidosis hypoglycemia; hypocalcemia Neutropenia, thrombocytopenia; pancytopenia Increase in specific metabolites Decreased

Table 3.11 (continued)

Clinical presentation	Disorders	Enzymes defect/ mutations	Investigations	Results
Cholestasis (neonatal or early childhood)	Peroxisomal disorders	Multiple (or all) peroxisomal enzymes	Plasma very long-chain fatty acids (VLCFA), phytanic/pristanic acid, plasmalogens Peroxisomal morphology in liver/fibroblasts	Raised VLCFA, phytanic acid Increase or decrease in pristanic acid Reduced plasmalogens Raised CSF protein Raised specific bile acids Complete absent/reduced/abnormal structure of peroxisomes
	CDG syndrome	Specific gene mutation for enzymes of protein glycosylation	Plasma transferrin isoforms in isoelectric focusing (IEF)	Specific IEF pattern
	Lysosomal storage disorders	Specific lysosomal enzymes gene mutation	Peripheral blood film Liver/bone marrow biopsy or skin fibroblasts Leukocytes/fibroblasts Urine glycosaminoglycans and oligosaccharides Chitotriosidase (serum, dried blood spot)	Vacuolated leukocytes Storage cells (vacuolated cells) Specific enzyme assay for enzyme activities Increased Markedly increased
	Niemann–Pick A and B disease	Sphingomyelinase deficiency	Bone marrow/liver	"Niemann–Pick," storage cells, reduced sphingomyelinase in white cells
	Neimann-Pick C	Defect of intracellular cholesterol transport	Filipin staining on skin fibroblasts Cholesterol esterification studies Mutational analysis	Positive Filipin staining of fibroblasts Unesterified cholesterol Mutation in *NPC1 and 2*
	Bile acid synthesis defects	Deficiency of: 3β-Hydroxy-Δ^5-C_{27}-sterol dehydrogenase Δ^4-3-Oxosterol 5β-reductase Oxysterol 7α-hydroxylase	Urine and plasma bile acids	Increase in conjugated bilirubin, transaminases, AP, PTT Normal γGT Decrease in calcium, cholesterol, vitamins A, D, E, K Increase in specific bile acids (bile, plasma, urine)
Isolated hepatomegaly or hepatosplenomegaly	Glycogen storage diseases type I, III, IV, VI, IX, XI	Specific enzyme gene mutation	Plasma Glucose challenge Urine oligosaccharides Urine organic acids Liver histology Enzyme analysis on liver/muscle/skin fibroblasts	Increase lactate, TGs, uric acid, transaminases; hypoglycemia Decrease in lactate Increased Increase in 2-oxoglutaric acid Distended hepatocytes with prominent glycogen and lipid vacuoles Reduced/absent enzyme activities
Lysosomal storage disorders	As above			

centers, the diagnostic value of ERCP has been superseded by magnetic resonance imaging (MRI) via performance of a magnetic resonance cholangiopancreatography (MRCP) which is noninvasive. Limitations of MRCP due to experiential lack of sensitivity mean ERCP is still valuable as a diagnostic tool, and ERCP also retains an important role in therapy [24].

Table 3.12 Ophthalmic lesions in liver disease

Disease	Lesions	Comments
Galactosemia	Cataracts	
Congenital infections	Chorioretinal scar/	Congenital toxoplasmosis, CMV, HSV, VZ infections
	Active chorioretinitis	Congenital toxoplasmosis, CMV, HSV, VZ infections
	Cataracts	Less specific than chorioretinitis, congenital rubella
Wilson disease	Kayser–Fleischer ring	Not specific for Wilson disease
Alagille syndrome	Posterior embryotoxin	Not specific for Alagille
	Optic disc drusen	
	Pigmentary retinopathy	
Niemann–Pick type A	Cherry-red spot	
Tay–Sachs disease	Cherry-red spot	

Molecular Biology

The development of molecular biology has revolutionized methodology for many complex diagnostic procedures, transforming many techniques into routine laboratory procedures [25], particularly in screening for rare neonatal diseases [26]. Progress in identifying specific genes and DNA sequencing has made possible the diagnosis of many inborn errors of metabolism and inherited disease (e.g., Alagille syndrome, Wilson disease, tyrosinemia type I) and led to the identification of specific mitochondrial disorders.

Advances in methodology for gene cloning and molecular cloning methods have been helpful in identifying viruses such as hepatitis C and G [27], while the polymerase chain reaction has been used to diagnose active infection and monitor patients with many different viral diseases, such as hepatitis C, cytomegalovirus (CMV), and Epstein–Barr virus (EBV). Diagnosis for autoimmune disorders has improved, with specific assays that use recombinant protein antigens (e.g., antinuclear antigens and liver–kidney microsomal antibodies). The rapid development of molecular techniques is certain to lead to further improvements in diagnostic methods and to a better understanding of pediatric liver disease.

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