Liver and biliary disease in infancy

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Abstract

Liver disease in infancy is a relatively rare but serious cause of morbidity and mortality. Since jaundice is a common finding in the neonatal period, the immediate priority is to differentiate between unconjugated hyperbilirubinaemia, which is generally a benign developmental phenomenon, and conjugated hyperbilirubinaemia (conjugated fraction >20%), which is always pathological. Conjugated hyperbilirubinaemia, suggested by yellow urine and stools that are not yellow or green in an infant of any age, is pathognomonic of liver parenchymal or bile duct disease. It warrants prompt investigation because some of its causes require urgent treatment. Genetic counselling can also be required.

Keywords α_1-antitrypsin deficiency; Alagille's syndrome; biliary atresia; infantile liver disease; MRCP; progressive intrahepatic cholestasis

Key points

- Conjugated jaundice in the neonatal period warrants urgent investigation and referral to specialized centres
- Biliary atresia is the most common severe liver disease of infancy; timely surgical intervention impacts on outcome
- Recent advances in genetics are enabling us to identify several types of hereditary cholestasis

Infantile cholestasis/neonatal hepatitis syndrome

The terms 'hepatitis syndrome of infancy' and 'neonatal hepatitis syndrome' were initially used to describe a group of disorders causing clinical and biochemical liver dysfunction, of which the most distinct is conjugated hyperbilirubinaemia. The frequent presence of inflammatory changes in liver biopsy material led to the use of the term 'hepatitis', although the cause is only occasionally infective (Table 1). 'Infantile or neonatal cholestasis' is probably a better name to describe this entity, which has a reported incidence of approximately 1 in 2500 live births. The most common causes, in order of frequency, are biliary atresia (BA), idiopathic infantile cholestasis and α_1-antitrypsin deficiency (A1ATD).

Bile production is dependent on active transport into the bile canaliculus of bile acids and other osmotic compounds, followed by passive movement of water. Active transporters at both the hepatic basolateral membrane and canalicular membrane play an important role in this. Genetic defects of these transporters are recognized and identify a range of familial intrahepatic cholestatic diseases (see below). In liver disease and sepsis, the expression of the transporters helps to protect the hepatocyte from the cytotoxic effect of bile acids.

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In the fetus and newborn, immature bile acid synthesis and bile acid transport result in decreased bile flow. Cholestasis leads to bile acid and conjugated bilirubin retention, apparent as jaundice, hypercholesterolaemia and pruritus, and to decreased

Causes of infantile cholestasis

Infections

Viral
- *Toxoplasma*
- Rubella
- Cytomegalovirus
- Herpes simplex virus
- Human herpesvirus-6
- Varicella zoster
- Hepatitis A–C
- Non-A–C hepatitis
- Echo-, adeno- and Coxsackie viruses
- HIV
- Reovirus type III
- Epstein–Barr virus
- Parvovirus (erythrovirus) B19

Bacterial
- Syphilis
- Listeria
- Malaria
- Tuberculosis

Endocrine causes
- Hypopituitarism
- Diabetes insipidus
- Hypoadrenalism
- Hypothyroidism
- Hypoparathyroidism

Chromosomal disorders
- Trisomy 18, 21

Toxicity
- Copper
- Parenteral nutrition

Bile duct abnormalities
- Biliary atresia
- Choledochal cyst
- Spontaneous perforation of bile ducts
- Gallstones
- Inspissated bile syndrome
- Neonatal sclerosing cholangitis
- Caroli's syndrome and disease
- Non-syndromic bile duct paucity

Metabolic cause
- Alagille's syndrome
- α_1-Antitrypsin deficiency
- Galactosaemia
- Tyrosinaemia
- Fructosaemia
- Progressive familial intrahepatic cholestases
- Cystic fibrosis
- Niemann–Pick type A, type C
- Gaucher's disease
- Wolman's disease
- Zellweger's syndrome
- Carbohydrate glycoprotein deficiency
- Neonatal haemochromatosis
- Primary disorders of bile acid synthesis
- Mitochondrial cytopathy
- Citrin deficiency
- Lysinuric protein intolerance

Miscellaneous causes
- Haemophagocytic lymphohistiocytosis
- ARC syndrome (arthrogryposis, renal tubular dysfunction, cholestasis)

Table 1

bile excretion into the intestine; this results in malabsorption of dietary long-chain fats and fat-soluble vitamins.

Clinical presentation

Most babies with infantile cholestasis present with prolonged jaundice, dark urine and pale stools within the first 4 weeks of life, but they can occasionally present as late as 4 months old.[1] The second most common presentation is spontaneous bleeding, usually secondary to vitamin K deficiency associated with fat malabsorption, which can also cause failure to thrive and rickets. Less commonly, babies present with hypoglycaemia or hypoalbuminaemia.

Review of the perinatal records, pregnancy and family and past medical histories helps to determine the possible role of intrauterine infections, exposure to toxins, drugs or prolonged intravenous nutrition, familial, genetic or metabolic conditions, or consanguinity. Hepatomegaly and splenomegaly are common clinical findings. Facial dysmorphic features or other stigmata of syndromic disorders, evidence of congenital heart disease, manifestations of intrauterine infections and cutaneous haemangiomas are of diagnostic value.

Management

Urgent investigations are necessary to identify disorders that have a specific treatment and prevent complications (Table 2). Standard tests of liver function are seldom helpful in the differential diagnosis. Infective, metabolic and endocrine causes must be excluded urgently as the prognosis can be modified radically by early treatment. Galactose and fructose must be excluded from the diet until galactosaemia and fructosaemia have been excluded. If the child has been given a blood transfusion, the parents should be tested for heterozygosity for galactosaemia because the enzymatic defect is detected in red blood cells.

Fat-soluble vitamins (A, D, E, K) must be prescribed to avoid deficiencies and their complications. They can be given orally, but intramuscular supplements are recommended in persisting cholestasis. The nutritional management of infantile cholestasis requires a high-calorie diet containing 120–150% of the estimated average daily requirement, with an increased percentage of fat as medium-chain triglycerides (triacylglycerols). A lactose-free formula should be used until galactosaemia has been excluded.

The second priority is to identify infants requiring surgical correction of bile duct pathologies such as BA, choledochal cyst or spontaneous perforation of the bile ducts. Stool colour is a helpful diagnostic aid: white or brown, but not green or yellow, stools suggest bile duct obstruction (Figure 1). Other investigations include ultrasonography of the liver and liver biopsy, both of which should be interpreted by experienced observers. Radionuclide scans are valuable only where excretion of radioisotope in the gut excludes complete bile duct obstruction, but do not help in differentiating between parenchymal or bile duct disease when no bowel excretion is observed. Phenobarbital (5 mg/kg per day) should be started 48 hours before the test to optimize biliary excretion.

Third-line investigations involve blood tests aimed at identifying rare metabolic or genetic disorders, suspected on the basis of specific clinical findings or because no other aetiology is recognized. These include blood tests for enzymology or genetics, bone marrow aspiration, and skin and muscle biopsy for mitochondrial respiratory chain defects.

Biliary atresia

BA is the end result of a destructive, idiopathic, inflammatory process affecting both intra- and extrahepatic bile ducts, leading to fibrosis and obliteration of the biliary tract and eventually biliary cirrhosis. It is the most common surgically correctable liver disorder in infancy, affecting 1 in 5000–19,000 live births worldwide; it is also the most frequent cause of liver transplantation in children.[2]

The cause of BA remains unknown but is thought to be multifactorial, the bile duct damage resulting from several

Investigations for infantile cholestasis

Urgent investigations

- Bacterial culture of blood and urine
- Urine microscopy and analysis for reducing substances
- Prothrombin time/international normalized ratio
- Full blood count and reticulocyte count
- Blood glucose, urea and creatinine
- Serum electrolytes
- Blood group and cross-match

Standard investigations

- Liver function tests including split bilirubin and γ-glutamyl transpeptidase
- TORCHES screen (toxoplasmosis, rubella, cytomegalovirus, herpes simplex, syphilis), HIV, hepatitis A, hepatitis C, hepatitis B
- α_1-Antitrypsin phenotype/genotype
- Thyroxine, thyroid-stimulating hormone, cortisol (for endocrinological problems)
- Lactate, pyruvate, ammonia
- Galactose-1-phosphate uridyltransferase (red blood cells)
- Immunoreactive trypsin/sweat electrolytes (for cystic fibrosis)
- Amino acids (serum and urine)
- Succinyl acetone, organic acids (urine) (for tyrosinaemia)
- Bile acids mass spectrometry (urine) (for bile acid synthesis disorders)
- Direct Coombs' test (if appropriate)
- Ferritin
- Cholesterol, triglycerides

Specialized investigations (tertiary centre)

- Ultrasound scan of liver
- Chest X-ray/echocardiogram
- Liver biopsy
- Cholangiography (selected cases) (magnetic resonance, endoscopic, percutaneous)
- Radionuclide hepatobiliary scanning after phenobarbital (limited value)

Second-line investigations (tertiary centre)

- Blood tests for rare metabolic disorders (white cell enzymology, transferrin electrophoresis, etc.)
- Bone marrow aspirate for storage disorders
- Skin biopsy for fibroblast culture and enzyme analysis
- Muscle biopsy (mitochondrial respiratory chain defect)

Table 2

Figure 1 Stool samples from two children with BA. Neither sample is green or yellow, as normal infant stools should be, suggesting complete bile duct obstruction. Stools can show some pigment, but if not bright yellow or green further investigation in a specialized centre is indicated.

possible contributing factors (genetic, infective, inflammatory and/or toxic). BA is a progressive disorder and in some cases stools are pigmented during the first week of life, only later becoming acholic. All infants with pale or acholic stools at whatever age should be promptly referred to specialized centres because early surgical treatment of BA is essential for a good outcome.

Biochemical findings are usually not helpful. An ultrasound scan revealing an absent or abnormal gallbladder with an irregular wall, or, in older infants, the triangular cord sign, is suggestive of BA. However, a normal gallbladder or absence of the triangular cord sign does not exclude BA. Histological examination of the liver by an experienced histopathologist leads to the correct diagnosis of BA in >90% of cases. In up to 20% of patients, there are other congenital anatomical abnormalities, including polysplenia, situs inversus and unusual vascular abnormalities such as absence of the inferior vena cava and a preduodenal portal vein. Cystic BA, associated with a cystic change of the biliary tree, can be detected on antenatal ultrasonography and requires early referral to a specialized centre.

After confirming the diagnosis, surgical treatment consists of a Kasai portoenterostomy. Here, the fibrous tissue at the porta hepatitis, replacing the atretic biliary structures, is transected and a Roux-en-Y loop of jejunum is anastomosed to the liver. The success of the operation is judged by the appearance of pigment in the stools and clearance of jaundice. Overall 5- and 10-year survival in the UK with native liver has been reported as 46% and 40%, respectively, with overall survival of 90% and 89%, respectively. The use of adjuvant corticosteroid treatment postoperatively has been associated with improved clearance of jaundice, but does not affect more long-term native liver survival.

The most common postoperative complication is cholangitis, with a reported incidence of about 30–40% at 5 years, most children having one single episode. Portal hypertension is already present in most patients at the time of initial surgery, but only approximately 15% develop upper gastrointestinal bleeding secondary to varices in childhood. These can usually be managed with banding or sclerotherapy.

For children with an unsuccessful Kasai portoenterostomy, or who develop progressive liver disease despite successful surgery, liver transplantation (LT) is the only therapeutic option.

α_1-antitrypsin deficiency (A1ATD)

A1ATD is the most common inherited cause of infantile liver disease. α_1-Antitrypsin is a glycoprotein that is synthesized by hepatocytes and alveolar macrophages, and acts as a protease inhibitor.

More than 90 alleles, controlled by the protease inhibitor (Pi) gene on chromosome 14q31–32.2, have been isolated and identified, the most common being PiM. The inheritance pattern is autosomal co-dominant. A1ATD, which is caused by a single-gene defect leading to significantly reduced or absent serum α_1-antitrypsin, is associated to the PiZZ, PiNulNul and PiZNul variants.[3] The prevalence of the PiZ allele in the European population is 0.5–2%. PiZZ A1ATD causes chronic liver disease in 10–20% of affected children. The cause of the liver disease is unknown, and genetic, environmental and physical factors are likely to be involved.

The clinical features of A1ATD are variable, some patients being asymptomatic, about 20% developing liver disease of variable severity and about 60% developing emphysema during adulthood. A1ATD should be suspected in all cases of infantile cholestasis and in unexplained liver disease in childhood. More than 50% of children with A1ATD have abnormal liver function tests, but only 10–15% develop overt liver disease, most commonly during the first 4 months of life. Clinical presentation can mimic BA. In about 10% of cases, serious bleeding diathesis is the presenting symptom; 1–2% of PiZZ patients present with cirrhosis in childhood or adult life, despite having no history of infantile liver disease.

The diagnosis is confirmed by determining the α_1-antitrypsin phenotype by isoelectric focusing or agarose electrophoresis. Serum α_1-antitrypsin concentration can be misleading as α_1-antitrypsin is an acute-phase reactant and the concentration can be within the normal range during the early hepatitic stage of the disease. Biliary features can be prominent on histology and similar to those seen in BA. The distinctive periodic acid–Schiff-positive diastase-resistant α_1-antitrypsin globules in periportal hepatocytes are detectable only after 12 weeks of life. A diagnosis of A1ATD should be excluded by determining the phenotype in all children with suspected BA before surgical intervention.

There is currently no specific treatment for A1ATD. Supportive dietary management with fat- and water-soluble vitamin supplements is indicated. If decompensation occurs, LT is the only therapeutic option. Antenatal diagnosis is available but genetic counselling is difficult because of the varying severity of the clinical phenotype and the difficulties of predicting the outcome.

Hereditary cholestasis

Severe forms of intrahepatic cholestasis with progressive hepatocellular damage occur sporadically or on a familial basis.[4] To date, >100 inherited diseases have been identified.

Familial intrahepatic cholestasis type 1 (FIC1) and bile salt export pump (BSEP) deficiency

FIC1 and BSEP deficiency are characterized by low serum γ-glutamyl transpeptidase (GGT). *ATP8B1* encodes FIC1, a widely expressed membrane P-type ATP-ase, and *ABCB11*, expressed

only in the liver, encodes BSEP. The FIC1 protein is widely expressed, its expression being high in the small intestine and pancreas, and low on the hepatocyte canalicular membranes.

Infants with FIC1 present within the first 6 months of life with cholestasis, which is of variable severity and sometimes episodic. Other characteristics are diarrhoea, fat-soluble vitamin deficiency, hearing loss and recurrent pancreatitis. Pruritus is a dominant feature after infancy. BSEP is a canalicular bile acid transporter expressed only in liver. Most children present during the first few months of life with isolated mild neonatal hepatitis. Pruritus is also a characteristic feature. In both FIC1 and BSEP deficiency, failure of bile acid excretion at the canalicular level is the reason for the low serum GGT and normal serum cholesterol.

Both are usually progressive diseases. Management consists of supportive medical treatment for the complications of cholestasis — fat-soluble vitamin deficiency and pruritus. Ursodeoxycholic acid (UDCA) can help by increasing hepatocytic excretion of endogenous bile acids and inhibiting their intestinal reabsorption, thereby limiting their return to the liver. Surgical partial external biliary diversion or ileal exclusion has been reported to arrest disease progression and relieve pruritus, but the results are not consistent. BSEP deficiency confers a high risk of hepatobiliary malignancy, particularly in individuals carrying two null mutations. LT is indicated in patients with decompensated cirrhosis or failed diversion and severe pruritus. Although the survival rate is excellent after transplantation, failure to thrive, pancreatitis and chronic diarrhoea usually persist in FIC1. Recurrence has been reported in BSEP deficiency as a result the recipient's production of antibodies against the BSEP protein present in the donor liver.

Familial intrahepatic cholestasis type 3 (FIC3): MDR3 deficiency

A third type of FIC, characterized, in contrast to type 1 and 2, by high serum GGT, is associated with multidrug resistance gene-3 (MDR3) deficiency because of mutations of the *ABCB4* gene. This type of intrahepatic cholestasis is also associated with cholestasis during pregnancy.

Novel disease-causing genes have more recently been reported, including *FXR* (*NR1H4*), *TJP2* and *MYO5B* as well as *DCDC2*.

Alagille's syndrome

Alagille's syndrome (AGS) was initially described as idiopathic bile duct paucity (Figure 2a) associated with chronic cholestasis, causing jaundice, pruritus, hypercholesterolaemia and xanthomas (Figure 2b). The facial features comprise deep-set eyes, a small pointed chin, mild hypertelorism, an overhanging forehead and a straight nose, which is on the same plane as the forehead in profile (Figure 2c). There are vertebral arch defects on spinal radiographs (Figure 2d), as well as cardiac (peripheral pulmonary artery stenosis) and ocular (posterior embryotoxon) abnormalities. Other common features are renal abnormalities, growth retardation, cerebrovascular abnormalities and pancreatic insufficiency. AGS is associated with mutations in one of two

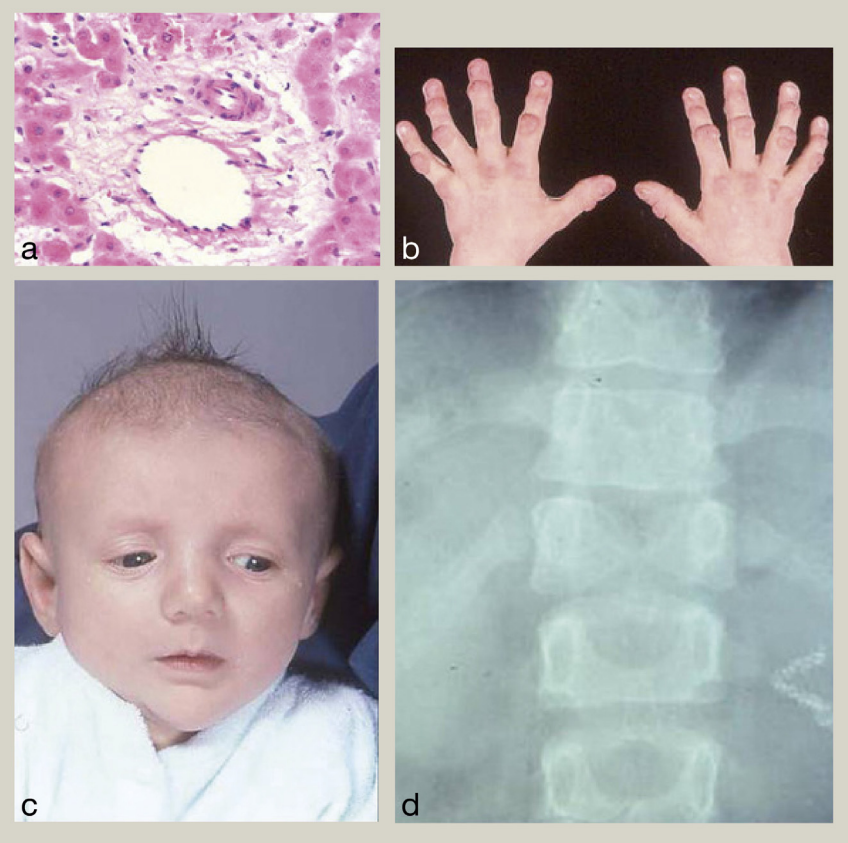

Figure 2 Alagille's syndrome. (**a**) Liver biopsy showing lack of a bile duct in the portal tract. (**b**) Large hand xanthomas. (**c**) Classical facial appearance (deep-set eyes, mild hypertelorism, overhanging forehead, small pointed chin). (**d**) Typical appearance of a 'butterfly' vertebra.

genes in the NOTCH signalling pathway: *JAG1* or *NOTCH2*. With its variable phenotype, the true frequency of AGS is estimated at 1 in 30,000 live births.[5]

Hepatic involvement in AGS is variable, ranging from asymptomatic elevations of hepatic transaminases to end-stage liver disease, which occurs in approximately 20–30% of patients.

Management consists of vitamin supplements, nutritional support and control of pruritus. Long-term prognosis is uncertain. LT can be an option in cases of continuing severe cholestasis and pruritus, but careful assessment of cardiac status is required before surgery can be considered. ◆

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TEST YOURSELF

To test your knowledge based on the article you have just read, please complete the questions below. The answers can be found at the end of the issue or online here.

Question 1

A 6-week-old baby presented with prolonged jaundice. He was the first child of non-consanguineous parents.
On clinical examination, he was jaundiced with no dysmorphic features. A tip of the spleen was palpable. The stools were pale and the urine dark.

Which blood tests are required as first-line investigations?

A. Serum bile acids
B. Genetic studies for cholestatic disorders
C. α_1-Antitrypsin concentrations
D. Clotting profile
E. Serum cholesterol levels

Question 2

A 4-month-old baby presented with a history of failure to thrive, pruritus and mild jaundice. The stools were pigmented. On clinical examination, some dysmorphic features were present as well as a heart murmur.

What is the most likely diagnosis?

A. Biliary atresia
B. α_1-Antitrypsin deficiency
C. Cytomegalovirus hepatitis
D. Progressive familial intrahepatic cholestasis
E. Alagille's syndrome