Diagnosis of Neonatal Cholestasis

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Key Words

Neonatal cholestasis • Acholic stools • Biliary atresia • Metabolic disease

Abstract

Cholestasis is frequent in neonates (1/2,500 live births) and in young children. It includes many etiologies with sometimes poor prognosis. In case of neonatal cholestasis, the most important point is to look at the stool color and to rule out biliary atresia which needs to be surgically treated before the 45th day of life. Biliary atresia represents almost 50% of cases of neonatal cholestasis, the other causes being numerous. Some cases can be treated with success, such as tyrosinemia type I or inborn errors of bile acid synthesis. However, in the majority of cases, there is no specific treatment, and the evolution of the disease is toward cirrhosis or liver insufficiency leading to liver transplantation. Nowadays, liver transplantation has good results, but it is a difficult procedure with frequent side effects. In the future, analysis and a better understanding of the mechanisms of the different cholestatic diseases could allow the development of other treatments such as liver cell transplantation or gene therapy, bringing new perspectives for children.

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Introduction

Cholestasis in children often appears in the first weeks of life, because the neonate is particularly predisposed by the biliary secretion immaturity. Neonatal cholestasis is frequent and its incidence is 1/2,500 live births. The identification of the cause of cholestasis is very important, because the most frequent one is biliary atresia which should be corrected surgically before 45 days of life [1]. Many other diseases can lead to cholestasis in the neonatal period. Moreover, 15 diseases account for 95% of cases, and the prognosis depends on the etiology. Cholestasis is the most frequent cause of liver transplantation in children, and liver transplantation dramatically changes the prognosis. Currently, an increasing number of genes involved in diseases causing neonatal cholestasis are known, and identification of a pathological mutation in a family can allow prenatal diagnosis for the more severe diseases. Also, in the future, identification of other genes involved in these diseases will lead to a better understanding of the mechanisms of cholestasis, and, probably, to new therapies other than liver transplantation.

Physiopathology of Cholestasis

Cholestasis is defined by manifestations linked to diminished or absent bile flux or by abnormal bile formation. It can be due to metabolic hepatocyte alterations

Fig. 1. Main steps of bile production in the hepatocyte. Bile production is an active process, involving the transport of bile acids and other osmotic compounds across a concentration gradient into the bile canaliculus. At the apical pole of hepatocyte: OATP = Organic anion transporting protein; NTCP = Na taurocholate cotransporting polypeptide. At the canalicular pole of hepatocyte: cMOAT = Canalicular multispecific organic anion transporter; BSEP = bile salt export pump; MDR3 = multidrug resistance protein 3.

(secondary to hepatocyte injury or to external factors such as infection or parenteral nutrition) or to intra- or extrahepatic biliary alterations.

Bile production is an active process, involving the transport of bile acids and other osmotic compounds across a concentration gradient into the bile canaliculus. This osmotic concentration induces the passive movement of water into the canaliculus. Active transporters are localized at the basolateral membrane of the hepatocyte for sinusoidal blood uptake (fig. 1): Na taurocholate cotransporting polypeptide (NTCP), organic anion transporting protein (OATP) and, at the canalicular membrane for biliary excretion, bile salt export pump (BSEP), canalicular multispecific organic anion transporter (cMOAT) and multidrug resistance protein 3 (MDR3). The expression of these transporters is modified by liver disease, injury or sepsis in order to protect the hepatocyte from the cytotoxic effects of elevated bile acid concentration. This regulation could probably explain why jaundice may be an early sign of sepsis. Bile flow is low in the fetus and newborn because of the immaturity of bile acid synthesis and transport processes. In animal models, NTCP and BSEP are present already before birth, but at a very low level in comparison with adults. The development process for human hepatocyte transporters has not yet been quantified, but plasma bile

acids do not fall into the normal adult range until 6 months of age. These observations strongly suggest the predisposition of the neonate to cholestasis [2, 3].

Diagnosis of Cholestasis

Cholestasis should be suspected if jaundice has not disappeared during the second week of life. Cholestatic jaundice is associated with pale stools and dark urines; there is no pruritus in the neonatal period. The liver may be enlarged, firm or hard. Sometimes, a splenomegaly is present. Acholic stools, hard liver and abdominal or thoracic situs inversus are very suggestive of biliary atresia. Biological features of cholestasis are hyperbilirubinemia, mainly conjugated, and often elevated γ -glutamyl transferase, as well as alkaline phosphatase activities and serum bile salts. It is important to differentiate jaundice of liver insufficiency and a cholestatic jaundice (in this situation, prothrombin time is normalized after vitamin K₁ injection). In cholestasis, there is liposoluble vitamin malabsorption and vitamin K₁ deficiency, predisposing to bleeding; an injection of vitamin K₁ should always be performed. Abdominal ultrasonography should be performed in all cholestatic infants, by an experienced radiologist. Intrahepatic bile ducts are rarely dilated, suggest-

ing gallstone or a choledochal cyst. A small and dysmorphic gallbladder is very suggestive of biliary atresia, but is not specific, because the gallbladder is empty when the cholestasis is severe. On the contrary, a normal gallbladder does not exclude biliary atresia, because of cystic forms of biliary atresia. The aim is not to miss biliary atresia. The diagnostic procedure should be performed as quickly as possible. If the stools are permanently acholic, a liver biopsy or a cholangiography must be performed to rule out or confirm biliary atresia.

Etiologies

Extrahepatic Diseases

Exclusively extrahepatic causes of neonatal cholestasis are rare (5%) and can easily be diagnosed by ultrasonography. The stools may be permanently or transiently acholic.

Lithiasis of the common bile duct may cause severe cholestasis, and the presentation may be intermittent acholic stools. Predisposing factors are hyperhemolysis or parenteral nutrition. Spontaneous elimination of the calculus is frequent. On the contrary, a percutaneous cholecystography can allow to wash out the stone. In principle, there is no recurrence.

Spontaneous perforation of the bile duct is very rare and occurs often at the junction of the common bile duct and cystic duct. Clinical presentation is biliary peritonitis, bile collection or biliary stenosis. The treatment is surgical.

A choledochal cyst is rarely discovered in the neonatal period, but is the second most frequent surgical cause of neonatal cholestasis. In the neonatal period, the clinical presentation is usually similar to biliary atresia, but can also be a sudden onset of cholestasis or cholangitis with abdominal pain and pancreatitis. The diagnosis is made by ultrasonography or tomodensitometry. The treatment is surgical with total ablation of the residual cyst to avoid later malignant transformation. The long-term prognosis is very good, with a normal liver function.

Extra- and Intrahepatic Causes

Biliary Atresia

Biliary atresia is the most frequent cause of neonatal cholestasis and accounts for almost 50% of cases. It is the

first indication of liver transplantation in children. The incidence is 1/10,000 live births. It results from a bile duct obliteration beginning in utero or in the very early postnatal period. The bile duct alteration (inflammatory destruction) involves the whole biliary tree in 80% of cases. The cause of biliary atresia is still unknown, but a genetic origin can be suspected in some cases because of the association of some heterotaxic elements such as abdominal or thoracic situs inversus, and polyspleny in about 20% of cases, and a high incidence ($\times 6$) in some populations [4]. The aim of the surgery is to re-establish a bile flow through an intestinal loop (Kasai procedure). It will be successful if the intrahepatic bile ducts at the hilum are not yet occluded. Because the inflammatory destruction seems to be progressive, the Kasai procedure should be performed as early as possible. Before 45 days of life, the child has an 80% chance of remaining without jaundice at the age of 3 years. If the Kasai procedure is performed after 45 days of life, the success of the surgery decreases dramatically. If the Kasai procedure fails, the evolution of the disease is toward biliary cirrhosis, and a liver transplantation has to be performed between 1 and 2 years of life. Even when the Kasai procedure is efficient, in a large majority of cases, cirrhosis is already present, with a risk of portal hypertension and intestinal bleeding. The child can also develop cholangitis, icterus and chronic liver insufficiency, as well as acute liver failure; a liver transplantation is often necessary later on. Only 10% of children are alive without liver transplant at the age of 20 years [5]. Because of the severity and the importance of early diagnosis, biliary atresia should systematically be suspected in neonatal cholestasis. The diagnosis is strongly suspected if the child permanently has acholic stools (particularly from birth) with a hard hepatomegaly, if there are heterotaxic elements, if there is a biliary cyst at the hilum, and if the gallbladder is small and dysmorphic after fasting. Ultrasonography can help the diagnosis, but the diagnosis is actually made by cholangiography realized either at a laparoscopy or directly at laparotomy, both followed with the Kasai procedure if necessary.

Neonatal Sclerosing Cholangitis

Neonatal sclerosing cholangitis is a very rare disease, different from primary sclerosing cholangitis as seen in older children and associated with inflammatory bowel disease. The clinical presentation is a severe neonatal cholestasis, with acholic stools, that can mimic biliary atresia. The diagnosis is made by cholangiography, which shows patent intrahepatic ducts but irregular

narrowing of extrahepatic or intrahepatic bile ducts. Usually, no intestinal disease is found in these patients. Frequently, there is a resolution of the icterus, but early cirrhosis and portal hypertension; a liver transplantation is necessary for few children [6]. Some observations suggest a genetic origin because of familial cases [7]. A few cases associated with ichthyosis are linked to a mutation in the *Claudin-1* gene, encoding for a tight-junction molecule [8]. Other cases of this neonatal ichthyosis-sclerosing cholangitis (NISCH) syndrome have been described and show a variable clinical expression in the skin and liver phenotype [9]. However, the majority of children with neonatal sclerosing cholangitis have no ichthyosis, suggesting that the disease is heterogeneous.

Sclerosing Cholangitis with Postnatal Onset

Cholangitis can be associated with other diseases such as histiocytosis X, immunodeficiency syndrome, chronic inflammatory bowel disease or autoimmune hepatitis and congenital psoriasis. However, in some cases, there is no associated disease [6].

Intrahepatic Causes

Intrahepatic cholestasis represents almost 50% of the causes of neonatal cholestasis and comprises a heterogeneous group of diseases.

Infectious Diseases

The classical causes are fetopathies (congenital rubella, toxoplasmosis, cytomegalovirus and syphilis infections), often associated with a low birth weight, neurological or hematological symptoms. Bacterial infections (the most frequent is urinary tract infection with *Escherichia coli*) can induce inhibition of canalicular bile acid transport and cause cholestasis.

Toxic Causes

The most frequent toxic agent for the liver in the neonatal period is parenteral nutrition. The mechanisms are probably complex, associating recurrent infections, inflammatory response, inappropriate content of the parenteral nutrition, absence of enterohepatic circulation and immaturity of bile transport [10]. In some cases, children with very severe cholestasis induced by parenteral nutrition may develop liver failure. Drug injury can be an additional factor to liver alterations. A polymorphism associated with a decreased hepatic *BSEP* expression was

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Fig. 2. Picture of an infant with Alagille syndrome. Note the typical features: prominent forehead, deep-set eyes, hypertelorism, small pointed chin.

significantly more frequent in drug-induced cholestasis in adult patients [11], suggesting that genetic factors could also play a role in neonates in inducing parenteral nutrition liver disease.

Ischemia

Ischemia in newborns is frequently accompanied by cholestasis, without symptoms other than uncomplicated cholestasis. Cholestasis is associated with several contributing factors related to the severity of the neonatal distress. Neonates born before the age of 35 weeks have an increased risk ($\times 3$) of neonatal cholestasis [12, 13].

Genetic Diseases

Alagille Syndrome

Alagille syndrome is an autosomal dominant disorder also called 'syndromic bile duct hypoplasia'. It affects all ethnic groups. It is characterized by neonatal jaundice, intrahepatic cholestasis and developmental disorders affecting the liver, heart, vertebra, eyes and face. Alagille syndrome represents 10–15% of neonatal cholestasis, and its frequency is 1/100,000 live births. The clinical diagnosis is made on the association of facial dysmorphism (prominent forehead, deep-set eyes, hypertelorism, small pointed chin; fig. 2), posterior embryotoxon, butterfly-shaped vertebra, peripheral stenosis of

the pulmonary artery (or another cardiopathy), and cholestasis due to paucity of the interlobular bile ducts. The inheritance is dominant, with a variable penetrance and a great heterogeneity in clinical manifestations, suggesting that several factors are important in the pathogenesis. The prognosis is worse in children who present with neonatal cholestatic jaundice, with rare resolution of jaundice. A majority of them will require liver transplantation in childhood [14]. However, severe liver complications are also possible in late-onset liver disease, requiring follow-up throughout life. A molecular diagnosis is possible: 70% of patients have a mutation in the *jagged-1* gene encoding for a Notch receptor involved in cellular differentiation and development. The mechanism is probably haploinsufficiency. There is no genotype-phenotype correlation. No clustering was observed, and the majority of mutations are familial specific mutations. Sporadic cases are the most frequent (70%) relative to the high rate of de novo mutations [15]. There is a high rate of new mutations that makes the molecular diagnosis difficult.

α_1 -Antitrypsine Deficiency

α_1 -Antitrypsine deficiency is a common recessive disorder, affecting 1/2,000 live births. The diagnosis is suggested by protein electrophoresis with an absent pic of α -globulins and confirmed by the specific dosage of serum α_1 -antitrypsine and phenotype protein analysis. The α_1 -antitrypsine protein is synthesized in the liver and exported to the lung. In the lung, the protein functions as an antiprotease, preventing alveolar damage caused by leukocyte elastase. The abnormal protein has a 'Z' phenotype and cannot be exported out of the liver and polymerizes in the endoplasmic reticulum. Intrahepatic accumulation of the mutant Z protein in the liver induces activation of autophagy, mitochondrial injury and caspase activation, and thus, hepatocellular injury. Only patients with the ZZ genotype develop cholestasis in childhood or lung and liver disease in adulthood. Liver disease can occur at any age. The majority of children are free of significant liver dysfunction, and only a small proportion of homozygous ZZ children (10–15%) develop cholestasis in the neonatal period. The variable clinical presentation suggests an important contribution of genetic and environmental modifiers. The heterozygous carrier state for the mutant Z gene, present in 1.5–3% of the population, is not a cause of liver injury but may be a modifier gene for other liver diseases [16–18]. The clinical feature of neonatal presentation can be very similar to biliary atresia. There is no specific management and the outcome is

variable: 50% of children will no longer have liver abnormality in adulthood, 10% will remain cholestatic and require liver transplantation, and some of the children (40–50%) have a compensated liver disease with mild alterations and may require liver transplantation in adulthood.

Cystic Fibrosis

Cystic fibrosis is one of the most frequent hereditary diseases in Caucasians (1/2,000 live births in Europe). The clinical presentation may be variable. Neonatal cholestasis is a typical but rare primary clinical manifestation in cystic fibrosis and is most often associated with meconium ileus. The mechanism is probably obstruction of bile ductules by abnormal mucoid secretions. Patients may have complete cholestasis, mimicking biliary atresia. In cystic fibrosis patients with neonatal cholestasis, one third have another condition, increasing the risk of cholestasis, such as α_1 -antitrypsin deficiency, hypopituitarism, perinatal asphyxia and total parenteral nutrition [19].

Progressive Familial Intrahepatic Cholestasis

Progressive familial intrahepatic cholestasis (PFIC) is a heterogeneous group of diseases that accounts for 10% of neonatal cholestasis. The clinical presentation is neonatal or childhood cholestasis with pruritus. The evolution is cirrhosis or liver insufficiency in childhood, often requiring liver transplantation. The inheritance is autosomal recessive. Three groups of diseases have recently been identified, with particular phenotypic features (see the following chapter).

PFIC-1 and PFIC-2 are characterized by early cholestasis with a frequent liver insufficiency and severe pruritus; the gamma GT activity is always normal. PFIC-1 is due to a mutation in the *FIC1* gene, encoding for an ATPase, present at the canalicular pole of the hepatocyte and playing a still unknown role in bile formation. Some patients may have impairment of other organs except for the liver, such as diarrhea, renal failure, pancreatitis and deafness. PFIC-2 is due to a mutation in the *BSEP* gene which is implicated in the export of bile salts in the canaliculi. The evolution is often more severe than in the PFIC-1 deficiency.

PFIC-3, due to a mutation in the *MDR3* gene, is a transmembrane hepatocyte phospholipid transporter. Compared with the first 2 diseases, PFIC-3 deficiency is characterized by less pruritus, frequent portal hypertension, late liver insufficiency and high gamma GT activity.

Half of the patients with PFIC have a good response to ursodeoxycholic acid. Some children may benefit from a treatment with partial external biliary diversion (except those with MDR3 deficiency). Partial responders or nonresponders will require liver transplantation [20–22]. If a molecular diagnosis is available for the family, a prenatal diagnosis is possible in some laboratories [23].

Inborn Errors of Bile Acid Synthesis

Inborn errors of bile acid synthesis are specific defects in the enzymes involved in the reactions leading from cholesterol to cholic and chenodeoxycholic acids [24]. At birth or in early childhood, affected individuals present with cholestatic jaundice, fat-soluble vitamin deficiency and acholic or fatty stools (steatorrhea). Serum transaminases are usually elevated, gamma GT activity is normal, and a conjugated hyperbilirubinemia is often present. Liver biopsies may reveal nonspecific changes, but giant cell transformation of hepatocytes, inflammation, fibrosis gamma GT activity is normal and canalicular and hepatocyte cholestasis are usual. Recognition of defects in bile acid synthesis relies upon mass spectrometric analysis of the urine and serum to establish an absence or marked reduction in certain primary bile acids, cholic and chenodeoxycholic acids, concomitant with the presence of excessive amounts of atypical bile acids and sterols, synthesized as a consequence of the enzyme deficiency.

Mutations of several genes have so far been identified (fig. 3) [25, 26]:

3 β -hydroxy-C27-steroid dehydrogenase oxydoreductase deficiency (HSD3B7); Δ 4-3-oxosteroid 5 β -reductase deficiency (AKR1D1); oxysterol 7 α -hydroxylase deficiency (CYP7B1); 2-methylacyl-CoA racemase deficiency; sterol 27-hydroxylase deficiency (CYP27A1); bile acid-CoA:amino acid N-acyltransferase deficiency (BAAT); cholesterol 7 α -hydroxylase deficiency (CYP7A1).

Early diagnosis is important because patients can be successfully treated with oral administration of cholic acid. Normalization in serum liver enzymes and bilirubin and resolution of the histologic lesion are consistent responses to bile acid therapy, and the need for liver transplantation in most cases can be circumvented. Bile acid biosynthetic defects, in particular 3 β -hydroxy-C27-steroid dehydrogenase oxydoreductase deficiency, may also cause late-onset chronic cholestasis; in these individuals, the clinical history generally reveals a pattern of mildly elevated transaminases in infancy, that often resolved, only to reappear later on with an early-onset of vitamin-D-deficient rickets.

Niemann-Pick Disease Type C

Niemann-Pick disease type C is a rare autosomal recessive lipid storage disorder characterized by progressive neurodegeneration. The clinical presentation is heterogeneous, and the age at onset varies between the perinatal period and adulthood. In the neonate, hepatomegaly and splenomegaly are frequent, associated with prolonged cholestatic jaundice. Usually, the icterus disappears spontaneously, but sometimes, a rapidly fatal liver failure develops. The age at onset of neurological symptoms and their evolution determine the severity of the disease. Niemann-Pick disease is found in 8% of infants evaluated for cholestasis; it should be considered in all infants with cholestasis, particularly those with splenomegaly or associated neurological symptoms. The diagnosis is suspected when lipid-laden foam cells are found in the bone marrow and liver. The mutated gene is involved in intracellular cholesterol transport and causes accumulation of nonesterified cholesterol in lysosomes. The diagnosis is established by demonstrating these anomalies in cultured fibroblasts. Molecular diagnosis is possible. Two genes are involved: *NPC1* and *NPC2*, allowing a prenatal diagnosis [27]. There is no specific treatment.

Mitochondrial Disorders

Mitochondrial disorders may manifest as neonatal acute liver failure, steatohepatitis, cholestasis or cirrhosis with chronic liver failure of insidious onset. Usually, there are significant neuromuscular symptoms, multi-system involvement and lactic acidemia. The liver disease is usually progressive and eventually fatal. Several molecular defects (mutations in nuclear genes such as *SCO1*, *BCSIL*, *POLG*, *DGUOK* and *MPV17* and deletion or rearrangement of mitochondrial DNA) have been identified in recent years with the possibility of genetic and prenatal diagnosis. Current medical therapy of mitochondrial hepatopathies is largely ineffective, and the prognosis is usually poor. The role of liver transplantation in patients with liver failure remains poorly defined because of the systemic nature of the disease with the possibility of the involvement of other organs than the liver [28, 29].

Galactosemia

Galactosemia is characterized by a deficiency in galactose metabolism resulting in the accumulation of galactose-1-phosphate. It is autosomal recessive and affects 1/35,000 births in Europe. The clinical symptoms appear during the first days of life and include vomiting, jaun-

Fig. 3. Bile acid biosynthetic pathways. The classic pathway of bile acid biosynthesis is only present in hepatocytes. The alternative pathway exists in all tissues. Only major regulatory steps and enzymes are shown. The classic pathway synthesizes 2 primary bile acids, cholic acid and chenodeoxycholic acid. Framed enzymes are those known to be involved in inborn errors of bile acid synthesis. CYP7A1 = Cholesterol 7 α -hydroxylase; HSD3B7 = 3 β -hydroxyl Δ 5-C27-steroid oxydoreductase; CYP8B1 = sterol 12 α -hydroxylase; AKR1D1 = Δ 4-3-oxosteroid 5 β -reductase; CYP27A1 = sterol 27-hydroxylase; CYP7B1 = oxysterol 7 α -hydroxylase; BAAT = bile acid CoA:amino acid-N-acyltransferase.

dice, lethargy, hepatomegaly, liver failure in a baby breast-fed or receiving a lactose-containing formula. If left untreated, the condition evolves rapidly towards hepatic and renal failure with sepsis due to Gram-negative bacte-

ria. Cataract develops after several days or weeks and becomes rapidly irreversible. The diagnosis relies on the detection of galactose-1-phosphate accumulation in erythrocytes (spot test), measurement of galactose-1-phosphate

uridyl transferase activity in erythrocytes, determination of a deficiency in one of the enzymes of the metabolic pathway and identification of the gene mutation. Three enzymes are involved in the galactose metabolic pathway: (1) galactose-1-phosphate uridyl transferase (the most common), (2) galactokinase, and (3) uridine diphosphate galactose-4-epimerase.

A prompt diagnosis is important in order to begin a lactose-free diet. Liver failure disappears in a few days. The lactose-free diet has to be maintained lifelong. If not, neurological complications (decreasing intellectual performances with age) and hypergonadotropic hypogonadism (ovarian dysfunction) appear during childhood. Despite a strict diet, long-term complications such as mental retardation, verbal dyspraxia, motor abnormalities and hypogonadism are frequent. It has been suggested that these complications may result from the endogenous galactose synthesis or from abnormal galactosylation. Novel therapeutic strategies, aiming at the prevention of galactose-1-phosphate production, should be developed [30].

Tyrosinemia Type I

Tyrosinemia type I is an autosomal recessive disorder due to fumarylacetoacetate deficiency, an enzyme involved in the catabolism of tyrosine. In the acute neonatal form, onset occurs between the ages of 2 and 6 weeks, with symptoms of hepatocellular insufficiency, including vomiting, diarrhea, jaundice, hypoglycemia, edema, ascites and bleeding. Septicemia is a frequent complication. The diagnosis is confirmed by the finding of δ -aminolevulinic acid and succinyl acetone in urine, and by enzymatic activity measurement in fibroblasts. The treatment is based on tyrosine-restricted diet and NTBC [(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione], which inhibits tyrosine oxidase and prevents the accumulation of toxic succinyl acetone [31]. Despite this treatment, some patients develop hepatoma with elevated α -fetoprotein and require liver transplantation [32]. Prenatal diagnosis relies on metabolite assay, enzymatic study or screening for the mutation when it is known.

Peroxisomal Disorders

Many peroxisomal disorders have an impact on bile acid synthesis [33]. The Zellweger syndrome which is defined by a reduced number of peroxisomes is characterized by dysmorphic craniofacial features, profound hypotonia, seizures, as well as liver and renal dysfunctions. Hepatomegaly and cholestasis with progressive fibrosis

are observed. The biochemical diagnosis relies on the study of very-long-chain fatty acids. This impaired metabolism results in the accumulation of toxic metabolites and damages developing cells. The disease is transmitted as an autosomal recessive trait. Most infants with Zellweger syndrome die within the first year of life secondary to apnea or respiratory disease related to infection or intractable seizure.

Carbohydrate-Deficient Glycoprotein Syndromes

Carbohydrate-deficient glycoprotein (CDG) syndromes are a group of disorders in glycoprotein synthesis characterized by multivisceral involvement. CDG syndrome type Ib is characterized by hepatic and intestinal manifestations (diarrhea, vomiting, hepatomegaly with liver fibrosis) and the absence of neurologic involvement. Liver fibrosis may evolve toward cirrhosis and liver insufficiency [34]. This form of CDG syndrome can be successfully treated with oral mannose. The biological diagnosis is based on the demonstration of abnormal glycosylation of serum glycoproteins, measurement of leukocyte enzyme activities and mutations in the corresponding genes. Prenatal diagnosis of CDG syndrome is possible.

Transient Neonatal Cholestasis

Transient neonatal cholestasis represents 5–10% of neonatal cholestasis. It is often called 'neonatal hepatitis', but no infectious agent is found. The evolution is always good toward resolution. The diagnosis should be made with caution, after a careful follow-up. When a liver biopsy is performed, multinucleated giant cell hepatitis is seen, with mild ductular proliferation and moderate portal inflammatory fibrosis [35]. The mechanism is probably multifactorial: bile acid secretion immaturity in the neonatal period, liver fetal injury (ischemia, infection), long-time fasting (parenteral nutrition) and possibly genetic factors predisposing to cholestasis [36]. The other causes of neonatal cholestasis have to be ruled out before this diagnosis is retained.

Practical Approach in the Diagnosis of Neonatal Cholestasis

In cases of suspected cholestasis, the first and most important point to assess is the color of the stools. If the stools are acholic, biliary atresia must be diagnosed or ruled out. If an obstructive pathology of the extrahepatic biliary tree is ruled out, the diagnosis of biliary atresia is

Fig. 4. Algorithm in the case of very pale or acholic stools. The first point is to know if the biliary tract is dilated or not. If there is no extrahepatic obstruction, Alagille syndrome, cystic fibrosis and α_1 -antitrypsine deficiency must be ruled out. If no evident etiology is found, a liver biopsy or a cholangiography must be performed.

Fig. 5. Algorithm in the case of pale but not acholic stools. An extrahepatic obstacle must be ruled out. In cases of a low gamma GT level, a PFIC-1 or PFIC-2 and inborn errors of bile synthesis are possible. In cases of a high gamma GT level, Alagille syndrome, cystic fibrosis and α_1 -antitrypsine deficiency must be ruled out. If no etiology is evident, a liver biopsy and cholangiography must be performed.

likely. A cholangiography and a liver biopsy must be performed. If the biliary tract is not patent, a Kasai procedure is to be performed. If the biliary tract is patent, another diagnosis and a possible neonatal sclerosing cholangitis have to be considered (fig. 4).

Biliary Atresia

In order not to miss biliary atresia, consider the following: (1) neonatal cholestasis is a biliary atresia until definitively demonstrated that it is not; (2) diagnosis of biliary atresia is an emergency; (3) do not first think of

Fig. 6. Consequences of cholestasis. In cases of cholestasis, a diminished bile flow is responsible of an increase in serum bile salts leading to pruritus and liver injury. The absence of bile in the intestine leads to lipid malabsorption and impaired growth, and a fat-soluble vitamin malabsorption leads to bleeding (vitamin K deficiency), rickets (vitamin D deficiency), neuropathy (vitamin E deficiency) and ocular disease (vitamin A deficiency).

breast milk jaundice; (4) ask parents about the stool color; (5) do not think that a normal growth rules out a severe liver disease; (6) do not stop the explorations after finding cytomegalovirus in urines; (7) do not think that bilirubinemia is always very high in biliary atresia; (8) do a liver biopsy if the diagnosis is unclear; (9) organize an operative cholangiography when biliary atresia is not ruled out and the time is running.

If the stools are pale but not acholic, the biochemical tests (related to the various causes of neonatal cholestasis) or a liver biopsy will help the diagnosis. Different parameters are important, such as dilatation of the biliary tract and the level of gamma GT activity (fig. 5).

Management of a Cholestatic Child

In addition to a possible specific treatment, treatment of the consequences of cholestasis is important to avoid complications. The major consequence is the impairment of lipid absorption due to the absence of bile in the intestine. Malabsorption of fat-soluble vitamins may result in bleeding (vitamin K deficiency), rickets (vitamin D deficiency), neuropathy (vitamin E deficiency) and ocular disease (vitamin A deficiency). Fat-soluble vitamins

should be given orally at large doses, as long as the cholestasis is not too severe and the level of vitamins A, E and D are checked regularly. If cholestasis is severe, or in case of poor response to the oral route, parenteral injections are mandatory.

Impaired lipid absorption and cholestasis may severely impair growth. Formulas enriched with medium-chain triglycerides may improve fat malabsorption and nutrition. In cholestatic infants, 180–200 calories/kg/day may be necessary, and enteral feeding could be used to maintain growth.

The use of ursodeoxycholic acid (a hydrophilic bile acid) is beneficial in cholestasis. The precise mechanisms of its action are not completely understood; there is probably an enrichment in the bile acid pool with more hydrophilic bile acids, and stimulation of bile flow. It improves biochemical parameters and controls fibrosis progression in PFIC. Its efficiency is not demonstrated in other cholestatic syndromes, but ursodeoxycholic acid seems to improve biochemical parameters and speeds the clearance of jaundice in transient neonatal cholestasis. The only side effect is diarrhea that responds to dose reduction. Its use is recommended at a dose of 20 mg/kg/day.

Pruritus is a debilitating symptom in severe cholestasis and is obvious only from the second semester of life. The most effective treatment is rifampicin, used at 5–20 mg/kg/day. The mechanism of action is unclear but probably involves the inhibition of hepatocyte bile uptake and the induction of microsomal enzymes. Phenobarbital and cholestyramine are less effective and have more side effects.

Vaccinations should be performed, especially if a liver transplantation is probable in the future (fig. 6).

Conclusion

The early diagnosis of neonatal cholestasis is important considering the severity of biliary atresia. Liver transplantation dramatically changed the prognosis of biliary atresia and a lot of other cholestatic diseases in children. Therapeutic progress in pediatric hepatology is

essentially linked to liver transplantation that gives good chance of surviving and a good quality of life. The long-term results for transplantation are good, with a survival rate around 90%, 5 years after transplantation. But it is a very difficult procedure with a lot of surgical and medical complications such as renal insufficiency and posttransplant lymphoproliferative disease. Specific medical treatments have been developed in some disorders such as NTBC treatment in type I tyrosinemia and cholic acid in bile acid synthetic disorders which allow avoiding transplantation in the majority of cases. Except for these 2 particular cases, the only medical treatment possible for cholestasis is a nonspecific treatment: ursodeoxycholic acid. The recent progresses in genetic diagnosis in cholestatic diseases could lead to a better comprehension of physiopathologic mechanisms and to the development of new therapeutic strategies such as liver cell transplantation or gene therapy.

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