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Review

Extrahepatic bile duct atresia from the pathologist's perspective: pathological features and differential diagnosis

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Abstract

Extrahepatic biliary atresia (EHBA) refers to stenosis or atresia of the extrahepatic biliary tree. It accounts for 25-30% of cases of neonatal cholestasis. If left untreated, EHBA progresses to biliary cirrhosis and is universally fatal within the first 2 years of life. Early diagnosis is crucial since surgical treatment (Kasai procedure) is the only treatment option. Histopathologic examination of liver biopsy specimens is a key element in the diagnostic work-up of infants with suspected EHBA. Pathologic diagnosis aims at excluding non-surgically correctable causes of neonatal cholestasis thereby leading to surgical exploration for confirmation of the diagnosis. All published data indicate that pathologists can diagnose EHBA with high sensitivity, high specificity and reasonable interobserver agreement. The most useful histologic features in the diagnosis of EHBA are portal tract changes including ductular proliferation and bile plugs in ducts and ductules. These lesions are not pathognomonic but can be seen in extrahepatic obstruction of any cause. Total parenteral nutrition (TPN)-associated cholestasis and alpha1-antitrypsin (A1AT) deficiency cannot be differentiated from EHBA without access to clinical data and may lead to false-positive diagnosis. False-negative interpretation may be caused by early age at diagnosis or by small/indequate specimens. The pathologist also plays a role in the examination of the resected fibrotic segment and

of explant specimens. Histopathology can yield prognostic information, being also an indispensable tool in research for the possible pathogenesis of this disease. A well-coordinated, multidisciplinary approach is required in the assessment of suspected cases of EHBA.

Keywords

Extrahepatic biliary atresia, liver histopathology, differential diagnosis, neonatal cholestasis.

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Introduction

Cholestatic jaundice, clinically defined by an increase in the level of conjugated bilirubin, is a common presentation of liver disease in neonates and infants [1, 2]. This is probably related to the relative immaturity of hepatic secretory and excretory function and of the biliary drainage system in early life [1, 3]. In infants with prolonged (> 2 weeks) jaundice, pale stools and dark urine, a thorough investigation is warranted, usually following proposed algorithms [1, 3]. Timing and expediency are crucial because a number of diagnoses are directly linked to early intervention [3]. The list of differential diagnoses is very broad, but close to 70% of cases are due to either neonatal hepatitis or biliary atresia [1, 3]. In addition to laboratory evaluation, ultrasound and hepatobiliary scintigraphy are used to identify correctable causes of jaundice. However, liver biopsy is often needed to determine if surgical exploration of the biliary tree is warranted [3].

Extrahepatic biliary atresia (EHBA) is the most common cause of cholestasis in the newborn and the most common indication for liver transplantation in children [1, 3]. Its incidence varies from 1 in 18,000 live births in Europe and North America to 1 in 5,000 in Taiwan [1]. EHBA can be defined as a progressive fibroinflammatory process involving a segment or all of the extrahepatic biliary tree leading to loss of patency of the lumen (stenosis) and obstruction to bile flow [3]. Despite considerable research efforts, the etiology and pathogenesis remain largely unknown [3, 4]. Anatomically, 3 main types of EHBA have been described. Type 3 (obstruction at the porta hepatis level) comprises > 95% of cases [1]. Clinically, 2 major forms of EHBA are recognized [1, 3]. The perinatal form accounts for the majority of cases. The embryonic or fetal form occurs in 10-20% of cases [3, 5]. Most of these children have the biliary atresia splenic malformation (BASM) syndrome [3, 5]. More recently, a cystic EHBA has been described, accounting for 10% of total cases in a recent series of a single center [6]. EHBA is amenable to surgical correction by the Kasai portoenterostomy [1, 4]. This operation has a better outcome if performed not later than 3 months of age [1, 4]. Early surgical restoration of bile flow also offers the prospect of normal growth and long-term survival without liver transplantation [4].

Histopathological features of extrahepatic biliary atresia

Liver biopsy

The liver biopsy is a cornerstone of the diagnostic work-up of infants with cholestatic jaundice and is generally considered the most reliable tool for the prelaparotomy diagnosis of biliary obstruction [7]. It is standard practice in most pediatric centers to obtain a percutaneous liver biopsy before surgical intervention [2-4].

Portal changes

Portal findings in EHBA are broadly similar to what is seen in large-duct obstruction due to other etiologies [7]. The portal tracts show variable edema and prominent ductular proliferation with small interanastomosing ductules usually located at the periphery of the portal tracts and accompanied by inflammatory cells, especially neutrophils ("ductular reaction") [1, 3, 7-9]. Bile plugs are frequently seen within the dilated ductular lumina (**Fig. 1**). In 10% to 48% of cases, the bile ducts are still in a configuration reminiscent of the embryonic ductal plate (referred to as ductal plate malformation, DPM) [1, 7, 10, 11] (**Fig. 2**). These cases, referred to as "early severe biliary atresia", show severe fibrosis at a very early

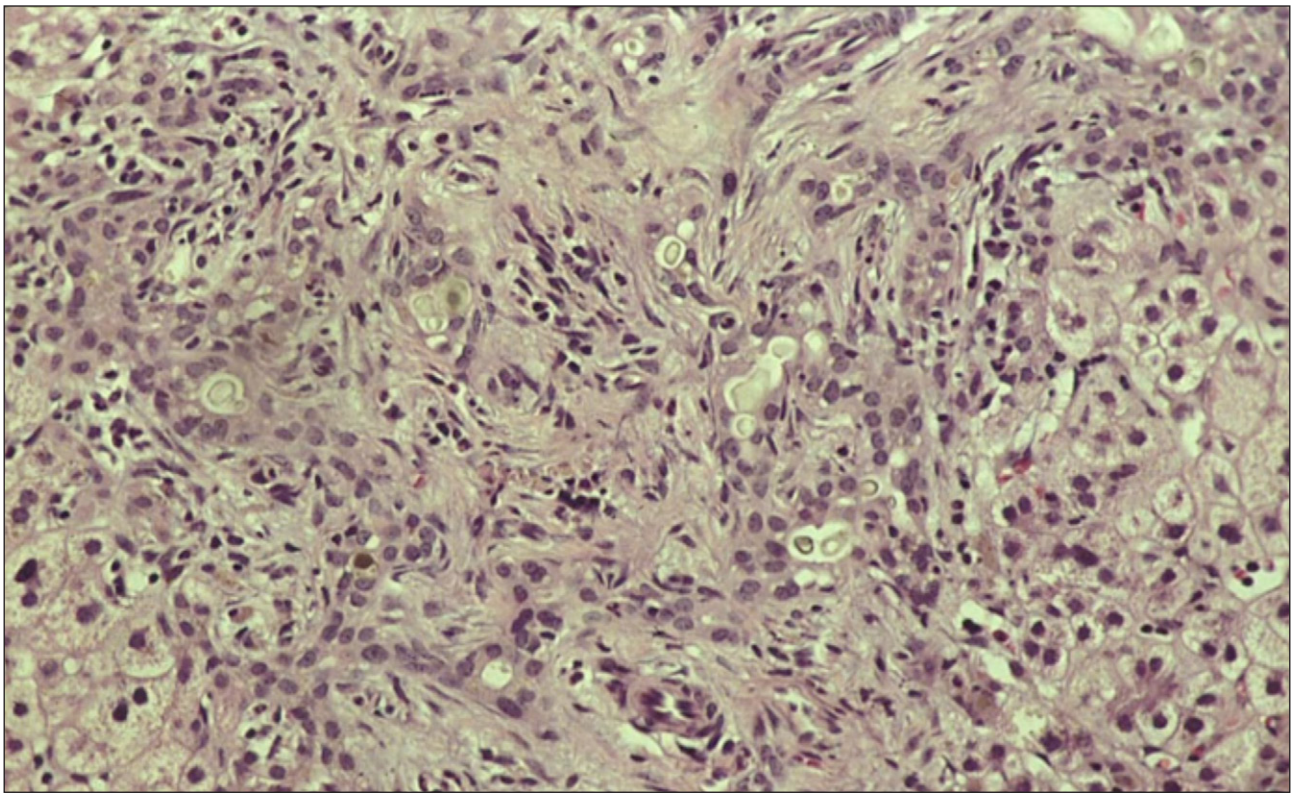

Figure 1. Extrahepatic biliary atresia (EHBA): portal tract showing ductular reaction with numerous bile plugs in ductules (hematoxylin-eosin [HE], photomicrograph courtesy of Prof. Dr. V. Desmet).

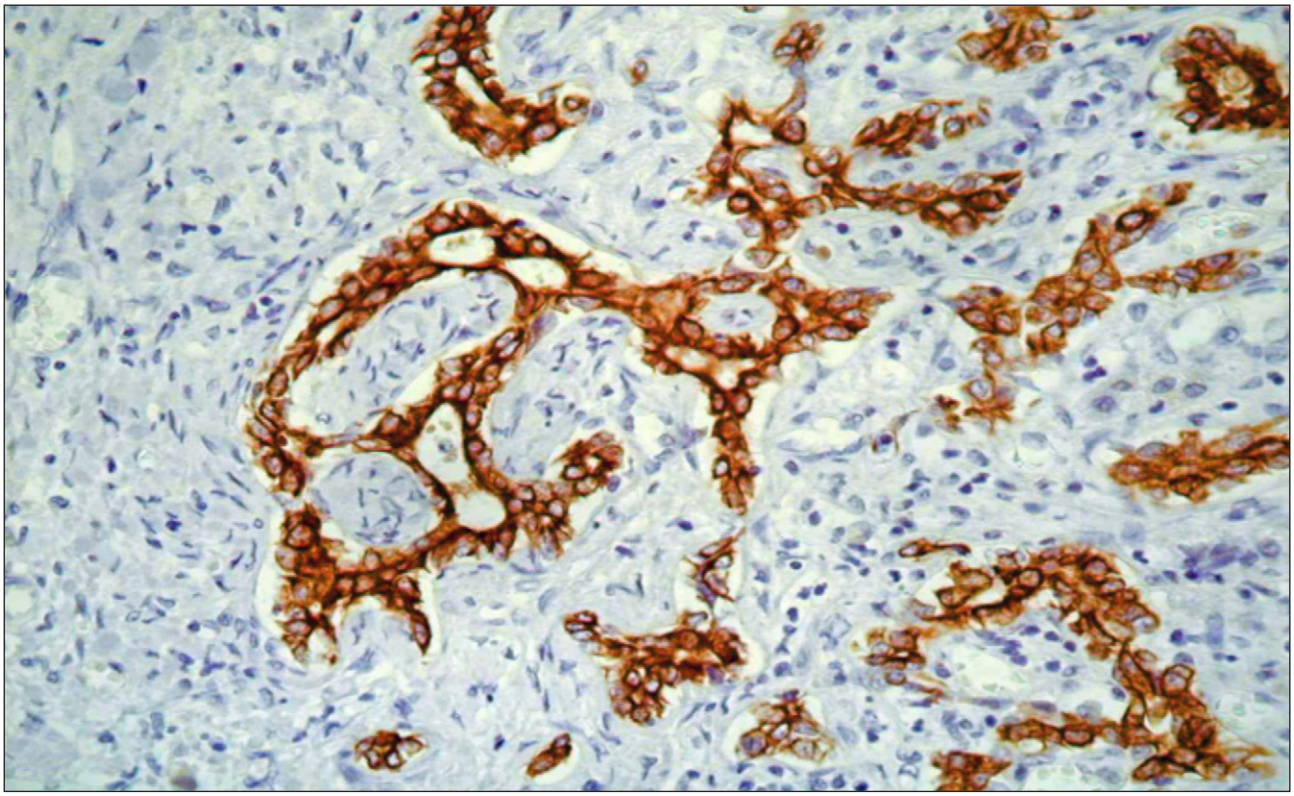

Figure 2. Extrahepatic biliary atresia (EHBA) with ductal plate malformation (DPM): ductules in ductal plate configuration are highlighted by a cytokeratin 7 immunostain (photomicrograph courtesy of Prof. Dr. V. Desmet).

age and have a worse prognosis [7, 10]. DPM may be difficult to differentiate from periportal ductular reaction in small biopsies from neonates [10]. This may lead to overdiagnosis of this variant of EHBA and may explain the wide variance in its reported incidence (10% to 65% of patients) [5, 7, 10, 12]. Portal-based fibrosis is a typical diagnostic finding. Fibrous expansion of portal tracts and portal-portal fibrous septa linking portal tracts ("biliary fibrosis") are expected to be present in most cases of EHBA, particularly those with biopsy in the second month of life or later. Progressive fibrosis results in biliary cirrhosis in 1 to 6 months. Injury and destruction of interlobular bile ducts leading to ductopenia are thought to occur later in the course of the disease but may be detected on initial biopsy in a subset of patients [7, 8]. This intrahepatic component of the disease may represent an important factor leading to the progressive liver dysfunction that occurs in a significant proportion of cases following a Kasai procedure and that may result in liver failure and need for liver transplant. Lymphocytic inflammation is usually mild. Extramedullary hematopoiesis (usually myelopoiesis) is occasionally seen [7, 8]. Immunohistochemical staining for "biliary type" cytokeratins 7 or 19 can be very helpful in highlighting the ductular reaction and in demonstrating the DPM or ductopenia [9, 10].

Lobular changes

Lobular changes are variable and are less useful in the differential diagnosis with nonobstructive causes of neonatal jaundice [7]. Canalicular and intracellular bilirubinostasis is present in nearly all cases (**Fig. 3**). A variable amount of extramedullary hematopoiesis (erythropoiesis) can be present. Giant cell transformation is seen in approximately 20% to 50% of cases, but is never as prominent as it is in neonatal hepatitis [1, 5, 13]. With progression of the disease, features of cholate stasis including periportal hepatocyte swelling, copper/copper binding protein accumulation in periportal hepatocytes and Mallory body formation can develop [7].

Biopsy requirements

An adequate needle biopsy should be an minimum of 2.0 cm long and 0.2 mm wide, not fragmented and should ideally contain 10 portal tracts [14]. A surgical wedge should be

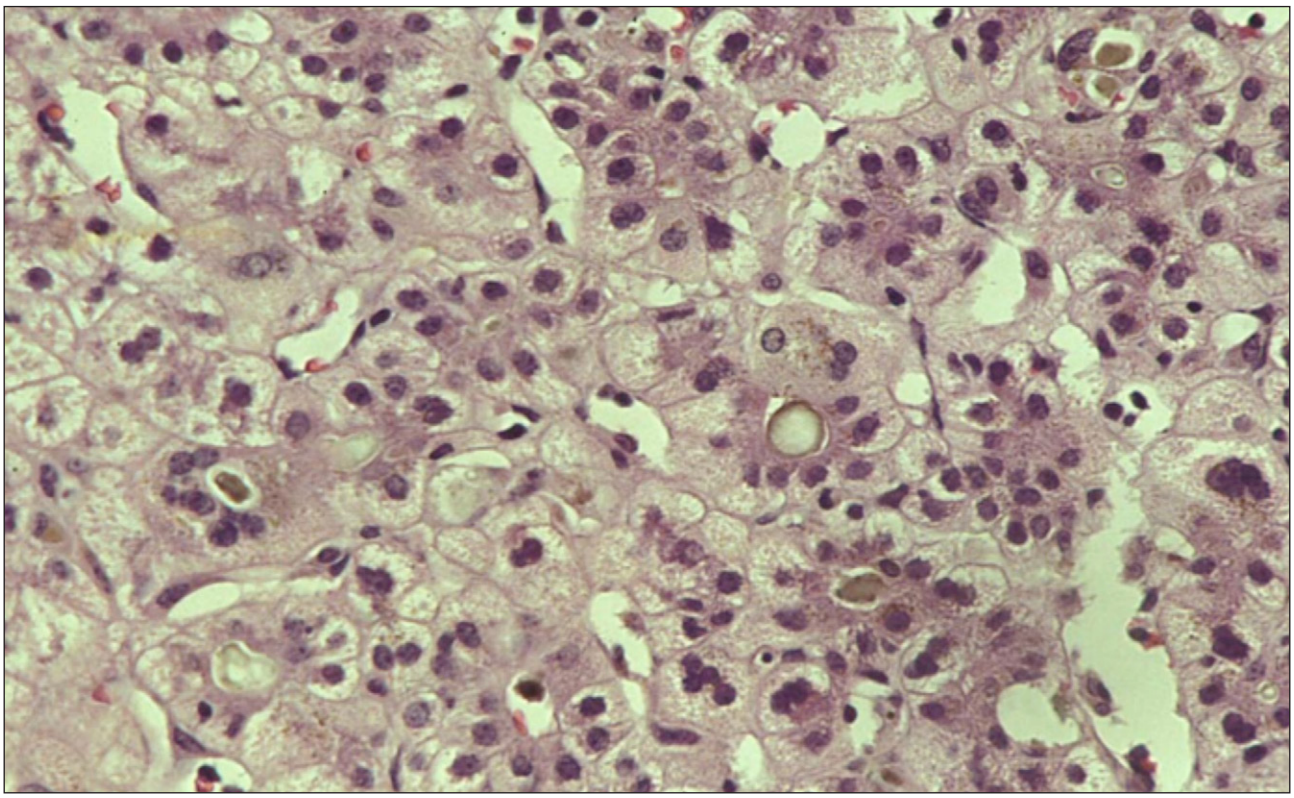

Figure 3. Extrahepatic biliary atresia (EHBA), lobular changes. Numerous bilirubin plugs are present in cholestatic liver cell rosettes (hematoxylin-eosin [HE], photomicrograph courtesy of Prof. Dr. V. Desmet).

sufficiently deep to include 6 complete portal areas independent of the liver capsule [3]. Because of the broad differential diagnosis of jaundice in children, it is advisable to not only fix the larger part of the specimen in formalin, but also to snap-freeze a part of the biopsy (to preserve mRNA) and to place a small portion of the tissue in special fixative for future electron microscopy [15].

Diagnostic pitfalls

Inadequate specimens (< 5-6 portal tracts) may lead to under-recognition of obstructive features [14]. Even when dealing with an adequate specimen, the pathologist should be familiar with normal and potentially misleading features of the livers of infants and children, including the presence of developmental residua of fetal histology, two-cell thick plates, hematopoietic elements, copper and hemosiderin [15]. Giant cells are commonly present in infants with liver disease, regardless of the etiology [15].

The histologic features of EHBA may evolve over time. Features of biliary obstruction may be poorly developed in cases in which the biopsy is performed before 4-6 weeks resulting in a falsely negative diagnosis [16]. In this situation, repeated biopsies are advisable if EHBA remains in the clinical differential diagnosis [7].

Accuracy of diagnosis

Some authors consider the liver biopsy the gold standard for the diagnosis of EHBA [17, 18] but relatively few studies have evaluated the accuracy of liver biopsies in this diagnosis. Roquete et al., in a retrospective series of 51 cases of EHBA and 45 cases of intrahepatic cholestasis from a single center, reported a sensitivity of liver biopsy for extrahepatic obstruction of 90.2%, a specificity of 84.6% and an accuracy of 87.8% [19]. They also reviewed 7 other studies reporting sensitivities ranging from 76% to 100%, specificities ranging from 75.9% to 100% and accuracies ranging from 86.5% to 96.8%. In a recent study by the Biliary Atresia Research Consortium (BARC, now the Childhood Liver Disease Research and Education Network or ChiLDREN) a set of 97 anonymous liver biopsy samples (49 cases of EHBA, 17 cases of ideopathic neonatal hepatitis and 31 other causes of neonatal jaundice) was sent to 10 pathologists [14]. A semiquantitative scoring system comprising 16 histological features was developed and then used by the pathologists who were blinded to clinical history, imaging results and laboratory data. Interobserver agreement was evaluated statistically [14]. There was moderate to substantial interobserver agreement in identification of bile plugs in ducts, giant-cell transformation, extramedullary hematopoiesis and bile duct proliferation [14]. Histologic features that best predicted EHBA on the basis of logistic regression included bile duct proliferation, portal fibrosis and absence of sinusoidal fibrosis. The pathologists' diagnosis of obstruction in clinically proven cases of EHBA ranged from 79% to 98% with a positive predictive value of 90.7% [14]. Not surprisingly, the authors found that distinguishing between EHBA and disorders such as total parenteral nutrition (TPN)-associated liver disease and alpha1-antitrypsin (A1AT) disease is not possible without adequate clinical information.

The Kasai specimen

The Kasai procedure typically yields a fibrotic segment of extrahepatic bile duct [7]. Numerous studies have detailed the morphologic appearance of the resected biliary remnant. Several investigators have attempted to correlate the size of the bile duct remnants at the porta hepatis with outcome of the portoenterostomy but their results are conflicting (probably due to inherent methodological problems) [3]. Up to now, prognostication based on the size of the remnants has little practical value [3]. However, systematic examination of Kasai procedure specimens remains important because histopathological documentation of fibrous obliteration of the extrahepatic biliary tree represents the final confirmation of biliary atresia [7].

Post-portoenterostomy liver biopsies and explant specimens

Liver biopsies following portoenterostomy are most frequently obtained for evaluation of persistent jaundice and recurrent fevers [3]. In untreated cases, progressive loss of intrahepatic bile ducts occurs as early as 5-6 months. Even when biliary drainage is obtained by portoenterostomy, loss of intrahepatic bile ducts is seen with concomitant sclerosis and atrophy of the portal venous system [8, 20, 21]. Cysts filled with inspissated bile develop in 20% of children. Cholangitis occurs in 50-90% of cases and worsen the patient's prognosis. Malignancies including

hepatocellular carcinoma, hepatoblastoma and cholangiocarcinoma have been reported in a small percentage of children with EHBA [3]. Examination of the explanted specimen usually shows typical features of biliary cirrhosis with broad fibrous bands and irregularly shaped regenerative nodules with a "jigsaw" appearance [7]. In patients who underwent a Kasai procedure and whose native livers survived for several years, prominent perihilar regenerative nodules measuring up to 14 cm can be present [22].

Histopathological differential diagnosis

From a histopathologic standpoint the most crucial distinction in cases of neonatal cholestasis is between obstructive and nonobstructive etiologies. The obstructive etiologies are mainly represented by EHBA, but also include choledochal cyst, tumors and stones [3]. They can be differentiated on the basis of clinical and laboratory data and ultrasonographic and cholangiographic findings [3].

DPM-like structures in biopsies of EHBA may result in a differential diagnosis with fibropolycystic diseases such as congenital hepatic fibrosis and Caroli disease [7, 11] This differential diagnosis can usually be solved on the basis of age of presentation, clinical manifestations and imaging features [7].

While the histopathologic features of EHBA are well described, there is unfortunately significant overlap with changes seen in non-obstructive neonatal diseases.

Cholestasis may appear within 2 weeks of TPN and may progress to severe liver disease [23]. The pathogenesis is unknown but likely multifactorial and possibly related to the immaturity of the bile excretory system [7]. Hepatocytic and canalicular bilirubinostasis are the most frequent and earliest histologic findings. After a few weeks, mild ductular reaction and ductal cholestasis can appear, increasing with disease progression. Periportal inflammation may be seen and giant cell transformation has been reported in one-third of cases [23]. Increasing portal fibrosis may be associated with perivenular fibrosis [24].

Neonatal sclerosing cholangitis presents in the first 2 weeks of life and may be histologically indistinguishable from EHBA [1]. The diagnosis rests on cholangiography demonstrating patency of the extrahepatic bile ducts and characteristic beaded appearance of the intrahepatic bile ducts. Many cases have been linked to a mutation in the claudin-1 (*CLDN1*) gene and are referred to as the neonatal ichtyosis-sclerosing cholangitis (NISCH) syndrome [25].

Paucity of bile ducts comprises 2 groups, a "syndromic" variant (Alagille syndrome) and a "nonsyndromic" type associated with multiple etiologies [1, 3]. Alagille syndrome is an autosomal dominant disease with intrahepatic paucity of bile ducts associated with heart and vascular abnormalities, spinal abnormalities, ocular abnormalities, facial irregularity and renal abnormalities [1, 3]. A mutation of the *JAG1* gene is present in 95%, and about 1% have mutations of the *NOTCH2* gene [3]. Infants present with jaundice, pruritus, increased total and direct bilirubin, often high gamma-glutamyltransferase (GGT) and elevated cholesterol and bile acid levels. Paucity of bile ducts refers to a reduced number of interlobular bile ducts. The normal ratio of bile ducts to portal tracts is between 0.9 and 1.8 [3]. Alagille based his orginal diagnosis of the syndrome on a ratio less than 0.5 [3]. Sampling is obviously an important issue. Some authors require a minimum number of 10 portal tracts, others consider 5 portal spaces sufficient in newborns. There is now ample evidence that the histopathological findings vary by the age of the patient and progression of the disease. Paucity may gradually develop and in a large series of cases of Alagille syndrome from King's College Hospital, 25% of liver biopsies even when taken after 1 year of age did not show paucity [26]. Findings by Libbrecht et al. [27] support the concept that intrahepatic bile ducts fail in Alagille syndrome because of lack of elongation and branching of the ducts that are present at the time of birth. Lobular bilirubinostasis is present. Ductular proliferation is usually lacking. It can occasionally be seen in early biopsies and then the main differential diagnosis is EHBA [1]. Early biopsies may show bile duct injury and duct sclerosis. Progression to cirrhosis appears slower than in other cholestatic diseases. Immunohistochemical staining for cytokeratin 7 or 19 hightlights the bile ducts and ductules and may be very useful in the interpretation of biopsies [10, 27].

A1AT deficiency is the most common cause of genetic liver disease in childhood and causes overt liver disease in 10% to 20% of children. Liver disease is almost exclusively associated with the PiZZ phenotype [1]. The typical clinical presentation is with neonatal cholestasis. The histologic features vary depending on the age of

the patient. It is important to keep in mind that the characteristic PAS-diastase resistant eosinophilic hyaline globules that are the hallmark of the disease may not be seen in the first 3 months of life [1]. The histopathological findings vary and include damaged bile ducts, ductular proliferation and ductular bilirubinostasis, duct paucity, portal mononuclear infiltrate, parenchymal giant cells and fibrosis [1, 8]. Cirrhosis may develop as early as 6 months of age. Immunohistochemical staining for A1AT has to be interpreted with caution as normal hepatocytes also make A1AT [1]. Electron microscopy shows characteristic electron-dense material filling the endoplasmic reticulum.

Progressive intrahepatic familial cholestasis (PFIC) is a group of autosomal recessive disorders usually presenting in infancy and childhood and rarely in adults [28]. Three forms have been recognized [28]. PFIC1 disease (*ATP8B1* disease) and PFIC2 (*ABCB11* disease) are characterized by normal or low GGT [1, 28]. MDR3 deficiency (formerly PFIC3) is caused by mutations in the *ABCB4* gene that encodes the multidrug resistant P-glycoprotein, that translocates phospholipid across the canalicular membrane. MDR3 deficiency may present with jaundice and acholic stools as early as the first month of life but more commonly in the first year of life or in young adults [1]. MDR3 deficiency is associated with elevated serum GGT. Liver biopsy findings vary but ductular proliferation/reaction is characteristic, similar to that seen in EHBA [1]. Hepatocellular but also canalicular and ductular cholestasis may be seen. Bile ducts may contain cholesterol clefts. Approximately 50% of patients develop cirrhosis (of the biliary type) and progress to liver failure before adolescence [1, 29]. Immunohistochemical staining for MDR3 is normally canalicular and may be diagnostic in cases when there is complete lack of staining for the protein [30].

Bile acid synthetic disorders are inborn errors of bile acid synthesis and are inherited as an autosomal recessive trait. They can cause life-threatening cholestatic liver disease usually presenting in infancy and progressive neurologic disease presenting later in childhood or in adult life. Sundaram et al. [31] have reviewed the enzyme defects, associated genes and clinical features. Clinically bile acid synthetic disorders often present early in life with conjugated hyperbilirubinemia, raised transaminases and usually normal GGT. The diagnosis is established by fast atom bombardment-mass spectrometry of urine and serum or by demonstrating the specific mutation using genomic DNA sequencing [1]. Liver histopathological features vary and include giant cell transformation of hepatocytes, portal inflammation and (rarely) ductular proliferation with bile plugs [32].

Neonatal giant cell hepatitis

Neonatal giant cell hepatitis (NGCH, also known as idiopathic neonatal hepatitis or idiopathic neonatal giant cell hepatitis) formerly accounted for more than 65% of neonatal and infantile cholestatic diseases [33]. Increased understanding of the pathophysiology of bile synthesis and transport along with advances in molecular genetics has led to the characterization of a subset of liver diseases that present in neonates and infants. As a result, the diagnosis of NGCH has decreased to 15% and is currently a diagnosis of exclusion [1, 33]. Giant cell transformation of hepatocytes is a nonspecific reactive change seen in a variety of cholestatic diseases in children [1, 15]. Histologically, in addition to syncytial hepatic giant cells, variable inflammation and predominantly lobular or canalicular cholestasis are seen. In a recent series, bile ducts appeared hypoplastic in 32% of cases but were not absent or reduced in numbers [34]. 18% of cases showed at least mild focal ductular proliferation and bile duct/ductular cholestasis was found in 7% of cases [34]. Extramedullary hematopoiesis is common [34]. NGCH can be related to hypopituitarism and some cases are associated with autoimmune hemolytic anemia [1].

Conclusion

Liver biopsy plays a pivotal role in the diagnosis of EHBA. Liver histopathology may also provide prognostic information. In cases that turn out not to be EHBA, the pathologist may find clues that point to an alternative diagnosis, again helping to guide the therapy. According to the available literature data, pathologists diagnose EHBA with high sensitivity, high specificity and good interobserver agreement. However, some differential diagnoses cannot be solved just by histology. Adequate clinical information should be available to the pathologist at the time of the biopsy, including the patient's age, the liver enzyme profile (in particular the level of GGT), the serum level of A1AT and any history of prior

TPN. Complex problems like neonatal jaundice can only be managed by an interdisciplinary team and require close collaboration between all members of this team.

Declaration of interest

The Authors declare that there is no conflict of interest.

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