Extrahepatic biliary atresia: current concepts and future directions

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Abstract

Objective: To provide an updated review on extrahepatic biliary atresia, focusing mainly on its etiopathogenesis, diagnosis, treatment and prognosis.

Sources: MEDLINE and PubMed databases were searched using the following keywords: *biliary atresia*, *etiopathogenesis*, *diagnosis*, *treatment*, *prognosis*, *children*.

Summary of the findings: Extrahepatic biliary atresia is the main indication for liver transplantation among pediatric patients. As to its etiology, cytomegalovirus, reovirus and rotavirus have been widely investigated as possible triggers of the immunomediated obstruction of the biliary tree. The immune response, especially the predominant T_H1 and interferon-gamma responses, genetic susceptibility and disorders related to the embryonic development of the biliary tree can play a role in the etiopathogenesis of extrahepatic biliary atresia. Yet today, portoenterostomy is the only available treatment, with better results when performed in the first 2 months of life. As to prognosis, all untreated children eventually die due to complications resulting from portal hypertension and liver cirrhosis, and most treated children have to undergo liver transplantation.

Conclusions: Extrahepatic biliary atresia is still the major indication for pediatric liver transplantation, and to change this scenario some more light should be shed upon the etiopathogenesis of biliary atresia in different disease phenotypes. Future research into the role of interferon-gamma and of other cytokines is necessary in order to assess whether these aspects should be potential targets for therapeutic intervention.

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Introduction

Extrahepatic biliary atresia (EHBA), characterized by obliteration or discontinuity of extrahepatic bile ducts, is still the major cause for liver transplantation among children nowadays.[1] Despite the amount of effort put in by researchers worldwide, surgery – Kasai portoenterostomy and its modifications – is the only available treatment.[2] All untreated children eventually die due to complications resulting from portal hypertension and liver cirrhosis, and most treated children have to undergo liver transplantation.[3]

The exchange and diffusion of information that can make the diagnosis of EHBA easier is of utmost importance, since

prognosis is improved when patients are surgically treated (by portoenterostomy) in the first 2 months of life.

Incidence and classification

EHBA affects neonates and infants, and its incidence is slightly higher in Japan (1:9,600 live births – LB)[4] than in the United States (1:14,000 LB)[5] and in the United Kingdom (1:15,000 LB),[6] with a small female preponderance (1.2:1).[7]

Obstruction of the bile duct lumen can involve any branch of the extrahepatic biliary tree, and the types of atresia are classified according to the site of obstruction,[8] as shown in Table 1.

Based on the period in which atresia occurs, it may be classified as embryonic or fetal and perinatal. The embryonic form accounts for 20% of cases.[9] In this form, the extrahepatic biliary tree might have undergone abnormal morphogenesis and is often associated with non-hepatic structural anomalies.[10] The polysplenia syndrome is the most common anomaly, being found in 8 to 12% of patients with atresia and is characterized by polysplenia/asplenia associated with a midline liver, interruption of the inferior vena cava, preduodenal portal vein, *situs inversus* and/or intestinal malrotation.[9] Other congenital malformations can be observed, such as cardiac anomalies, annular pancreas, immotile cilia syndrome, duodenal atresia, esophageal atresia, polycystic kidney disease, cleft palate and jejunal atresia.[11]

In the perinatal form, bile ducts are patent at birth, but an inflammatory and sclerosing reaction, caused by perinatal injury, results in the obstruction of the biliary tree.[7] This form accounts for 80% of cases of atresia, but it is not usually associated with malformations.[9]

Etiopathogenesis of extrahepatic biliary atresia

In 1885, atresia was reported as an autopsy finding[12] and, despite numerous studies since then, its etiopathogenesis has not been fully determined yet. The application of immunology, genetics, and animal models to study biliary atresia have begun unraveling the contribution of infectious, immune, autoimmune, genetic, epigenetic, vascular and morphogenic processes in the pathophysiology of biliary obstruction.

Infectious processes

The seasonal oscillation in the incidence of atresia, shown by Yoon et al., led to the assumption that atresia could be caused by environmental factors, probably by a virus, in the perinatal period.[5] However, the seasonal pattern of this disease was not confirmed by subsequent studies.[13] Nevertheless, a great deal of effort has been put in the isolation of hepatotropic viruses from children with biliary atresia. The presence of hepatitis B virus was reported in Japan,[14] but not confirmed by Balistreri et al. in the United States.[15] Other pathogens have also been identified in patients with atresia, such as human papillomavirus,[16] respiratory syncytial virus,[17] herpes virus,[18] cytomegalovirus (CMV),[19] reovirus type 3[20] and rotavirus.[21] Of these, the latter three have received greater attention.

With regard to CMV, Tarr et al. assessed 23 patients with biliary atresia using liver histology, serology and culture and found five (24%) CMV-positive patients.[22] Likewise, a Brazilian study detected positive IgM for CMV in 28.5% of patients with EHBA or choledochal cyst.[23] Fischler et al. detected CMV DNA in the liver of 50% of children with atresia whose mothers were CMV-positive.[19] However, these findings were not confirmed by other researchers, who did not find CMV in the biliary remnants of patients with atresia.[24]

Table 1 - Classification of EHBA according to the site of extrahepatic biliary obstruction.

Type	Prevalence	Characteristics
Type 1	~ 5%	Obliteration of the common bile duct, while proximal ducts are patent. The gallbladder usually contains bile.
Type 2	~ 3%	Atresia of hepatic ducts; the gallbladder does not contain bile and the transection of proximal remnants shows two distinct bile duct lumens.
Type 3	> 90%	Atresia involving the right and left hepatic ducts. Obstruction to the level of the *porta hepatis*. Absence of proximal lumens at the *porta hepatis*.

Reovirus was associated with biliary atresia when the virus caused similar signs and symptoms in weaning mice.[25] Later, Morecki et al. reported a high prevalence of positive serologic results for reovirus in patients with atresia,[20] but this finding was not replicated in a subsequent study.[26] The presence of reovirus in liver tissue and bile ducts, detected by polymerase chain reaction (PCR), in patients with atresia or choledochal cysts,[27] provided striking evidence of a potential role of reovirus in the development of atresia.

Similarly to reovirus, rotavirus has been investigated as etiologic agent of EHBA, after group A rotavirus inoculation was found to induce EHBA in newborn mice,[28] and murine models have then been used by several of the currently available studies.[29] However, discrepant results regarding the presence of this virus in infants with atresia were described in other studies.[21,30]

Thus, no study so far has managed to definitively prove the role of a specific virus as etiologic agent of EHBA, or to explain why some viruses, which affect millions of children, cause bile duct injury in only a small percentage of them. One alternate or complementary biological process that may participate in virus-induced injury to the biliary system in the pathogenesis of biliary atresia is immune dysfunction.

Immune dysfunction

The role of immune dysfunction has been based upon the assumption that the biliary epithelium may express inappropriate antigens on its surface that can be recognized by lymphocytes after viral or toxic damage. There could be an immune cascade, which could produce inflammation and biliary fibrosis.[11]

Sokol et al. suggested that, from the molecular standpoint, viral antigens may cross-react with biliary antigens, triggering an immune response against the virus, and also against biliary antigens.[31] Therefore, persistence of immune injury to bile duct cells may lead to disease progression.

The abnormal expression of the human leukocyte antigen (HLA)-DR in the biliary epithelium in patients with atresia is another evidence of participation of the immune process, since its presence suggests that these cells are acting as antigen-presenting cells and directly activating T lymphocytes.[32] Moreover, several authors noticed increased expression of LFA-1, an intercellular adhesion molecule, also known as integrin β_2, in the cells of the inflammatory infiltrate of the portal space, and also increased expression of LFA-1 ligand, ICAM-1, in the endothelium of patients with atresia.[33-35] Elevated ICAM-1 and VCAM-1 levels are associated with advanced liver disease.[36] This body of evidence suggests that adhesion molecules may play a

remarkable role in the inflammatory reaction in biliary atresia, possibly by the retention and activation of circulating leukocytes.

Interestingly, Bezerra et al. showed activation of proinflammatory genes in children with EHBA, with an increase in interferon (IFN)-gamma and osteopontin, which indicates T_H1 response, as well as low levels of expression of immunoglobulin-related genes, pointing to an inhibition of the T_H2 response.[37] A subsequent study in patients with atresia revealed periductular lymphocyte infiltration, with predominance of T_H1 and cytotoxic T lymphocytes.[38]

Shivakumar et al. confirmed the role of IFN-gamma in knockout mice and observed, in the first stage of the study, that the mice did not develop biliary atresia after rhesus rotavirus (RRV) inoculation. Interestingly, the administration of recombinant IFN-gamma into the knock-out mice resulted in the development of bile duct obstruction due to the accumulation of inflammatory cells.[39]

The results of the afore-mentioned studies are consistent with those obtained by Carvalho et al. in a study that assessed a transcriptome of bile ducts in an animal model.[40] These authors demonstrated the predominance of a proinflammatory process, with IFN-gamma activation and sequential expression of a hierarchical network of genes related to this cytokine, showing the predominance of T_H1 response in animals with atresia (Figure 1).

Recently, Feng et al., in an animal experiment, have suggested that biliary obstruction is mediated by the active form of NF-κB.[41] Thus, the immune dysfunction hypothesis suggests that a perinatal or postnatal event, probably a viral infection, may trigger an immunopathological process, which results in fibrosing obstruction of extrahepatic bile ducts, which had been well-formed in the embryonic period. In this case, EHBA would be the final stage of this inflammatory process.

Autoimmunity

The progressive nature of liver injury in patients with atresia, the presence of lymphocytes in the liver and the association with certain types of HLA suggest a possibly autoimmune, persistent attack against the bile ducts.[42]

As to the prevalence of HLA, Silveira et al. found a high prevalence of HLA-B12 and of A9-B5 and A28-B35 haplotypes in children with atresia, especially in those without associated malformations.[43] However, a Spanish study did not show any differences in HLA I and II between atresia patients and healthy children.[44] Quite recently, Yuasa et al. detected the association of HLA-DR2 and of HLA-A24-B52-DR2 haplotypes with perinatal biliary atresia.[45] These results may indicate that one or more genes close to the HLA locus play a role in the pathogenesis of atresia, or that HLA-DR2 on the surface of the
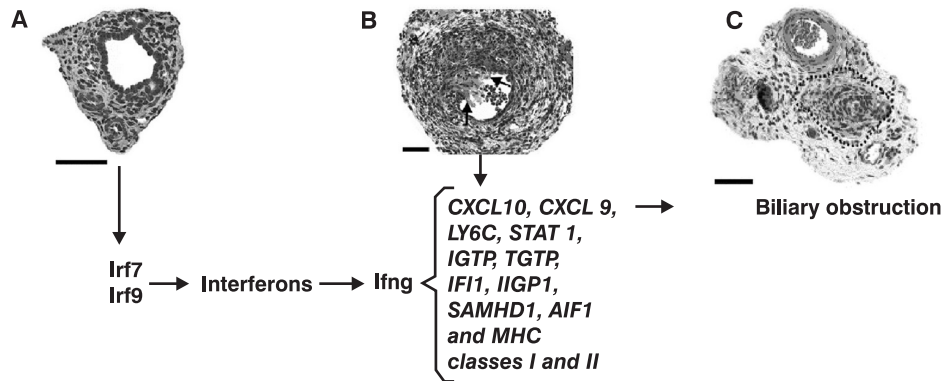
Black bar: 100 μm.
Source: Carvalho et al.,[40] modified.

Figure 1 - Histological cross-sections of extrahepatic bile ducts of rotavirus-infected mice (animal model of biliary atresia), showing initial cholangitis, with presence of discrete inflammatory infiltration in the extrahepatic bile ducts of animals sacrificed at 3 days (A); remarkable inflammatory infiltration that partially obstructed the bile duct lumen, and injury to biliary epithelial cells (black arrows) in animals sacrificed 7 days after infection (B); and bile duct obstruction (dashed circle) by inflammatory cells and extracellular matrix deposition in animals sacrificed at 14 days (C). Similarly to the histological findings, there was a time pattern in gene activation, with elevation of interferon regulatory factors (IRF7 and IRF9) on day 3, followed by increased expression of IFN-gamma and of IFN-induced genes, on day 7

biliary tract can be directly associated with pathophysiological mechanisms of this disease. Furthermore, children with EHBA may develop autoimmune hepatitis in the post-transplant period,[46] which could translate into susceptibility to autoimmune diseases.

Genetics and hepatic morphogenesis

Atresia is not believed to be an inherited disease, but genetic factors might be involved in its pathogenesis. Reports of familial cases[47,48] provide evidence in favor of this hypothesis, although the risk of familial recurrence is extremely low. The behavior among different races also plays a role, since according to an epidemiological study, the incidence of atresia was 5.7 times higher in Polynesia than in metropolitan France.[13] The most widely investigated genes are those related to laterality (*inversin*) and to the development of bile ducts. In this context, the association of embryonic atresia with the polysplenia syndrome shows possible laterality defects during embryogenesis,[49] which motivated studies about the genes involved in the laterality of *inv* mice. In these mice, a spontaneous mutation in the *inversin* gene, on chromosome 4, resulted in total abdominal *situs inversus*, obstructive jaundice and death in the first week of life.[50] The detailed analysis of the hepatobiliary system of *inv* mice revealed extrahepatic biliary obstruction and intrahepatic ductular proliferation.[51] Nevertheless, the lack of inflammation or necrosis in the liver parenchyma of these mice is not compatible with the histological characteristics observed in infants with biliary atresia.

Moreover, the human *inversin* gene was mapped on chromosome 9q, and no mutation in this gene was detected in a case series of patients with biliary atresia and laterality defects.[52]

Another gene that may play a role in EHBA is Jag-1,[53] although its influence on the development of atresia has not been definitively confirmed. Recently, the genetic inactivation of hepatocyte nuclear factors (HNF), such as HNF-1β[54] and HNF6,[55] has caused morphological anomalies in intrahepatic bile ducts and in the gallbladder. HNF-1β was associated with paucity of intrahepatic bile ducts, whereas HNF6 was related to ductal plate malformation and to the presence of intrahepatic cysts,[56] as shown in Table 2.

Altogether, these data suggest that mutations in the genes that regulate hepatobiliary development may play a role in extrahepatic biliary atresia, but the implication of these specific genes in the pathogenesis of atresia in humans remains unclear. Another pending question is how ductal plate malformation may lead to EHBA.

Epigenetic factors

The role of epigenetic factors in the pathogenesis of atresia was assessed by Zhang et al. in a study that investigated a transcriptome of children with either perinatal or embryonic atresia.[57] These authors found an increased expression of genes related to chromatin regulatory factors (SMARCA-1, HDAC3 and RYBP).[57] Because these genes influence epigenetic processes, the authors speculated a potential role for epigenetic factors on the pathogenesis of bile duct obstruction.

Decrease in arterial blood supply to the liver

Association between EHBA and occlusion of portal vein and hepatic artery suggests that an intrauterine ischemic event may exert some influence upon the development of bile ducts, and may play a role in the pathogenesis of atresia.[58,59]

In short, there have been several hypotheses on the etiopathogenesis of EHBA in the literature. Most authors have focused on the bile duct injury triggered by a perinatal insult, probably viral in nature, that is perpetuated by the immune response targeting the biliary tract in patients with genetic susceptibility. Table 3 summarizes the major mechanisms involved in the pathogenesis of biliary atresia, and Figure 2 shows the interaction between these different mechanisms.

Clinical picture

The clinical signs that characterize EHBA are jaundice, acholic stools, choluria and hepatomegaly, which are observed both in the embryonic and perinatal forms. However, the age of onset and the presence of associated symptoms allow for the differentiation between the two clinical forms.

Children with the embryonic form of atresia usually have the onset of jaundice in the first 3 weeks of life. Since physiologic jaundice may precede cholestatic jaundice in this age group, patients usually do not have a jaundice-free period. These patients often have low birth weight, and additional investigation may show association with other malformations.[9] In perinatal atresia, patients have adequate birth weight and appear well but develop light-colored stools

and jaundice between the second and eighth weeks of life. In this stage, stools, which were initially light-colored, become gradually acholic, whereas the urine becomes choluric.[11] It should be highlighted that jaundice may be mild, despite bile duct obstruction. The change in skin color might not be so evident in dark-skinned patients, with only mild scleral jaundice. Since at the time of symptom onset, the child usually has a good health status and adequate weight, mild jaundice is often missed and the diagnosis is established later.

Other signs may be present, such as steatorrhea. As a consequence of reduced fat absorption, the patient may present with malnutrition and signs and symptoms resulting from the deficiency of fat-soluble vitamins, such as hemorrhage, including intracranial hemorrhage due to vitamin K deficiency.[8]

In the most advanced stages of the disease, one can observe splenomegaly, collateral circulation, ascites, upper gastrointestinal bleeding due to the rupture of esophagogastric varices and other signs and symptoms resulting from portal hypertension and from liver cirrhosis.[7]

Diagnosis

The differential diagnosis encompasses a long and heterogeneous list of diseases[61] (Table 4). In many of them, such as atresia, long-term survival and quality of life depend on early treatment. Therefore, neonatal cholestasis can be seen as a pediatric gastroenterological emergency.

In the evaluation of the infant with cholestasis, the first step is to define whether jaundice is secondary to an

Table 2 - Molecular circuits that control the morphogenesis of the biliary system

Gene	IHBD	EHBD	Gallbladder
Jagged/Notch pathway	Abnormal	No findings	No findings
Hes1	No findings	Hypoplasia	Agenesis
HNF6	Ductal plate malformation IH biliary cysts	Abnormal	Agenesis
HNF1β	Rarefaction of small IHBD Dysplasia of large IHBD	Undefined	Abnormal epithelium Dilated cystic duct
Foxf1	Normal	Undefined	Small or absent Without epithelial cells
Foxm1b	Agenesis	Undefined	Undefined

EHBD = extrahepatic bile ducts; IH = intrahepatic; IHBD = intrahepatic bile ducts.
Source: Balistreri et al.[56]

obstructive process. Afterwards, in the spectrum of parenchymal diseases, special attention should be paid to treatable causes (infectious and metabolic). With regard to EHBA, the definitive diagnosis is based on the fibrosing obstruction of the extrahepatic biliary tree during exploratory laparotomy with cholangiography, since no available diagnostic method has a sensitivity and specificity of 100% for the diagnosis of atresia.[7] However, an array of clinical, laboratory, imaging and histological information should be assessed together, in order to select the patients who are going to be submitted to laparotomy.

As expected, from the laboratory standpoint, the patients show an increase in total bilirubin (TB), with predominance of direct bilirubin (DB) or conjugated bilirubin. Nonetheless, interestingly, TB is seldom greater than 12 mg/dL, and may be as low as 5 to 8 mg/dL; and DB is often lower than 8 mg/dL, despite total obstruction of bile ducts.[9] With regard to liver enzymes, the levels of gamma-glutamyl transferase (GGT) and alkaline phosphatase (AF) are higher than hepatocellular enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Special attention should be paid to GGT, since AF is originated in bones.[63] The elevation of bile acids is universal in these patients.[9] Liver function, assessed by albumin and clotting function, is normal in the initial stages of the disease, and hypoalbuminemia and coagulopathy may be observed in patients with end-stage cirrhosis. Importantly, the international normalized ratio (INR) may be abnormal due to vitamin K deficiency.[8]

Echographic examination of gallbladder characteristics showed a sensitivity of 91.9%, specificity of 96.7%, positive predictive value of 89.5%, negative predictive value of 97.5% and accuracy of 95.6% for the diagnosis of atresia.[64] Another important echographic finding is the presence of the triangular cord (Figure 3). By analyzing the presence of this cord in patients with atresia, Tan Kendrick et al. observed a low percentage of false-negatives and no case of false-positives for the diagnosis of atresia,[65] and Park et al. demonstrated high specificity and positive predictive value of 95%.[66] Given that the presence of the triangular cord is highly suggestive of EHBA, Kotb et al. suggested a new algorithm for the diagnosis of patients with neonatal cholestasis.[67] According to this algorithm, patients with the triangular cord sign should be referred to intraoperative cholangiography, whereas those patients in which the triangular cord is not identified should be submitted to liver biopsy.[67] It should be highlighted that the absence of this sign is not sufficient to rule out the diagnosis of EHBA.[11] Echography also plays an important role in the assessment of associated anomalies, such as polysplenia, and of other diagnostic possibilities, such as choledochal cyst. It should be recalled that cysts of the extrahepatic biliary tree may be

Table 3 - Mechanisms implicated in the pathogenesis of biliary atresia

Mechanisms	Evidence
Viral infections	Detection of viruses (CMV, rotavirus, reovirus, among others) in children with biliary atresia. Animal model of atresia induced by rotavirus inoculation in newborn mice.
Immune dysregulation	Increased expression of intercellular adhesion molecules. Increased frequency of HLA-B12, B8, DR3 alleles. Hepatic profile with predominant T_H1 response. Prevention of inflammatory obstruction of bile ducts in IFN-gamma-deficient mice.
Toxins	Associated cases at the same time and in the same region.
Defect in prenatal circulation	Intrauterine devascularization results in extrahepatic bile duct strictures.
Morphogenetic defects	Coexistence of other malformations. Defects in the remodeling of the ductal plate. Mutations in laterality genes (CFC1, ZIC3) in patients with atresia and laterality defects. Epigenetic factors: increased expression of regulatory genes in children with embryonic atresia. *inv* mouse: model or bile duct obstruction and *situs inversus*.

CMV = cytomegalovirus.
Source: Bezerra et al.,[60] modified.
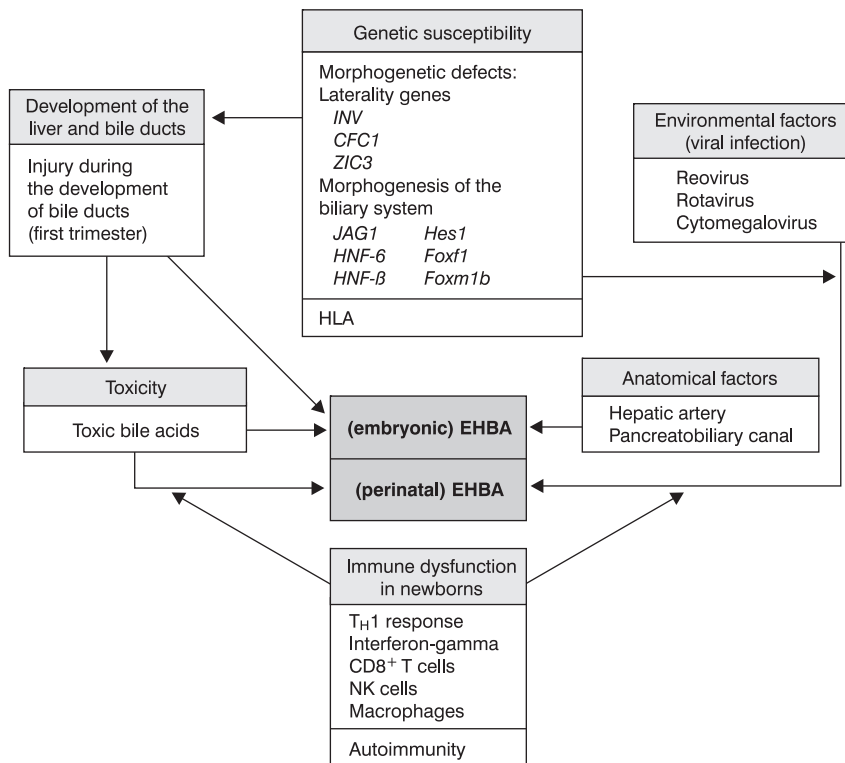
EHBA = extrahepatic biliary atresia; HLA = human leukocyte antigen; NK = natural killer.

Figure 2 - Interactions of probable pathogenic mechanisms of EHBA

observed in 5% of patients with atresia. Some cysts contain mucus, whereas others contain bile.[11] The latter finding may be mistaken for the diagnosis of true choledochal cyst, which may be established with (percutaneous or surgical) cholangiogram.

Technetium-99m diisopropyl iminodiacetic acid (Tc-99m DISIDA) scintigraphy is of limited value. In cases in which a radiotracer is detected in the intestine, one may say that bile ducts are patent, which rules out the possibility of bile duct obstruction. However, failed excretion of the isotope into the intestine, with its urinary elimination, has a specificity of 50 to 75% for the diagnosis of atresia, despite a high sensitivity (95%). This is because cholestatic parenchymal diseases may have the same pattern.[68]

Endoscopic retrograde cholangiopancreatography (ERCP) has been recommended by some services,[69] but it is not performed on a routine basis for the differential diagnosis of neonatal cholestasis, since it requires appropriate material and qualified personnel, in addition to being an invasive and costly exam. Magnetic resonance cholangiography may be useful, especially if the bile ducts are patent.[70] In a study carried out by Norton et al., this exam showed 82% of accuracy, 90% of sensitivity and 77% of specificity for the diagnosis of atresia.[71] As a matter of fact, the roles of ERCP

and of magnetic resonance cholangiography in the diagnosis of atresia have still been under discussion, and laparoscopy combined with intraoperative cholangiography is still recommended for infants with suspected atresia.[72]

Liver biopsy plays a key role in the diagnosis of EHBA. The aspects observed in the histopathological analysis are: expansion of portal spaces, due to ductular proliferation and inflammatory infiltration; bile plugging in bile ductules; formation of portoportal bridges; and giant cell transformation (Figure 4). The major role of histology is actually to define whether there is obstruction or not. In this case, proliferation of bile ducts and the presence of plugging in the ductules are the most specific findings for the diagnosis of atresia. With these parameters, the accuracy, the sensitivity and specificity are 90.5%, 100% and 75.9%, respectively.[73] It should be noted that when the biopsy is performed at an early age, the result can be false-negative, since characteristic findings, especially diffuse ductular proliferation, may appear only after 9 weeks of life. Thus, liver biopsy should be repeated if the patient does not show clinical improvement until the diagnosis is established or if the possibility of atresia is ruled out.[74]

In short, if the biopsy suggests obstruction, laparotomy with operative cholangiography is indicated, since only this

Table 4 - Differential diagnosis of neonatal cholestasis

I. Intrahepatic causes
    Infection-associated cholestasis
        Virus (cytomegalovirus, herpes simplex, hepatitis B virus, HIV, B19 parvovirus, among others)
        Bacteria (urinary tract infection, sepsis, *Listeria*, syphilis, among others)
        Protozoa (toxoplasmosis)
    Metabolic diseases
        Urea cycle disorder (neonatal cholestasis associated with citrin deficiency, arginase deficiency)
        Metal metabolism disorders (neonatal hemochromatosis, non-Wilsonian hepatic copper toxicosis)
        Lipid metabolism disorders (Niemann-Pick type C disease, Wolman disease, cholesterol ester storage disease)
        Carbohydrate metabolism disorders (galactosemia, fructosemia, type 4 glycogenosis)
        Amino acid metabolism disorders (tyrosinemia)
        Mitochondrial hepatopathies
    Hereditary forms of intrahepatic cholestasis
        Membrane transport or secretion disorders
          Deficiency of bile acid transporters – BSEP deficiency (progressive and persistent: PFIC2; benign and recurrent: BRIC2)
          Phospholipid transporter deficiency– MDR3 deficiency (PFIC3)
          Ion transporter deficiency - CFTR (cystic fibrosis)
          FIC1 deficiency (progressive and persistent: PFIC1, and benign and recurrent Byler disease: BRIC1)
          Neonatal ichthyosis– sclerosing cholangitis syndrome
          Arthrogryposis
          Aagenaes syndrome (lymphedema-cholestasis syndrome)
          Alpha-1-antitrypsin deficiency
        Bile acid biosynthesis or conjugation disorders
          3β-hydroxysteroid Δ5-C27 steroid dehydrogenase/isomerase deficiency
          3-oxosteroid 5β-reductase deficiency
          Oxysterol 7 α-hydrolase deficiency
          Familial hypercholanemia
          Secondary deficiencies (peroxisomal disorders: Zellweger syndrome)
        Defects in embryogenesis
          Alagille syndrome (*Jagged 1 gene mutation*)
          Ductal plate malformation (ARPKD, ADPLD, Caroli disease)
        Unclassified
          McCune-Albright syndrome
          Villin functional defect
          Indian childhood cirrhosis
    Endocrine syndromes
        Hypothyroidism
        Panhypopituitarism
    Genetic syndromes
        Down's syndrome
        Other trisomies
        Turner's syndrome
        Zellweger syndrome
    Storage diseases
        Gaucher's disease
    (Toxic) Drugs and toxins
        Endotoxemia, cholestasis associated with parenteral nutrition, chloral hydrate, antibiotics, other drugs
    Hypoxia/hypoperfusion
    Other
        Neonatal lupus, Caroli disease, bile sludge syndrome, histiocytosis X, macrophage activation syndrome (hemophagocytic lymphohistiocytosis)
    Idiopathic
        Idiopathic neonatal hepatitis, nonsyndromic paucity of bile ducts
II. Extrahepatic causes
    Extrahepatic biliary atresia
    Choledochal cyst
    Spontaneous perforation of bile ducts
    Choledocholithiasis
    Neonatal sclerosing cholangitis
    Bile duct stenosis
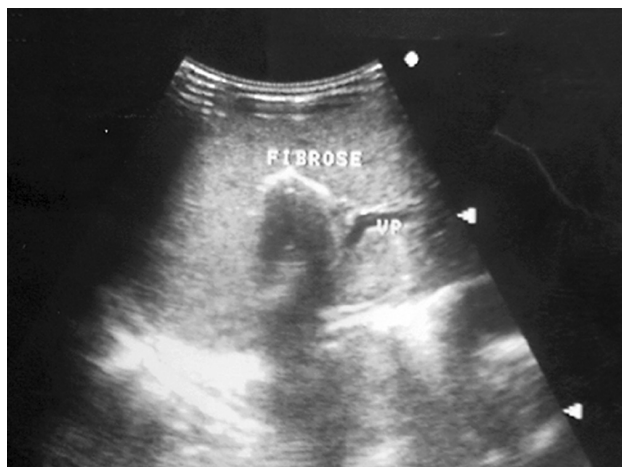   External compression of bile ducts (masses or tumors)

ADPLD = autosomal dominant polycystic liver disease; ARPKD = autosomal recessive polycystic kidney disease; BRIC = benign recurrent intrahepatic cholestasis; BSEP = bile salt export pump; CFTR = cystic fibrosis transmembrane conductance regulator; MDR3 = multidrug resistance 3; PFIC = progressive familial intrahepatic cholestasis.
Source: Balistreri et al.,[62] modified.

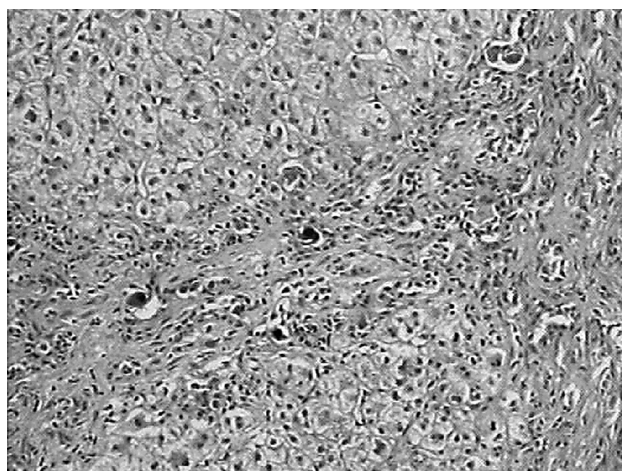**Figure 3** - Triangular cord sign (fibrotic area)

Figure 4 - Liver histology of a patient with biliary atresia showing expansion of portal spaces, due to ductular proliferation and inflammatory infiltration, bile plugging in bile ductules and hepatocellular giant cell transformation

procedure can confirm or rule out the possibility of atresia. In fact, the establishment of diagnosis is still a challenge, and the combined analysis of all information allows for higher accuracy.

Surgical treatment: portoenterostomy

Still today, the only therapeutic alternative for these patients is portoenterostomy, introduced by Kasai & Suzuki in 1959. In this surgical procedure, biliary drainage is established by a Roux-en-Y anastomosis to the hepatic hilum (*porta hepatis*), with a 40 cm^2 loop of duodenum. To obtain a satisfactory bile flow, according to Schweizer et al., it is important that the dissection be performed beyond the bifurcation of the portal vein branches.[75]

The patient's age is a key factor that influences response to portoenterostomy. Satisfactory biliary drainage is observed in up to 80% when the patient is submitted to early portoenterostomy, whereas this rate is around 10 to 20% in infants operated at 4 months of life.[76] In addition to patient's age at portoenterostomy, some postoperative complications may influence outcome in children.

Complications

During the course of the disease, children may present with complications from the disease itself, such as the consequences of chronic cholestasis (steatorrhea, malnutrition, fat-soluble vitamin deficiency, delayed neuropsychomotor development, jaundice, pruritus, portal hypertension and secondary biliary cirrhosis), as well as those related to portoenterostomy (ascending cholangitis),[9] as shown in Figure 5.

After portoenterostomy, the most frequent early complication is ascending cholangitis, and its treatment is crucial for the maintenance of bile flow and for the prognosis of the patient. There appears to be a relationship in which a larger the number of episodes of cholangitis correlates with a higher the risk for sclerosis and loss of intrahepatic bile duct remnants, with consequent progression to liver cirrhosis.[77] Ascending cholangitis occurs in 40 to 60% of surgically treated children, but it is most frequently observed in patients with satisfactory biliary drainage in the first year after surgery. Its pathogenesis is not fully known, but it may encompass bacterial translocation. Moreover, after portoenterostomy, the ampulla of Vater does not act as a barrier against the migration of bacteria. The major etiologic agents are *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumanni* and *Salmonella typhi*.[78,79] Clinically, cholangitis is characterized by the presence of fever, irritability, loss of appetite, vomiting, jaundice, choluria and acholic stools. However, the patient may present with only a sudden onset of jaundice or acholic stools, without other signs or symptoms. Thus, a high suspicion should be present so as to allow for early diagnosis. Obviously, other potential sources of infection, such as urinary tract and respiratory tract infections, should be carefully considered.[80] There is a high frequency of febrile diseases in children (e.g.: those caused by viruses), and bacterial cholangitis may occur during or these infections because of the lack of protective factors normally present in the bile flow. Therefore, it is not always an easy task to determine whether the symptoms are due to an unrelated infection or with bacterial cholangitis. It has been therefore recommended that the combination of fever, lighter color of the stools, jaundice and/or abnormalities in liver enzymes in the first postoperative year should be treated as cholangitis.[9] Furthermore, if after 24 to 48 h of antibiotic therapy, the patient does not show clinical and/or laboratory improvement, the use of corticosteroids, such as methylprednisolone for 5 days[9] should be considered. As to laboratory findings, episodes of cholangitis include
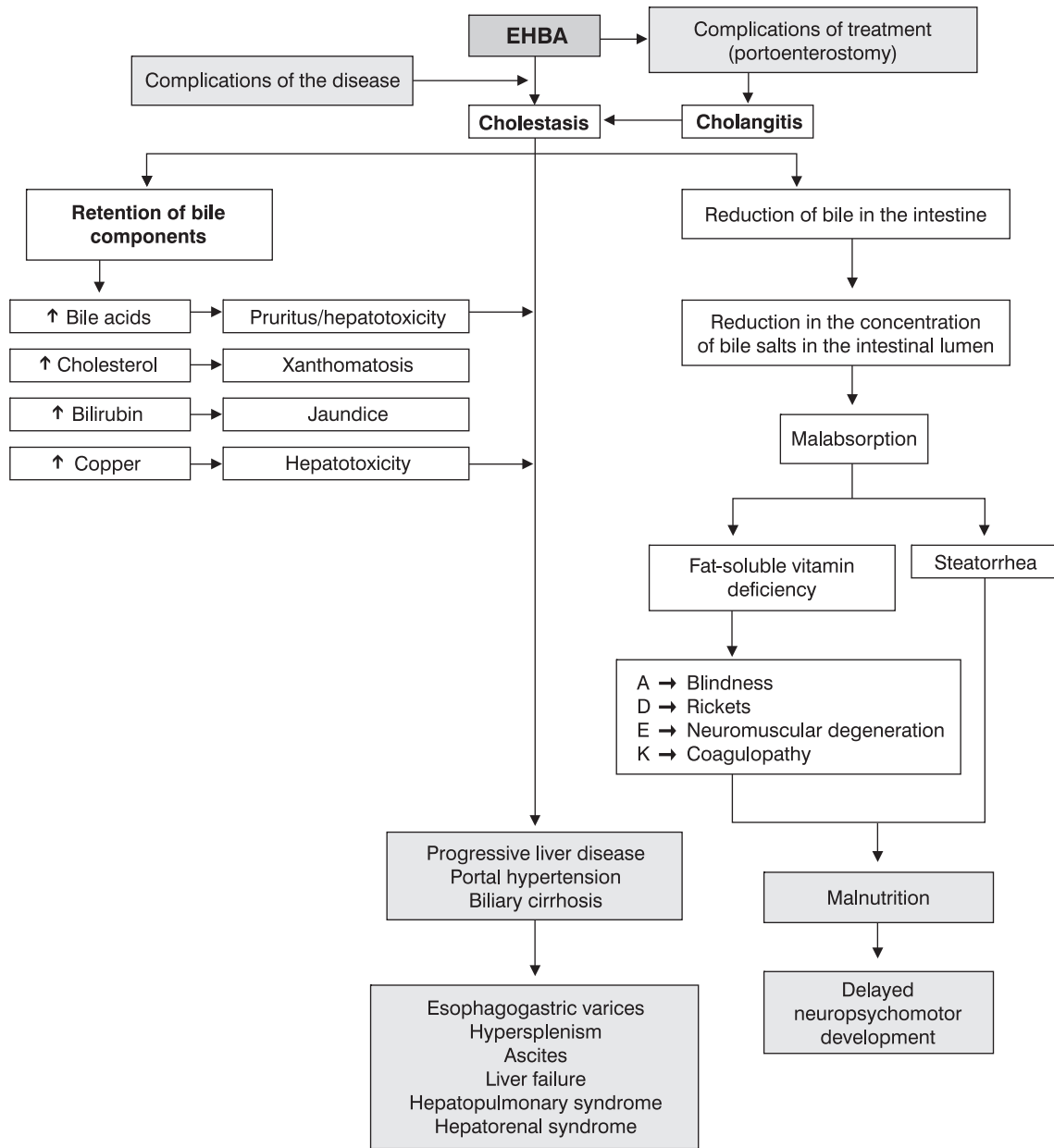
EHBA = extrahepatic biliary atresia.

Figure 5 - Complications of EHBA

leukocytosis, increased levels of DB, aminotransferases, GGT and AF; it should be recalled, however, that after portoenterostomy, the serum levels of these enzymes usually escalate 1 to 5 times above normal values. Consequently, a patient with bacterial cholangitis shows enzyme levels above the previous levels of enzymes for individual patients. Diagnostic confirmation may be obtained by blood culture and liver histology, but treatment should be initiated immediately, often before any of these results are available.[8] Treatment consists of broad-spectrum antibiotic therapy, which is effective against gram-negative bacteria and enteric microorganisms. Empirical treatment with ceftriaxone is

recommended.[80] Sulfamethoxazole and trimethoprim or neomycin are efficient prophylactic agents, with no difference between these drugs.[81] Refractory cholangitis may develop in some patients, in whom multiple parenchymal cysts can be observed, which are correlated with a dismal prognosis.[82] In these cases, the prolonged use of parenterally-administered antibiotics is indicated when signs of infection are observed,[82] and the indication for aspiration or drainage should be assessed.[83]

Portal hypertension is the second most frequent complication of atresia. Its presence depends on the degree

of liver fibrosis at portoenterostomy and on its response to portoenterostomy.[84] In infants without good drainage, the progression of fibrosis is quick. In these cases, varices develop early in the first year of life. Infants with satisfactory biliary drainage may present with fibrosis and portal hypertension, but this usually occurs later.[9] During the course of the disease, the patient may have collateral circulation, splenomegaly, hypersplenism, ascites, spontaneous bacterial peritonitis, upper gastrointestinal bleeding due to the rupture of esophageal and/or gastric varices, hepatic encephalopathy, hepatorenal syndrome, hepatopulmonary syndrome and liver failure.[9]

Delayed psychomotor development is observed in patients who present with progressive chronic liver disease. It is apparently associated with malnutrition, and in order to avoid this undesirable consequence, nutritional support should be maintained and liver transplantation should be indicated before the disease reaches an advanced stage.[85]

Pruritus may be present, although it is not intense as in other diseases, such as progressive familial intrahepatic cholestasis. The most common therapeutic options include ursodeoxycholic acid[86] and rifampicin.[87] As shown in Figure 6, rifampicin increases bilirubin secretion by inducing glucuronyl-transferase (UGT1A1) and bilirubin glucuronide carrier protein (MDR2). Since rifampicin induces CYP3A4, it makes the conversion of hydrophobic bile acids into hydrophilic compounds easier, which then undergo conjugation followed by excretion through MDR3.[62] Ursodeoxycholic acid increases the expression of several carriers, including BSEP, MDR3 and MRP4, which act upon the excretion of bile acids, phospholipids and conjugated organic anions, respectively.[62]

Management of patients after portoenterostomy

After portoenterostomy, the aim of therapeutic measures is to mitigate complications, promoting good nutritional status, stimulating choleresis, preventing infections (cholangitis) and persistent inflammation.

Nutritional therapy

The first step consists of nutritional assessment, considering the triceps skinfold and arm circumference.[89] Of note, weight is not the best parameter in patients with chronic liver diseases, since visceromegaly and ascites may shroud malnutrition.

Maintaining the nutritional status is essential to a good outcome, but it is a challenge, mainly in cholestatic children with progressive liver disease. In these children, macronutrient, micronutrient and fat-soluble vitamin deficiencies should be prevented. Breastfeeding may and should continue after portoenterostomy. For non-breastfed infants and for those who have difficulty gaining appropriate

weight, nutritional therapy includes the use of infant formulas with medium-chain triglycerides. In several cases, nasogastric tube feeding and supplementation with fat-soluble vitamins are necessary.[11]

Ursodeoxycholic acid

Ursodeoxycholic acid is routinely used to promote choleresis, as an attempt to avert fibrosis and progression of liver disease.[86] There are no studies confirming the efficacy of ursodeoxycholic acid, but as it is well-tolerated and offers potential benefits, as shown in Figure 6. Therefore, it is often used in the dose of 10 to 20 mg/kg/day.

Antibiotics

The major indications for antibiotic therapy in the postoperative period are prevention and treatment of ascending cholangitis. No common agreement exists on the best management regarding primary prophylaxis. If the patient receives steroids in the postoperative period, the use of antimicrobials is mandatory because it will also prevent *Pneumocistis carini* infection. Therefore, sulfamethoxazole combined with trimethoprim is recommended by different researchers.[81,90]

Steroids

The remarkable inflammatory process observed at the *porta hepatis* raised the hypothesis that immune mechanisms may play a role in the pathogenesis and progression of the disease after surgery, which motivated studies that assess the use of steroids in an attempt to prevent inflammatory cholangitis; to minimize intrahepatic bile duct injuries; to maintain the bile flow; and to reduce progression to fibrosis.[9]

In a retrospective review, the use of corticoid therapy for 8 to 10 weeks after surgery seemingly improved the outcome, when compared to historical controls.[91] The use of steroids for a short time period, for 1 to 2 weeks after surgery, has also been recommended by some authors.[92] With regard to the dose, Kobayashi et al. have recently stated that high doses of prednisolone are associated with better biliary drainage, since patients were jaundice-free much earlier and remained so for a longer time.[93] However, no randomized controlled trials have been published so far confirming the benefit of steroids in patients with atresia; meanwhile, patients receiving corticosteroids must be carefully followed up in order to avoid side effects, such as excessive irritability, hypertension, opportunistic infections, among others.

Prognosis and liver transplantation

Prognosis depends on the treatment used and on the postoperative outcome. If portoenterostomy is not performed, fibrosis will implacably progress to end-stage cirrhosis and death in the first year of life in 50 to 80% of
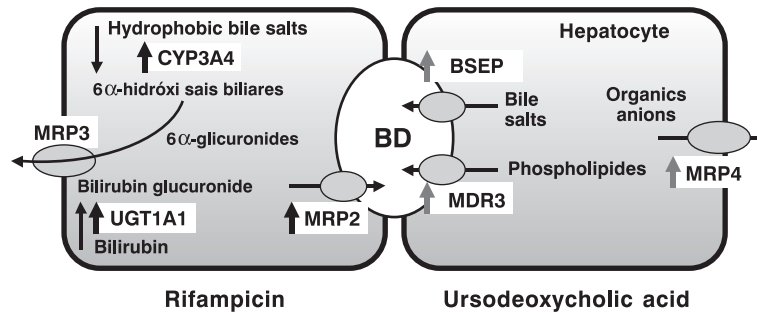
Source: Marschall et al.,[88] modified.
BD = bile ductule; BSEP = bile salt export pump; MDR = multidrug resistance protein; MRP = multidrug-associated protein.

Figure 6 - Effects of rifampicin and ursodeoxycholic acid on hepatobiliary transport and on the enzymes that take part in the metabolism of bile acids and bilirubin

children and up to the third year of life in 90 to 100% of patients.[76,94] Patients submitted to surgical treatment often have one of the following three outcomes: 1) satisfactory response: the patient shows clinical improvement, but mild liver enzyme abnormalities; 2) partial response: the patient shows satisfactory biliary drainage, but presents with progressive liver fibrosis; 3) therapeutic failure: the patient has an outcome that is identical with or worse than that of untreated patients.

Thus, the follow-up of patients should be rigorous, as there may be progression of liver injury to cirrhosis, despite satisfactory biliary drainage.

Factors that influence prognosis are the following: patient's age at the time of surgery; extension of liver fibrosis at surgery; degree of intrahepatic bile duct injury; number of episodes of ascending cholangitis; surgeon's expertise; site of bile duct obstruction; and the type of atresia (embryonic or fetal).[9] As to age, the patients submitted to the Kasai procedure at an early age (< 60 days) have a better prognosis,[79,95] as shown in Table 5.

After the third month of life, Kasai portoenterostomy is still indicated, because even with a lower success rate, the need for liver transplantation can be postponed. Nevertheless, these patients have to be carefully evaluated for portoenterostomy on an individual basis. Preoperative assessment should identify children with advanced liver disease, in whom Kasai portoenterostomy would not yield good results and to whom the delay in liver transplantation would be harmful.[76]

In addition to the influence of age, several studies have aimed to correlate the size of bile duct remnants at the *porta hepatis* with the outcome after portoenterostomy. Chandra & Altman noted better drainage with proximal bile duct remnants greater than 150 µm,[96] which was not demonstrated by other authors.[97] More recently, Baerg et al.

observed that the need of phototherapy in the neonatal period and bile ducts at the *porta hepatis* smaller than 200 µm are associated with the necessity for liver transplantation after portoenterostomy.[98]

Table 5 - Correlation between patient's age and percentage of biliary drainage after portoenterostomy

Patient's age at portoenterostomy (days)	Biliary drainage after portoenterostomy (%)
< 60	70-80
60-90	40-50
90-120	25
> 120	10-20

Source: Sokol et al.[9]

Still with regard to intrahepatic bile ducts, as the disease is progressive, a study examined the liver in detail and found out that unoperated patients with biliary atresia present with progressive intrahepatic paucity of bile ducts and that this aspect is variable among those submitted to surgery. After Kasai portoenterostomy, liver histology is not necessarily homogeneous, and two regions may be observed: a perihilar, regenerative, non-cirrhotic region (segment 4), with bile ducts alongside the artery in the portal space; and a peripheral, cirrhotic region with paucity of bile ducts. It has been postulated that survival after Kasai portoenterostomy depends upon the anatomical extension of the area with perihilar hyperplasia and upon the capacity of this region to

maintain liver function in the presence of progression to cirrhosis in more peripheral areas.[99]

As far as the site of biliary tree obstruction is concerned, patients with proximal patent bile ducts and distal obstruction (type I atresia) have a better prognosis than those with proximal atresia extending into the *porta hepatis*.[100,101]

Patients with embryonic atresia seem to have a worse prognosis when compared to those with the perinatal form of the disease.[95] The unsatisfactory outcome of children submitted to Kasai portoenterostomy at an age less than 30 days probably reflects the different pathogenesis of embryonic or fetal atresia.[102] Agenesis of bile ducts, which possibly results from primary agenesis of the hepatic diverticulum, is rare and requires liver transplantation, even before portoenterostomy.[103]

In terms of predictive factors, TB levels in the postoperative period are an excellent predictor of long-term survival.[104] Levels below 1.0 mg/dL, within 3 months after the surgery, correlate with good prognosis, and the necessity for future transplantation is unlikely.[105] This finding has a significant practical value, since it may help identify patients who need more intensive drug therapy and nutritional support during the progression of the disease.

Despite the great advances in pediatric hepatology in the last few years, only 11% of adolescents and young adults with atresia submitted to portoenterostomy show minimal signs of chronic liver disease, and are therefore regarded as "cured."[3] Between 70 to 80% of children with EHBA need liver transplantation in their first 2 decades of life,[94] which makes it the major indication for transplantation among pediatric patients, accounting for 50% of transplantations performed in children.[1] No other disease, even among adults, accounts for such a high indication for transplantation.

The timing for transplantation and the patient's nutritional status influence the post-transplant outcome. Improvements in transplantation techniques and the appropriate referral of patients allow for a striking increase in the survival rate.[106] Currently, the long-term survival of transplant recipients with atresia corresponds to 80 to 90%.[9] If, on the one hand, treatments for atresia have not been greatly improved, on the other hand, liver transplantation has become an effective treatment for pediatric patients[106] due to improvements in surgical techniques and in immunosuppressive regimens.

Referral of patients to specialized centers

The aspects described here highlight the importance of early referral of these children to specialized centers, but this does not usually happen. The possibility of physiologic or breastmilk jaundice may hinder and delay diagnosis, except if bilirubin levels are measured in order to detect direct hyperbilirubinemia.[11] Even in industrialized countries, 14 to 29% of patients with biliary atresia are referred for assessment only after the third month of life.[76,79] Therefore, every patient with jaundice should be assessed after 14 days of life,[107] since at this age, the diagnosis of physiologic jaundice can be totally ruled out. It should be underscored that the color of stools and urine must be considered when examining a jaundiced infant.

New perspectives

New perspectives on treatment are mainly based upon the role of immune dysfunction in biliary atresia, which is still not fully understood, even though it has been the object of numerous studies. There is a necessity for future research into interferon-gamma, other cytokines, and regulatory T cells, which inhibit the immune response mediated by effector T cells (CD4$^+$CD25$^+$ cells provide contact-dependent immunosuppression; T_H3 cells release TGF-β; and T_R1 cells produce IL-10),[108] using both animal models and humans, in order to assess whether these aspects could be potential targets for therapeutic intervention.

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