Abstract

Objective: To emphasize the importance of precocious diagnosis of extrahepatic biliary atresia and its direct relationship with the surgical re-establishment of the biliary flow before the second month of life. To discuss several complementary methods with the aim of selecting the ones that present better evidence, and avoiding delays in diagnosis and worse prognostic.

Source of data: Bibliographical researching regarding the period of 1985-2001, in Medline and MdConsult, using the following key words: neo-natal cholestasis; extrahepatic biliary atresia; neo-natal hepatitis. National and foreign articles were also selected based on the bibliography of consulted publications, and when necessary, for better understanding of the theme, opinions emitted in theses and textbooks were referred.

Summary of the findings: The revision of the consulted bibliography led to the assumption that early diagnosis of EHBA and surgical treatment to reestablish the biliary flow up to 60 days of life are fundamental in order to achieve good results. Among several complementary methods of diagnosis, cholangiography by MR, US and the hepatic biopsy are the ones that provide the largest success indexes.

Conclusions: The referring of patients bearers of EHBA to centers of references in Brazil, is still made tardily, probably due to lack of enlightenment of the doctors of primary attention, allied to bureaucratic and technological difficulties. The experience in England in relation to the “Yellow Alert” program, allowed that the number of children referred to surgical treatment before the 60 days of life increased significantly. Among the complementary methods, the MR cholangiography, ultrasonography and hepatic biopsy should be used, depending on the technological resources of the diagnosis units.


Extra-hepatic biliary atresia:
diagnostic methods

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Introduction

The clinical expression of extrahepatic biliary atresia (EHBA) is that of a cholestatic jaundice, caused by perinatal inflammatory processes originating in the biliary ducts, causing progressive sclerosis and obstructions even of the inter-hepatic biliary tree. In the United States, around 400 to 600 cases of EHBA occur annually and, despite all of the advances in the understanding of this infirmity, such as cholangiography by magnetic resonance imaging and sequential mass spectrometry, non-surgical confirmation of diagnosis remains a challenge.

In children, cholestasis is basically observed during the neonatal period, with 70% to 80% of cases corresponding to EHBA or neonatal (NH). At King’s College Hospital (London), over a period of 10 years (1989-1999), around 998 children were assessed due to cholestasis, of whom 22% had EHBA and 44.4% idiopathic NH. Other important causes of cholestasis were deficiencies of a-1 antitrypsin (8.1%), Alagille syndrome (4.5%), choledochal cysts (2.4%), related to total parenteral nutrition (2.3%) and progressive familial intra-hepatic cholestasis, Byler syndrome (2%).

Depending upon the geographical region being studied, statistical data related to the etiology of cholestasis may be different, as, for example, in South Africa where syphilis is responsible for 22% of cases.

Extrahepatic biliary atresia occurs in approximately 1:10,000-1:15,000 live births, with a slight predominance among the female sex (1.4:1). Despite being observed on all continents, it is more prevalent among Asians, and in the USA affects the Afro-American population more frequently, among whom the incidence is almost twice that among Caucasians. Approximately 10% to 20% of children carrying EHBA present associated congenital defects, such as situs inversus, ventricular or atrial septal defects, absent inferior vena cava, a pre-duodenal portal vein, intestinal malrotation, polysplenia or asplenia, among others. Chromosome anomalies are found in 1% to 2% of EHBA cases, being observed frequently in trisomy 13 and 18.

Depending upon the absence or presence of associated malformations, EHBA is subdivided into post-natal and embryonic forms. The first, in which atresia is isolated, is more common, and the second is associated with other malformations.

The fact that the disease rarely affects pre-term newborns and is discordant with monoytotic twins may reinforce the impression that is an infectious, ischemic, toxic or metabolic agent active at end of gestation, but, at present, there is no single factor implicated in the etiology of EHBA. The hypothesis that NH and EHBA were a single disease, in different phases of evolution has been ventured, however this has never been confirmed.

Clinical manifestations

Extrahepatic Biliary Atresia whenever jaundice with cholestatic characteristics continues for more than 14 days after birth. Signs are often evident between the second and sixth weeks of life, for which reason babies which are jaundiced after this age should undergo laboratory analysis, particularly serum fractionated bilirubin assay. In the event that conjugated bilirubin is found to be above 2 mg/dl (34.2 µmol/l) or 20% of total bilirubin, rapid investigation and/or referral to tertiary centers of excellence is indicated, for definitive diagnosis and choice of treatment. This is because good results with EHBA, as well as for other treatable causes, are associated with early intervention. The Kasai portoenterostomy, for example, if performed by the 60th day of life, produces around 80% of good results, but these levels are considerably reduced by a treatment delay of only 10 days.
Campion et al., retrospectively analyzing 21 cases of EHBA, found that despite the existence of signs of cholestasis at 12 days of life, only three children were submitted for surgery by the 45th day. Around eight children assessed for the first time on or about the 21st day of life, due to presenting only one cholestasis indicator, were not submitted for any type of laboratory analysis on the occasion of this consultation.¹³

Babies with EHBA, in the majority of cases, presented birth weight appropriate for gestational age and did not initially present weight gain detriment. The primary clinical symptoms are jaundice, choluria, acholic stools and hepatomegaly. At onset, jaundice is mild, in proportion to direct serum bilirubin levels, which are found at around 6 mg/dl (102.5 µmol/l), and is frequently assumed to be physiologic jaundice. In general, total bilirubin levels are between 6 and 12 mg/dl (102.5 µmol/l and 205.2 µmol/l), with the conjugated fraction being 50% to 60% of the total. According to Fitzgerald, when the conjugated bilirubin level is persistently lower than 4 mg/dl (68.5 µmol/l), the absence of total obstruction of the biliary ducts can be confirmed.¹⁴

Choluria is a frequent aspect, but it may not be easily perceived because of the increased urinary output of this age group, diluting the biliary material. The coloration of stools is an important element of diagnosis, since, in cases of total obstruction, they should not contain pigments which could turn them yellow or green. In 30% of EHBA cases, pigmented stools may persist for some weeks after birth.⁹ Hepatomegaly may be present at birth, or may become evident with evolution. The increase in firmness of the liver occurs progressively, and it is very often difficult to assess this clinical parameter during the first weeks of life.

The clinical form of EHBA is not always that which is habitually observed; five cases are described as associated with meconium peritonitis, in which a diagnosis of atresia was not made during surgery for the intestinal perforation.¹⁵

**Diagnostic approach**

In the presence of cholestasis during the first weeks of life, the investigation of four clinical symptoms is important when considering the existence of EHBA, they are: birth weight, observation of stool color for 10 consecutive days, the time when stool discoloration began, the clinical aspect of hepatomegaly. When normal birth weight, signs of the progression of cholestasis, a hardened liver and early discoloration of stools which becomes permanent are found, a diagnosis of extrahepatic biliary obstruction is suggested.¹⁶ In the face of such findings, a diagnostic investigation geared towards EHBA is obligatory and must be rapid. With this objective, Lai et al. formulated a rapid investigation protocol in which liver biopsy, coloration and duodenal fluid peak radioactivity, hepatobiliary ultrasound and persistent acholic stools were used as parameters for the separation of EHBA cases from NH ones. This approach produced the correct diagnosis in 96.8%, although four NH cases (3.17%) were subjected to unnecessary surgery.¹⁷

Other methods used in the diagnosis are varied, with preferences depending upon the investigating group and in the face of the technological reality of the different services.

**Auxiliary examinations**

**Laboratory tests**

When investigating a child with icterus, functional analysis tests, such as albumin and bilirubin, are evaluated in conjunction with exams known as hepatic lesion markers, such as aminotransferases, alkaline phosphatase and gamma glutamyl transferase. As time is an important factor in EHBA prognosis, the examinations indicated should be performed as part of a wide-ranging approach, with the intention of ruling out other etiologic possibilities. Thus congenital infection serology is indicated (TORCHS), sweat chloride assay and protein electrophoresis, in which a normal a1 fraction practically excludes an a1-antitrypsin deficiency.

Radiography is used in the diagnosis of EHBA as a differentiating element, since, in the presence of defined parameters we can clarify the etiology of cholestasis. Alagille syndrome may be suspected if a vertebral image showing “butterfly wing” is seen, while dextrocardia and situs inversus are commonly found with biliary atresia. Congenital toxoplasmosis, or cytomegalovirus may cause cerebral calcification, and, in syphilis, we find symptoms of periostitis and osteochondritis to be highly indicative.

In a laboratory investigation aimed specifically at the diagnosis of EHBA, Fitzgerald suggests that the analysis of alkaline phosphatase (AP) and of gamma glutamyl transferase (GGTP) provides information suggestive of obstructive disease, and that allied with other secondary methods permit a high rate of correct EHBA diagnosis.

Alkaline phosphatase is produced by the epithelial cells of the bile ducts and serum levels are increased in cases of extra-hepatic obstruction, cholangitis and intrahepatic cholestasis. Since AP is also produced in the bones, associated bone conditions may cause difficulties in the interpretation of results. In such a situation a 5’-nucleotidase assay, together with serum concentrations of calcium and phosphorous can reduce doubts.

Gamma glutamyl transferase is a protein of the biliary capillaries, which, in cholestasis cases presents elevated serum values. In the case of high AP levels and GGTP above 600 U/l, biliary atresia or another obstructive duct lesion, primary duct reduction syndrome, or even a1-
antitrypsin deficiency would be the main diagnostic candidates.

In cases of normal serum values for AP with GGTP below 100 U/l, a diagnosis of progressive familial intrahepatic cholestasis, or of an innate error of biliary acid synthesis is possible. When the results for AP and GGTP are low, it is probable that a primary hepatocellular disease is present, such as idiopathic neonatal hepatitis.14

Retrospectively analyzing GGTP values used for differentiation between EHBA and NH performed before ten weeks, Liu et al. observed that serum levels were elevated significantly more in EHBA cases (622.5 ± 211.9 U/l with EHBA and 168.8 ± 100.3 U/l with NH). Using a value of 300 U/l of GGTP as a divider between diagnoses, the authors obtained an 85% level of accuracy for the diagnosis of EHBA and, when the criterion used was serial GGTP assessment, an increase of 6U/l/day permitted correct diagnosis in 88% of cases.18

Ultrasound (US)
A rapid, non-invasive investigative method, and, when performed by a well-trained professional, provides excellent results. It is extremely useful in the diagnosis of choledochal cysts and also in verifying the absence of the gallbladder, which may suggest a diagnosis of EHBA, although it is not a reliable symptom. Nevertheless, if changes in gallbladder volume occur post-feeding in serial US analysis EHBA can be ruled out. Despite difficulties in identification due to its small volume, the contractility of the gallbladder in atresia sufferers can be observed in 19% to 22% of cases due to a patent bile duct.

The improvement in US specialists and the improved definitions of the images produced by late-generation apparatus now permits the identification of a triangular cord.21 Tan Kendrick et al., assessing 60 children, whose final diagnosis was EHBA, with no false positive cases.22 In Brazil, at the UFMG university hospital, the triangular cord signal as an EHBA diagnostic element achieved a sensitivity of 62.5% and specificity of 100%.23

False negative triangular cord results may occur in some EHBA cases due to hepatic radicles, such as hypoplastic or aplastic ducts or fibrous hepatic ducts, even at early stages. In cases of non-visualization, Kotb et al. recommend the performance of a liver biopsy.24 The authors are unanimous in stating that, for precise interpretation of the triangular cord, the ultrasonography professional must be experienced and an enthusiast of this method.

Duodenal intubation
A simple, little used method which, when negative for bile, suggests an obstructive process. A nasogastric tube is put into the distal portion of the duodenum and the liquid collected for 24 hours. If no bile fluid is seen the test is prolonged for a further 24 hours. The administration of magnesium sulfate at 25%, with a dosage of 1 ml/kg,25 or cholecystokinin, endovenously, can be performed when biliary fluids are negative, 24 hours after the collection of the duodenal liquid.26

In a recent publication (2001), the duodenal tube test as a diagnostic instrument for EHBA showed a sensitivity level of 97.3%, and specificity of 93.7%, a positive predictive value of 92.3% and a negative predictive value of 98.5%. The authors of this study indicated operative cholangiography in cases of duodenal tube test results negative for bile.27

The tube test is also used in investigation of the radioactivity of gastroduodenal juices, after the performance of scintigraphy using DISIDA Tc99m, with the intention of increasing the specificity of this test. With this methodology, Jaw et al. found large peaks of radioactivity, i.e. over 1,500 cpm/100mcl/mCi dose in NH cases, while, in EHBA cases, the maximum activity found was below 500 cpm/100mcl/mCi dose.28

Scintigraphy with Tc99m
The most frequently used radioisotope is DISIDA Tc99m (Tc99m linked to 2-6- diisopropyl), which has a very short half-life, low gamma ray emissions, very good concentration in the liver, non-conjugated excretion in the bile and a low renal excretion level. Literature refers to false positive/negative levels of 10%. The BRIDA Tc99m isotope (Tc99m linked to 2.4.6-trimethyl-3-bromo) is also recommended, offering the advantage that 98% of the dose administered is eliminated by the liver, while with DISIDA hepatic elimination is 85%.29 During the week before the examination, the patient should receive phenobarbital at a dosage of 5 to 10 mg/kg/day, associated or not with cholestyramine, at a dosage of 0.25 mg/kg/day. Phenobarbital is a potent microsomial enzyme inducer and increases bile flow facilitating the excretion of conjugated bilirubin. It is necessary to fast for 2 to 4 hours before the procedure.

The DISIDA Tc99m test is not recommended when conjugated bilirubin levels are over 20 mg/dl (342 μmol/l). In such cases BRIDA Tc99m should be employed, since, even with high levels of bilirubin, it maintains hepatic
Liver biopsy

Although there are no pathognomonic aspects in the histological analysis of the hepatic material collected by percutaneous or wedge biopsy, the sum of findings provides an important supplement for the formulation of a definitive diagnosis. By means of a histopathological analysis of liver biopsies performed upon 78 children with cholestasis, Zerbini attempted to identify parameters which could differentiate extrahepatic cholestasis from intrahepatic. The following were described as findings suggestive of extrahepatic cholestasis: portal duct proliferation, cholestasis in newly formed ducts, pronounced canalicular cholestasis, biliary thrombi in the portal area and accentuated portal and peritubal fibrosis. Discrete or absent ductule proliferation and the non-existence of portal fibrosis would rule out the possibility of extrahepatic cholestasis.31 It is important to emphasize that a liver biopsy performed before the fourth week may not demonstrate proliferation of ductules or fibrosis, commonly observed in biliary atresia cases. In such situations a second biopsy some time later is recommended.32

In Mowat’s sample, the main histologic findings indicative of abnormalities of the biliary tract were: widening of the portal space; numerous bile ducts; elongated, distorted and angular bile ducts; lymphedema; inflammatory infiltration and growth of the fibrosis in the portal space. When the disease is of a hepatocellular nature, the main elements found in histopathology were infiltration of mononuclear cells in the hepatic lobules, lipophanerosis and unequal hepacyte coloration.9 Santos et al. evaluated histopathological exams of 46 children with cholestasis, and found portal and periportal ductal proliferation, portal cholestasis in neoducts and porto-portal bridges to be factors indicative of EHBA.35 At the Universidade de São Paulo, the infant surgery group observed that liver biopsy offered a reliability level greater than 95% for diagnostic confirmation of EHBA, even abandoning radiological investigation of bile ducts during surgery.34

At the best investigative centers, liver biopsies offer sensitivity and specificity of 85% to 95%.26,35 It is important to refer to the fact that ductal proliferation is also seen in galactosemia and α-1-antitrypsin deficiency, although of a lesser intensity than is observed with EHBA.

Electronic microscopy of histologic cuts of the liver show that in EHBA cases inflammatory abnormalities and structural derangements predominate in the portal space, whereas with NH hepatocytic lesions predominate.36 An immunohistochemical study revealed a malformation of the ductal plate, remaining in its fetal configuration, in 38% of EHBA cases. This finding is considered a sign of a bad prognosis.37

Cholangiography

If, after the investigations described, there is still doubt about the possibility of EHBA, the performance of cholangiography is indicated. The type of cholangiography to be realized will depend upon the resources available at the diagnosing unit, which themselves should be capable of performing the surgery indicated in EHBA cases.

Retrograde Endoscopic Cholangiography (REC)

The development of modern, latest generation infant duodenoscopes, with optical fibers has made REC a diagnosis method with a low level of complication. After the application of the radiological contrast into the papilla of Vater, we can observe whether or not there is progression through the bile and pancreatic ducts. If there is not normal progression, we may observe three types of image: (a) bile duct not seen; (b) distal common bile duct and gallbladder seen, no sight of the principal hepatic duct; (c) opacity of the distal common bile duct, gallbladder and principal segment of the hepatic duct, with bile lakes at the porta hepatitis.26

Cholangiography by magnetic resonance imaging (CMR)

CMR is used as an important instrument in differential diagnosis of EHBA and NH in developed countries. According to services which employ this method, CMR should be incorporated as a routine procedure in cases of neonatal cholestasis.

With the objective of excluding the diagnosis of EHBA, Jaw et al. assessed 16 children with jaundice and 10 controls, between three days and five months old, using CMR. The Results show a 100% level of correct diagnosis. The results presented 100% of correct diagnosis. The gallbladder was identified as were the common bile duct and the common hepatic duct in the 10 controls and 10 jaundiced children, of whom nine were proven NH carriers and one presented intrahepatic bile duct paucity. The remaining six jaundiced patients presented EHBA. All of the jaundiced patients were also submitted for scintigraphy (99mTc-DISOFENIN), wherein four of the 10 patients without obstructions had
false positive results. Peng et al., using the same technique (CMR), assessed 15 patients (newborns and infants) with cholestasis, being six children with EHBA and nine with NH. The researchers observed only false positive EHBA diagnosis.  

**Intraoperative Cholangiography (IC)**

Intraoperative cholangiography is performed when other propaedeutic methods do not permit a definitive diagnosis. As patients with intrahepatic cholestasis may have their condition aggravated by anesthetic products, hemodynamic alterations and infections, the investigation which precedes IC should be as thorough as possible, in attempt to achieve a non-invasive diagnosis.

Intraoperative cholangiography should be performed at a medical center which is capable of performing the Kasai portoenterostomy immediately if necessary. Since IC will be feasible in only 17% to 25% of EHBA cases because of the impossibility of introducing the contrast medium by way of the gallbladder, it is indispensable that the pediatric surgeon be sufficiently experienced to confirm the existence or absence of pervious structures for the flow of bile from the liver to the duodenum.

**Diagnosis confirmed**

Once a EHBA diagnosis has been confirmed, the following conduct is indicated: (a) the Kasai portoenterostomy is the first surgical treatment indicated; (b) liver transplant is indicated in cases of Kasai portoenterostomy failure; (c) the liver transplant should be delayed by as long as possible, with the intention of allowing for maximum patient growth; (d) the liver transplant should not be performed until the occurrence of severe aggravation of the cholestasis, hepatocellular decompensation or severe portal hypertension; (e) multiple attempts to correct an unsuccessful portoenterostomy are not recommended, since the performance of the transplant becomes more difficult and dangerous.

**“Yellow Alert”**

This was a campaign begun in England in 1993, to clarify to primary care doctors the importance of etiologic investigation of jaundice in two week old babies. Jaundice beyond the 15th day of life is observed in 15% of the children, according to research carried out by Kelly and Santon. In the group of 1,170 children evaluated, no significant hepatic disease was highlighted. Despite criticisms of the costs involved in this investigation, “Yellow Alert” enabled the proportion of children submitted for surgical treatment before the passage of the first 60 days of life to grow from 15%, in 1974, to 60% in 1995. A French national study assessing the prognosis of EHBA in the period between 1986 and 1996, showed that timely treatment, anatomical conditions favorable to the flow of bile and the absence of associated malformations were indications of good results, which could then be further improved when surgery was performed at centers with experience in the procedures indicated.

In Brazil, in research at university hospitals in the cities of Salvador, São Paulo and Porto Alegre, only 10% of cases were submitted for surgery before the 60th day of life.

**Conclusions**

It is accepted that the Kasai portoenterostomy, when performed before the 60th day of life offers good results, i.e. reestablishes bile flow in 80% of cases, permitting, in this manner, patient growth and, if necessary, a later liver transplant. Nevertheless, in Brazilian university hospitals, only 10% of cases were referred for surgery before the 60th week of life. The program known as “Yellow Alert”, instituting in England in 1993, permitted that the proportion of children referred for early surgery, before 60 days of life had passed, increased by 45%. It is probable that the lack of clarification for primary care doctors, allied to bureaucratic and technological difficulties, contributes to late referral to tertiary care centers. Similar clarification campaigns should be stimulated in our locale.

Among the many different auxiliary methods of diagnosing EHBA, US is indispensable as it is a non-invasive method, in which the identification of the “triangular cord” displays good sensitivity (85%) and specificity (100%). Another examination which deserves to be highlighted, in view of its high right of correct EHBA diagnosis is cholangiography by RM, which should certainly be incorporated into investigation protocols.

Within the Brazilian reality, liver biopsy remains a valuable diagnostic technique, accessible in major cities. A histological finding of portal and periportal ductal proliferation, cholestasis in newly formed ducts, biliary thrombi in portal areas and portal fibrosis are highly suggestive of EHBA, providing a reliability of more than 95%.
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