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Early Screening and Diagnosis of Biliary atresia among Newborn

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Abstract: Biliary atresia (BA) is the main cause of obstructive jaundice in the newborn, and it's defined as an obliterative disorder of the intra and extrahepatic biliary tree dependent on an inflammatory-destructive process of unknown etiology. Research looking into the metabolomics profile of infants with biliary atresia has identified a different profile as compared to other children with neonatal cholestasis. This is not currently in clinical practice but may be a useful investigation in the future. Biliary atresia is the most common cause of pediatric end-stage liver disease and the leading indication for pediatric liver transplantation. Affected infants exhibit evidence of biliary obstruction within the first few weeks after birth. Early diagnosis and successful surgical drainage of bile are associated with greater survival with the child's native liver. Unfortunately, because noncholestatic jaundice is extremely common in early infancy, it is difficult to identify the rare infant with cholestatic jaundice who has biliary atresia. Hence, the need for timely diagnosis of this disease warrants a discussion of the feasibility of screening for biliary atresia to improve outcomes. Published analyses indicate that newborn screening for biliary atresia by using serum bilirubin concentrations or stool color cards is potentially life-saving and cost-effective. Further studies are necessary to evaluate the feasibility, effectiveness, and costs of potential screening strategies for early identification of biliary atresia.

Keywords: *biliary atresia, screening, diagnosis*

Introduction.

Biliary atresia (BA) is the main cause of obstructive jaundice in the newborn, and it's defined as an obliterative disorder of the intra and extrahepatic biliary tree dependent on an inflammatory-destructive process of unknown etiology [1]. The atresia of the biliary tract begins in the embryonic/perinatal period and has a variability in the atretic processes from case to case. It is fatal if left untreated with a reported survival of less than 10% at 3 years of age [2]. The condition is recognized as one of the most rapidly progressive liver diseases known to man. It is the leading cause of liver-related death in children and the foremost indication for liver transplantation in the pediatric population [3]. BA clinically manifests in the first few weeks of life with jaundice

and acholic pale stools, the prototypical clinical features of an obstructive-type jaundice, associated with the biochemical hallmark of serum conjugated (direct) hyperbilirubinemia [4].

The current standard of care for BA is sequential surgery with an initial Kasai hepato-portoenterostomy (KPE), in which the obstructed bile duct is resected and a loop of the small bowel is brought to the porta hepatis of the liver to restore bile flow, followed by liver transplantation for those in whom the KP fails or who progress to cirrhosis and liver failure at a later pediatric age or into adulthood. Without any surgical intervention, all infants with BA will die by three years of age [5].

Epidemiology

BA is a worldwide disease affecting multiple ethnicities. In a recent comprehensive review, the incidence of BA was shown to range widely among countries reporting population-based data, from approximately 1:5000 newborns in Taiwan to 1:20,000 in Europe, Canada, and areas in the USA. The highest rates (1:3500) were reported from French Polynesia [6, 7]. Although these reports suggest variability in biliary atresia prevalence globally, this could be due to differences in diagnostic practice or surveillance methods or to race/ethnic or genetic factors, environment, or infectious pathogens [7].

Etiology

The etiology of biliary atresia is unknown. Theories suggest a multitude of etiological and causative factors that are both genetic and acquired. Since about 3% to 20% of children with biliary atresia have some associated syndrome or another congenital abnormality, and as biliary atresia is more common in certain geographic regions, it is likely that some genetic component is present in the pathogenesis of the disease although no single etiology has been found so far. Only a few familial cases are described and no increase in the incidence has been noted in the case of twins [8]. The extrahepatic bile ducts first become visible as an out-pouching of the foregut at 20 days of gestation, and the intrahepatic bile ducts become visible at 45 days, which form from the primitive hepatocytes. Porta-hepatis is the place of the interface between the extra and intrahepatic bile ducts, and the successful union is crucial for the development of the patent biliary system. The non-syndromic isolated type of biliary atresia might result from faulty remodeling in fetal life at the hepatic hilum. This is supported by the fact that there are similarities in the cytokeratin staining of the bile ducts in patients with biliary atresia and first-trimester fetal bile ducts strengthening the possibility that biliary atresia could occur due to the failure of the bile duct remodeling at the hepatic hilum with the persistence of fetal bile ducts [8, 9]. Other theories favor a possible acquired, inflammatory, and infectious cause for the pathogenesis of the disease. Rotavirus and reovirus type 3 [10]. There have also been studies that show immune-related damage to the ductules of patients with biliary atresia due to an increase in the expression of intercellular adhesion molecule (ICAM)-1 in the bile ductules. Another an acquired etiology with seasonal clustering of the cases especially in the winter months and also because 50% of the children with the disease have pigmented stools earlier in life which then later became clay-colored [11].

Classifications

Biliary atresia is not a single disease that results from a specific etiology, but rather it is a phenotype that results from different etiologies. It is broadly classified as syndromic and non-syndromic isolated varieties. [12] grouped specific entities of biliary atresia based on the similarities they share [12].

Biliary Atresia Splenic Malformation Syndrome (BASM)

Biliary atresia is associated with polysplenia, vascular anomalies including a pre-duodenal portal vein, interrupted vena cava, azygous continuation, cardiac malformation, malrotation, and situs inversus. In this type, malformation occurs early in embryogenesis and accounts for the other anomalies. Maternal diabetes seems to play a role, and there is a female predominance [13].

Cystic Biliary Atresia

In this type, there is the obliteration of the biliary system with cystic dilatation. The incidence is reported to be around 10%, and it carries a better prognosis [14].

Cytomegalovirus (CMV) IgM Positive Biliary Atresia

This type represents about 10% of the cases, and most of them are non-Whites. These children are present with higher bilirubin and aspartate aminotransferase (AST) levels and more inflammatory infiltrates in the extrahepatic biliary apparatus on histology. This group has the worst prognosis [15].

Isolated Biliary Atresia

This represents the largest group, but the etiology is unknown [16].

Japanese Biliary Atresia Society (JBAS) Classification

In this classification, there are three basic types depending on the site of extrahepatic bile duct obstruction and do not include intrahepatic bile duct obstruction. In 1976, [17] published a new classification of BA [18]. He stated that most of the cases of BA are due to the obstruction of extrahepatic bile ducts. Cholestasis and liver damage are secondary effects of extrahepatic bile duct obstruction. There are some cases that might have intrahepatic obstruction but since the JBAS did not have sufficient data to accurately identify this type of obstruction, they focused only on the extrahepatic bile duct obstruction at that time [17]. In addition to the basic types, the detailed forms are classified according to the histological findings on examination of the lower bile duct and the hilar bile ducts. The above three factors (the state of the common bile duct, the state of the common hepatic duct, and the state of the ducts at the porta hepatis) can be combined into more than 80 types, but some of them include unlikely combinations. There are over 50 combinations that can be realized even if the unlikely combinations are excluded, but they can be easily expressed by combining the three factors, and, conversely, it is possible to easily imagine the morphology of the extrahepatic bile ducts from that simple expression. He states that this is an advantage of this classification [17, 19].

Investigations

Blood Investigations

Typical biochemical variables are shown in Table II.

Table I: The typical biochemistry of a child with biliary atresia [20].

	Typical concentration at presentation	Normal range
Conjugated bilirubin (μmol/L)	>100	<20
Alkaline phosphatase (IU/L)	>600	<500
γ-Glutamyl transferase (IU/L)	>100	20–40
Aspartate aminotransferase (U/L)	80–200	15–40
Alanine aminotransferase (U/L)	80–200	10–55
Albumin (g/L)	Normal at presentation	37–56
Prothrombin time (seconds)	Normal at presentation	9–13

The conjugated bilirubin is typically >100 μmol/L; however, lower levels can be seen and should not be falsely reassuring.

With the commencement of ursodeoxycholic acid and adequate nutrition, the bilirubin may fall, but this is not sustained and should not be falsely reassuring [21]. Synthetic function (albumin and prothrombin time) is usually normal at presentation unless there is vitamin K deficiency or a late presentation with cirrhosis. Cholesterol may be raised, but triglycerides are usually normal [20].

Bile Investigations

The Japanese and Chinese have reported continuous attempts to aspirate bile from the third part of the duodenum using a nasoduodenal tube. If there are no bile secretions over a 24 h period, then this is strongly suggestive of biliary atresia [22, 23].

Metabolomics

Research looking into the metabolomics profile of infants with biliary atresia has identified a different profile as compared to other children with neonatal cholestasis. This is not currently in clinical practice but may be a useful investigation in the future [24].

Imaging

a) Ultrasonography

Ultrasonography (US) is usually the next step. This typically shows absence of biliary tract dilatation with lack of display of the gallbladder. One feature that has been suggested as specific is the so-called "triangular cord sign" illustrating the cone-shaped periportal fibrous mass cranial to the bifurcation of the portal vein [25] (Figure I).

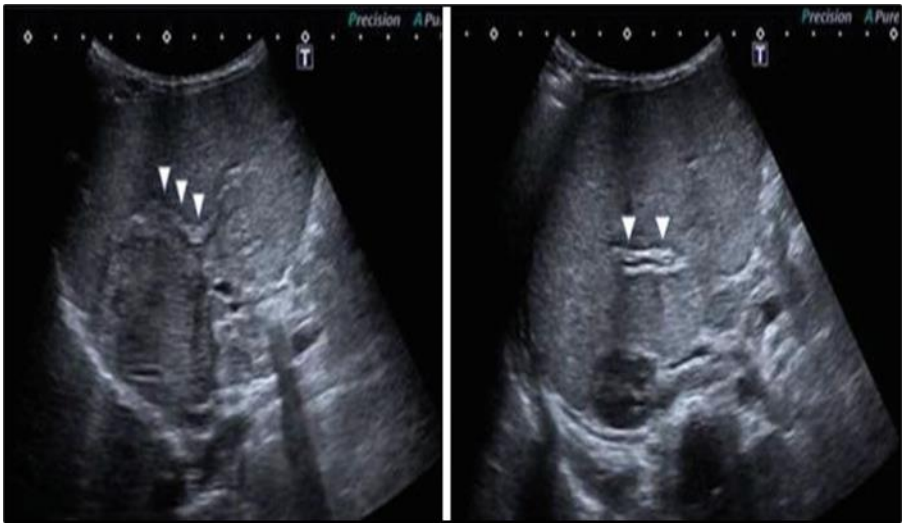

Figure I: Triangular cord sign: hyperechoic area, tube-shaped, anterior to the porta hepatis (arrowheads) representing the fibrotic residual of the biliary tree [1].

There is no single pathognomonic preoperative finding of BA, but reasonable suspicion necessitates progression to more invasive tests. In our practice, percutaneous liver biopsy is always performed after exclusion of medical causes of cholestatic jaundice (e.g., α-1 antitrypsin deficiency, Alagille syndrome) [1, 26] (Figure III). Ultrasonography and histology establish the diagnosis accurately in more of 85% of cases of BA. Key histological features include bile duct proliferation, a small cell infiltrate, portal fibrosis, and absence of sinusoidal fibrosis [27].

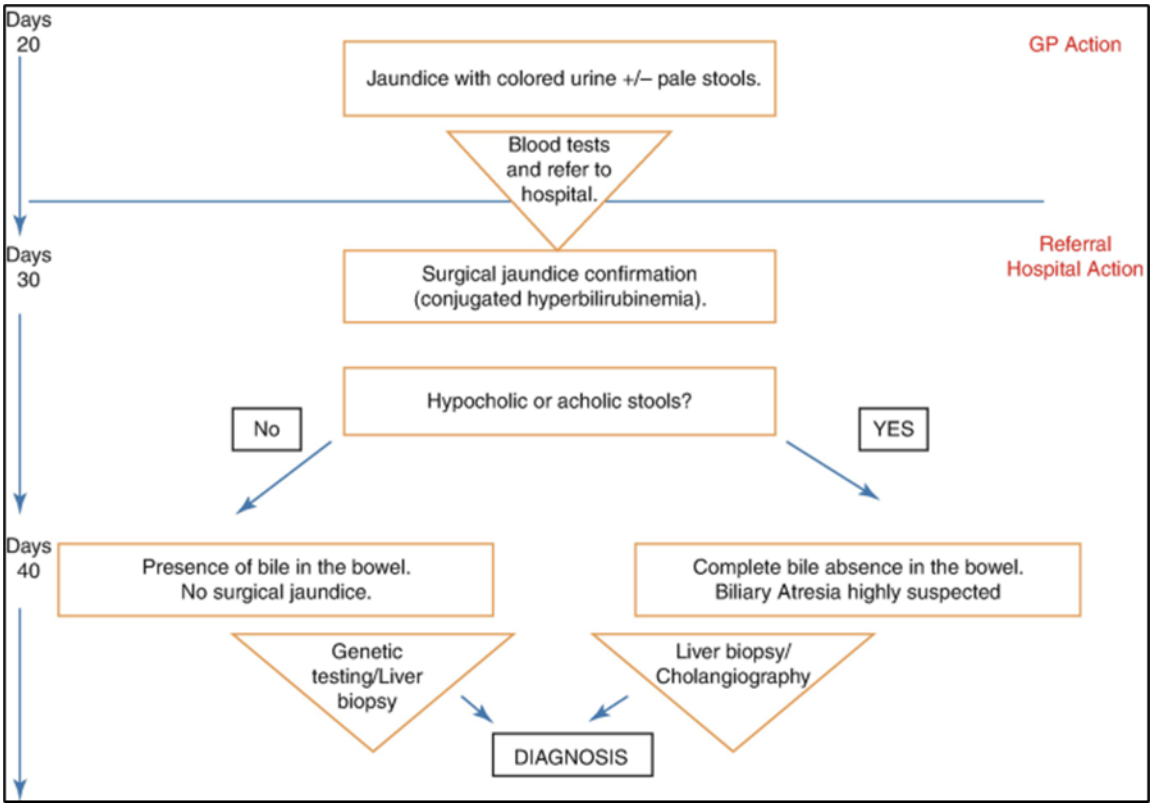

Figure II: Flowchart showing a timely and correct approach to the patient with suspected biliary atresia [1].

b) Radionucleotide excretion scan
Use code with caution.
It is useful if there is uncertainty of stool colour. Historically it was a standard test for bile excretion when biliary atresia was suspected; however, if the stool is visually pale, then it adds little to the diagnostic investigations. The injected isotope is taken up by the liver, but the usual excretion pattern into the intestine is not seen in biliary atresia by 24 h [20]. However, in infants with severe cholestasis from any cause, there may be no excretion within 24 h either. This scan is a sensitive test for biliary atresia, but it is not specific. To aid uptake of the radionucleotide, infants should be given phenobarbitone for 3 days prior to the scan; in a recent study, priming with phenobarbitone or ursodeoxycholic acid, however, did not change the excretion pattern of the test compared with no drug augmentation [28].

c) Endoscopic retrograde cholangiopancreatography (ERCP):
Use code with caution.
It can be used to visualise the biliary tract if diagnosis is uncertain. It is safe and feasible in young infants and may avoid an operative cholangiogram [29].

d) Magnetic resonance cholangiopancreatography (MRCP):
Use code with caution.
Currently this technology may not be detailed enough to identify the luminal patency of infant biliary tract that may only be 1 mm in diameter. It may evolve with technical advances to become a useful test [26].

e) Operative cholangiogram:

This is the gold standard and definitive test. Dye is injected into the biliary tree under direct vision at laparotomy or laparoscopically, and the patency of the bile ducts is assessed [30].

Liver Histology

A percutaneous liver biopsy provides information regarding extrahepatic biliary obstruction. Typically the findings are those of: [20]

The findings occur in any large duct obstruction and are not specific for biliary atresia; however, it may be useful for cases in which there is diagnostic uncertainty to exclude other causes of jaundice. The histological features also develop over time and may not be typical if sampled early [31]. Although histological confirmation prior to operative cholangiogram may be desirable, an infant with jaundice and pale stools with no other diagnosis will always require this definitive test. A liver biopsy is typically taken at the time of Kasai to provide information on the extent of fibrosis [32].

Management

Medication

The infant should receive fat-soluble vitamin supplementation and ursodeoxycholic acid before surgery and until clearance of jaundice following the operation. Fat-soluble vitamins are absorbed along with the long-chain fats which require bile salt micelles. In biliary atresia vitamin deficiency is possible as bile is not present in the intestine. Table III provides recommended initial drug doses for vitamins and ursodeoxycholic acid.

Nutrition

Poor absorption of long-chain triglycerides (LCT) occurs due to reduced or absent bile salt micelles. A change of feed from primarily LCT to one containing 60–65% medium-chain triglycerides (MCT) enable absorption of fats without the need for bile salt micelles. This means that the infant will absorb more calories and weight gain will be more effective. For those infants who are breast-fed, an MCT supplement can be provided alongside the breast-feeding which should be continued if possible. If an infant is not feeding well, then early instigation of nocturnal nasogastric tube feeding will enable good nutrition to continue [33].

Peri-Operative Cholangiogram

Peri-operative cholangiogram will definitively diagnose biliary atresia by the failure of passage of dye into the intrahepatic and extrahepatic biliary system [34].

Surgery

Without a Kasai portoenterostomy and liver transplant, if necessary, biliary atresia is fatal within the first 2 years of life. A successful Kasai is defined as normalisation of bilirubin levels within 6 months of the procedure. The success of the procedure is dependent on the age at operation, extent of liver damage (fibrosis at time of Kasai, ongoing inflammation and episodes of cholangitis) and experience of the centre. Those infants who have concomitant CMV infection have a poorer outcome with reduced clearance of jaundice, native liver survival and increased mortality [35, 36]. Fibrosis and cirrhosis are more likely to develop with long-standing obstruction; hence, the hypothesis that the earlier the Kasai, the better the outcome. This is indeed true for those with biliary atresia splenic malformation (BASM) or rarer forms of biliary atresia. However, in isolated biliary atresia cases, even having a Kasai at 100 days can lead to a 45% 5-year survival with the native liver [20]. A UK study of 93 cases of biliary atresia in 15 centres showed a significant difference in success rate in those centres that operated on five or more cases each year (61% vs 14% 5-year survival with native liver) and led to centralisation of the service [37]. Re-auditing after 4 years showed a 51% 4-year survival with the native liver and 89% survival overall. Ten years following centralization, the median age at Kasai was 54 days with 55%

undergoing a successful Kasai. Survival with the native liver at 5 years was 46% and 40% at 10 years. The overall patient survival however continued to be 89% at 10 years [38]. A systematic review of 153 infants showed that those who received post-operative steroids had an improvement in clearance of jaundice. This, however, has not been seen in other smaller studies [12].

Pre-operative: [39]

Correct coagulopathy (vitamin K).

About 5–10 of patients has associated cardiac anomalies that may need correction before KPE.

In most cases, an attempt to preserve native liver using portoenterostomy is a better strategy than primary liver transplantation. However, latter should be considered in "old" infants (>100 days), especially those with obvious cirrhosis (ascites, portal hypertension).
- Operative: [40]
1. Confirm diagnosis ± cholangiogram.
2. Porta hepatis dissection—facilitated by extra-abdominal delivery of liver.
3. Excision of all extrahepatic remnants to the level of liver capsule, facilitated by retraction of portal vein confluence. Clearance proceeds from bifurcation of the right vascular pedicle to insertion of umbilical vein on left portal vein.
4. Roux loop (~40 cm) reconstruction and portoenterostomy (6/0 PDS).

Liver Transplantation
Liver transplantation is an option offered if liver cirrhosis is far advanced or if a Kasai porto-enterostomy has failed. Role of Liver Transplantation is discussed in next section [41].

Complications

Ascending cholangitis is the most frequent complication after KPE, especially in the first postoperative year, and is probably due to the restoration of direct communications between intrahepatic bile ducts and the small bowel. Clinical presentation of cholangitis is with fever, jaundice, and abdominal pain. Acholic stool and deterioration in liver function tests should also be present [20]. Early diagnosis is very important to prevent the loss of remaining patent bile ducts and to preserve the native liver function. In patients unresponsive to antimicrobial treatment a percutaneous liver biopsy may be cultured to identify the causative organism, but this is uncommonly required. Cholangitis should be treated aggressively with intravenous antibiotics against Gram-negative organisms [30]. A prophylactic regimen with oral antibiotics, such as amoxicillin, trimethoprim, and cefalexin, might be considered in all children who have undergone KPE in order to prevent cholangitis in the first months after the operation. In cases of children with recurrent cholangitis, following clearance of jaundice, liver scintigraphy may detect a Roux-loop obstruction. This is important, as it is surgically correctable [1]. Portal hypertension (PH) and esophageal varices are two serious complications after KPE, and they are due to progressive liver fibrosis causing sustained elevation of portal venous pressure. Progressive hepatosplenomegaly, gastrointestinal bleeding, ascites, encephalopathy, and hepatopulmonary syndrome may all be signs of PH (Figure V). Among adult survivors with native liver, the incidence of PH varies from 50% to 90% [42].

Screening and Stool Color Card System

It has been reported that the Kasai portoenterostomy, performed at an early stage of BA, improves BA prognosis. Therefore, some strategies have been attempted for early detection of BA [43]. In order not to delay the time of KP, it is necessary to make a diagnosis as early as possible, and neonatal / infant "screening" for BA has been proposed. According to the World Health Organization [44], screening is defined as the presumptive identification of unrecognized disease in an apparently healthy, asymptomatic population by means of tests, examinations, or other procedures that can be applied rapidly and easily to the target population [45]. At the present time, no specific biomarkers exist for the diagnosis of BA. The screenings mainly use clinical features, such as pale-pigmented stool and product materials generated from pathophysiologies, such as high serum bilirubin and bile acid levels. However, these symptoms and laboratory findings are not specific to BA but are also found in cholestatic liver diseases. Thus, early detection screening for BA is only possible for cholestatic liver diseases, including BA [46]. Detection of BA using stool color card has been demonstrated to be effective and inexpensive. The strategy of early detection by SCC and followed by early Kasai portoenterostomy showed a significant improvement in the long-term native liver survival rate [47].

Stool Color Card Screening

First stool color card screening was launched in the Tochigi Prefecture in Japan in 1994. The stool color card has seven scales of stool color to help caregivers evaluate their children's stool color. As stool color gets darker, the number of stool color increases as well. Stool colors number 1 to 3 are defined as abnormal, indicating acholic stool. Colors 4 to 7 are defined as normal. After the 1-month health checkup, the completed card with stool color number by caregivers was mailed to the public health center. Cases with the suspected acholic stool were referred for further examination [48]. During 12 years between 1994 and 2011, a total of 313,230 infants were screened, and 34 patients were diagnosed with BA. Twenty-six out of 34 patients were identified by screening. The sensitivity and specificity at the 1-month checkup were 76.5% (95% CI 62.2–90.7) and 99.9% (95% CI 99.9–100). The mean age at the time of KP was 59.7 ± 19.4 days, which was earlier than that before the introduction of stool color card screening (70.3 days, 1987–1992). At the same period, JBAR reported that the percentage of age at the time of KP after 80 days was 25.3%, even though Tochigi's data was 11.8% ($P < 0.05$). The survival rate with native liver for the 34 patients was 87.6%, 76.9%, and 48.5% (at 5, 10, and 15 years after KP, respectively) and was better than that of historical controls. Stool color card screening, thereafter, expanded to 16 other administrative districts in Japan (Figure VI) [49]. [50] also found the reducing trend of the age of BA diagnosis has been started before this program launching in 2004. They concluded that earlier diagnosis and operation for BA were not entirely due to the stool color card screening alone; however, the stool color card has the potential benefit of increasing BA awareness in both caregivers and physicians. The liver transplant rate in three year-ranges was 32.7, 41.0, and 29.6%, respectively, and were 25.6% among those patients who received KP within 60 days of age and 32.3% over 60 days of age [50]. In reference to Taiwan's good results using stool color card screening for BA, Argentina [51], Switzerland [52], and other countries implemented similar regional or nationwide stool color card screening programs. Swiss stool color card screening pilot study was started in 2009. Seven different colors of photographs (4 normal and 3 abnormal) are printed on the card. The cards were handed out to the caregivers after their child's birth by the attending pediatrician or midwife. The card and the baby's stool color are checked at 4 weeks of age. Home-based screening program in British Columbia in Canada was performed on 87,583 patients between 2014 and 2016. During the period of the program, six patients were identified as BA. Five in six were received this program; however, only three received KP within 60 days. The other two patients were identified as acholic stools by their families, but their general practitioners did not give further tests until late referral. In general, early KP is considered as one of the factors of good prognosis of BA [53]. In a Canadian cohort study, the native liver survival of the patients who received KP before 30 days of age had better through 10 years of observation [54].

In a French cohort study, KP within 30 days of age brought higher 5-year native liver survival. Thus, earlier KP has been advocated to be performed around 30 days of age [55]. Stool color card screening resulted in early detection of BA and fast KP but could not find any BA patients before 30 days of life. The stool color card is a simple and inexpensive screening method. In areas where BA has not been screened, the introduction of the stool color card has been reported for its effectiveness of early diagnosis of BA and early KP and improved survival in native livers. However, aiming to detect BA at an earlier stage to improve prognosis, only the pale stool as a result of advanced bile duct obstruction might be somewhat difficult to achieve [46]. Despite the referring physicians' unfamiliarity with the SCC and the mothers' relatively low education level at our center; SCC proved to be a simple, efficient, highly sensitive, specific, and applicable method for early diagnosis of BA. Therefore, SCC screening might increase motherś (as well as physicianś) awareness of BA, and it is recommend that it be more publicized and used as a mass neonatal screening tool in low/middle-income countries such as Egypt [56].

Serum Direct or Conjugated Bilirubin

Measurement of increasing direct or conjugated bilirubin is a most logical way for detecting BA by considering its basic pathology of intra and extrahepatic bile ducts, which are gradually obstructing after birth [57]. In 2003, [58] measured conjugated bilirubin in neonates younger than 28 days of life in the United Kingdom. A total of 30,079 neonates were tested, and 23,415 samples (84.7%) could be analyzed. The remainders were inappropriate samples because of hemolysis (8.6%) and insufficient volume. Eleven patients were diagnosed with liver disease, and two of them had BA. The sensitivity, specificity, and a positive predictive value of this test were 100%, 99.59%, and 10.3%, respectively. They concluded that serum conjugated bilirubin concentration might be a reliable marker for neonatal liver disease but not very accurate for detecting BA [58]. [59] reported that 34 BA patients had elevated serum direct or conjugated bilirubin within the first 96 hours of age in 2011 [59]. In 2016, [59] initiated a prospective study using two-step screening with direct or conjugated bilirubin. They screened a total of 11,636 newborns at 4 Houston hospitals. One hundred and twenty-one infants were considered positive by screening in test 1 within less than 60 hours of life. The measurement of direct or conjugated bilirubin was repeated in test 2 at 2 to 3 weeks of age, and 11 infants continued to have positive test results. The 2 infants were diagnosed with BA and had positive results in both tests. Thus, sensitivity, specificity, and positive predictive value were 100%, 99.9%, and 18.2%, respectively [60]. They expanded their evaluation of the two-step BA screening strategy and investigated 124,385 newborns at 14 south Texas hospitals in 2020. There were 1354 infants with positive screening results in test 1 within 60 hours of age. After test 2, 119 infants continued to have positive results. 7 out of 119 infants were finally diagnosed with BA. This test had a sensitivity of 100%, a specificity of 99.9%, and a positive predictive value of 5.9% [61]. Serum direct or conjugated bilirubin measurements are widely available and inexpensive. This measurement requires to draw blood because the concentration of serum direct or conjugated bilirubin, which has photosensitivity, is not measured with a dried blood spot. Serum direct or conjugated bilirubin screening appears to result in a feasible strategy for earlier detection and treatment of BA within 30 days of age [61].

Serum Bile Acid Measurement

Serum bile acid (SBA) has also been elevated with BA and cholestatic disease in neonates. SBA is a more stable material and be able to be measured using the dried blood samples collected in the Guthrie test. [62] reported the possibility of SBA for BA screening. They compared cholestatic hepatobiliary disease patients (61 BA, 52 Idiopathic neonatal hepatitis, 17 Alagille syndrome, 15 α antitrypsin deficiency, 32 other diseases) and 708 normal newborns. The level of SB in BA was surely elevated. However, the sensitivity and specificity were 78.7% and 96.3%, respectively [62]. In 2012, [63] investigated SBA in 8 BA patients, 17 neonatal jaundice, and 292 normal infants at 3–4 days of age. Elevation of SBS in BA infants at 3–4 days of age might suggest the early

occurrence of the bile duct obstruction around birth. Both the sensitivity and specificity were lower than expected 79.1% and 62.5%, respectively. Samples of SBA are easier to obtain and deliver than those of direct bilirubin. SBA screening has potential for screening of BA, but so far is not acceptable for BA screening with inadequate specificity [63].

Urinary Sulfated Bile Acid

Bile is synthesized in liver cells and discharged into the bile duct. In case of bile obstruction, as seen in BA or other cholestatic hepatobiliary diseases, water-soluble sulfated bile acid synthesized from bile reflux into the serum and then secreted in the urine as urinary sulfated bile acid (USBA). Compared to the blood tests, USBA has less invasion of sampling and the advantage of safe handling of the samples. Urine samples were collected in small plastic bottles by the caregivers at 14–30 days of age and kept frozen at −4 degree Celsius until USBA assay. Method of measurement was performed by the automated analysis with the direct enzymatic assay. USBA value was corrected with urine creatinine in consideration of immaturity of renal function. This test is commercially available at a cost of US$5 [64]. Subsequently, [65] measured both serum and urine bile acid levels at various times in BA patients and showed that they were correlated. BA and cholestatic hepatobiliary diseases screening with USBA launched in Okinawa and Nagasaki Prefecture in Japan and continues to the present. In Nagasaki Prefecture, a total of 54,840 infants were screened with USBA between 2010 and 2018. This number represents about 60% of the number of births in the region. Elevated USBA levels were found in 1187 infants (2.16%) in the initial examination, and subsequent blood tests indicated that cholestatic liver disease was suspected in 56 infants. During the period, nine cases of BA have been diagnosed, and seven of them received USBA examination. Unfortunately, one out of seven patients were determined to be normal on the first USBA test. Later, blood test suspected BA because of prolonged jaundice, and the patients underwent KP at 111 days of age. This case was tested at 11 days of age, earlier than the protocol for USBA examination, suggesting that early neonatal USBA testing might be less likely to produce abnormal results than serum direct bilirubin testing [65]. According to the 2018 JBAR report, preoperative USBA measurement was performed in 27 BA patients, and two of them were false-negative with normal values. Although the timing of the USBA tests in those two cases is not clear, the occurrence of false negatives is major problem for screening, so the timing of the USBA tests needs to be discussed in the future [66].

Modified Color Card Screening

At present, the stool color card is the most widely used screening and seems to be superior in terms of economy and repeatability, but the problem is that it is a subjective judgment. In the mosaic stool mixed with light and a dark portion or the odd color stool not matched to the stool color card, sometimes it is difficult to classified to normal or abnormal stool [49]. [67] verified the reliability of pale stool identification among pediatric professionals. They prepared three colors (five normal, three indeterminate, and four acholic stool) which were photographed by the digital camera with computer calibration for ambient light. The pediatric professionals included 36 pediatric doctors and 45 pediatric nurses in three different hospitals. Suspected BA stools were correctly identified in 62.8% (doctors; 62.7%, nurses; 62.9%) and failed to recognize in 37.2%. The results showed that even experienced professionals could not recognize stool color associated with BA [67]. Two years' study in Canada, the color card was revised from 6 colors (3 normal and 3 abnormal) to 9 colors (3 normal and 6 abnormal) before starting the second year because of missing a BA patient by stool photo failure. In the Initial stool color card, only the color was printed. Currently, widely used color cards have not only the color but also the shape of the stool and help with judgment [68]. In the 2018 JBAR, 58 out of 152 BA patients (38%) at before 1 month old, 53 out of 151 patients (35%) at 1 month old, and 17 out of 131 patients (12.8%) after 1 month old declared to be having normal stool color before BA diagnosis. These stool colors judged to be normal belong to the lightest normal stool color. The difference in color between the lightest normal stool color

and the darkest abnormal stool color is subtle, and it is a true challenge for any professionals. However, there would still be a significant number of BA patients in the lightest normal stool color group [66]. Between 2015 and 2016, caregivers were recruited to interview their infants' stool colors at the 1-month regular health checkup. A total of 2028 interviews were divided into two groups by age at examination. The mean ages at the examination for stool color were 14 ± 2 and 31 ± 4 days of age, respectively. The proportion of infants with the lightest normal stool color were 62.8% and 52.6%, respectively. Stool color seems to be darker with growth, although half of the caregivers recognized their infants' stool color belongs to the lightest normal group at 1 month of age. Nobody rules out the possibility that this lightest normal group includes patients with BA [46]. Recently, several researchers have attempted to objectively judge the stool color through camera and computer programs. [69] first reported PoopMD as a mobile application for the identification of infant acholic stools. PoopMD is based on color hexes that were captured from the Taiwan stool card and account for variations in hue and brightness [69]. [70] announced the stool color evaluation system named Baby Poop in 2017. This program was integrated with pattern recognition and machine learning process utilizing the class-featuring information compression (CLAFIC) method. Baby Poop installed modified artificial intelligence (AI) technology. Judgment result displayed as not only "normal" or "caution / high probability of acholic stool" but also "indeterminate" for unrelated colors to images of the training data. Baba Poop was launched in 2016 as a free iPhone application for individual use [70]. AI technology is widely recognized for its evolution through deep learning and is advancing in all industrial fields. The field of medical care has been no exception, and AI has already been for disease diagnosis in practical use. If AI's high ability to distinguish the characteristics of substances can be applied to the screening field, it might replace the current stool color card screening diagnosed by the human eye [71]. Even though developing an AI program is expensive, once the program is created, AI screening has the advantage of not having to pay for annual printing as color card and low maintenance costs. Moreover, since the stool color of newborns and infants changes daily depending on their stool property and the condition of their digestive system, it is risk to make a judgment of stool color at a temporary point. Digital technology might facilitate daily observation and diagnosis, because nowadays, most of the young mothers carry mobile phones in their pouch—even if they do not carry the maternity booklet with stool color cards [49, 57].

Limits of Screening for BA and Future Prospects

As recounted above, so far, there are no perfect BA screening methods. Each screening tried to efficiently find out BA patients among the patients with cholestasis. Since there are no accurate BA assays currently, every screening strategy has produced a lot of false-positive cases and several false-negative cases as a result. Therefore, it is a matter of course for the occurrence of false-positive by current BA screenings [49, 68]. BA screening means the screening for patients who have cholestatic diseases, including BA. The important point is how the BA patients are picked among the patients with cholestasis. Repeated tests should be required to eliminate false positives, which cause a burden on caregivers and an increase in cost [72]. As noted in the previous literature, the stool color card strategy iscost-effective when compared to no screening at all. It is necessary to further investigate to determine the cost-effectiveness among different screening modalities rather than comparing no screening to a single modality. Also, it is worth mentioning that when calculating costs associated with screening, not only direct but also indirect costs should be all taken into account [73]. Each screening has its advantages and disadvantages. Serum direct bilirubin assay has a potential for early detection of BA. However, several blood draws are needed to narrow down the patients with cholestasis. Stool color card screening is inexpensive and easily repeated; however, it is not good for temporary examination and seems more suitable for gradual clinical changes [74]. Most reported screenings attempted to analyze their feasibility, potentiality, and economic efficiency by only one method in which they have been involved in. Even preoperative differential diagnosis of BA requires many examinations such as blood test, ultrasonography, Computed tomography (CT), and scintigraphy, i.e., taking advantage of respective screening characteristics,

"Hybrid screening" might bring earlier diagnosis, earlier KP, and reduction of screening cost [5]. Screening is one of the factors that have decreased the ages at diagnosis and KP in BA and contributed to the improvement of prognosis. The screening will continue to be needed for early diagnosis of BA, and we expect collaborations between researchers and clinicians to lead a development of further effective screening methods [75].

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