Review Article

Recent advancements in laboratory screening, diagnosis, and prognosis of biliary atresia: a literature review

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Objective: The purpose of this study is to compile and describe the recent advancements in non-invasive laboratory studies utilized in the screening, diagnosis, and prognosis of biliary atresia (BA) patients.

Background: The diagnosis of BA can be difficult to distinguish from other neonatal cholestatic liver diseases and currently the only definitive diagnostic study is an invasive surgical procedure. Because improved patient outcomes are dependent on earlier age of diagnosis and treatment, it is imperative to identify effective non-invasive methods for the early diagnosis and prognosis of BA.

Methods: A comprehensive literature review was completed, synthesizing the findings of a computerized database search, including search words pertaining to BA, screening, diagnostic, and prognostic laboratory markers. Excluded literature focused on imaging or procedural modalities.

Conclusions: Traditional laboratory values for the diagnosis of cholestasis, such as total and conjugated bilirubin, have shown improved efficacy and earlier diagnosis on BA, when used as a newborn screening tool. Recent advancements in non-invasive molecular markers, such as gamma-glutamyl transpeptidase (GGT), matrix metalloproteinase-7 (MMP7), interleukin-33 (IL-33) and stool proteomics show promise as effective diagnostic markers in BA, especially when used in conjunction with multiple predictive factors in diagnostic models. Additionally, predictive indexes for prognostic outcomes have improved over recent years aiding in surgical planning, timing of liver transplant and novel therapeutic modalities. Continued investigation and widespread implementation of improved screening, diagnostic and prognostic markers in BA is required to improve outcomes for these patients.

Keywords: Biliary atresia diagnosis; biliary atresia screening; gamma-glutamyl transpeptidase (GGT); matrix metalloproteinase-7 (MMP7); interleukin-33 (IL-33)

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Introduction

Biliary atresia (BA) is a neonatal cholangiopathy associated with fibrotic obliteration of the extrahepatic biliary tree leading to profound cholestasis and progressive liver failure (1,2). The incidence of BA is variable, estimated to range from 1 in 3,000 to 1 in 18,000 live births, and is more common in Taiwan, China, and Japan (3-6). Although relatively rare, BA is the most common cause of liver failure in children and is the leading indication for pediatric liver transplant (5,7-11). Unfortunately, without treatment, BA is universally lethal by 2 years of age (6,12). Aside from liver transplantation, the only treatment for BA is the Kasai portoenterostomy (KPE), a surgical procedure

in which an intestinal Roux limb is directly anastomosed to the portal plate in an attempt to reestablish biliary drainage. The success rate of KPE is around 50%, however even with initial successful surgical drainage the majority of patients continue to have progressive intrahepatic liver fibrosis leading to cirrhosis and liver failure, ultimately about 80% of BA patients will require liver transplant by 10 years of age (11).

Importantly, it has been shown in multiple studies that earlier age at time of KPE positively correlates with transplant free survival for BA infants (13-15). Therefore, early, and accurate diagnosis and treatment of BA is paramount in improving outcomes for these infants. Etiologic variability, low incidence, and a paucity of non-invasive sensitive and specific methods of diagnosis make it difficult to distinguish BA from other causes of neonatal cholestasis. Currently, intraoperative cholangiogram is the gold standard for definitive diagnosis of BA, however this is an invasive procedure. It can be difficult to identify which patients with neonatal cholestasis are more suspicious of having BA and therefore benefit from undergoing the risks of this procedure. Recently, there have been advances in serum and stool markers that have shown promise in improving specificity and sensitivity of diagnosis including gamma-glutamyl transpeptidase (GGT), matrix metalloproteinase-7 (MMP7), and interleukin-33 (IL-33).

While imaging modalities such as ultrasound, hepatobiliary scintigraphy, magnetic resonance cholangiopancreatography (MRCP) and transient elastography are also used in aiding BA diagnosis, we seek to illuminate and compare serum and stool laboratory values for their potential of expediting and simplifying BA diagnosis. Further, prognosis for transplant-free survival is difficult to predict aside from earlier time to surgical drainage (15). Therefore, we present the following article in accordance with the Narrative Review reporting checklist (available at https://dx.doi.org/10.21037/dmr-21-52), in order to highlight recent studies investigating molecular markers that aim to improve BA diagnosis and shed light on factors indicated in patient prognosis.

Methods

A comprehensive literature review was completed by searching PubMed, MEDLINE, Google Scholar, and Cochrane Review. The search terms included singular and combinations of the following: BA, Diagnosis, Prognosis, Screening, Biomarkers, Total bilirubin (TB), Conjugated bilirubin, Alkaline phosphatase (ALP), Liver function tests (LFT), GGT, MMP7, IL33, Long/short term outcomes, Liver transplant. Publication dates were limited to Jan 1990 through May 2021. Only English language literature was included.

Traditional laboratory values in diagnosis and screening of BA

Neonatal cholestasis is secondary to impaired bile flow and characterized by elevated TB and conjugated bilirubin in infants. Elevated TB at >2 weeks of age is considered abnormal and elevated conjugated bilirubin is never physiologic, both require investigation (16). Although it may vary slightly between institutions, cholestasis is usually defined as conjugated bilirubin >1.0 mg/dL (>17 μmol/L) when TB is <5 mg/dL (<85 μmol/L) or conjugated >20% of the TB when TB is >5 mg/dL (>85 μmol/L) (16). This threshold is decreased in the first five days of life in which abnormal conjugated bilirubin can be defined as >0.3 mg/dL (0.5 μmol/L) or >10% of the TB (16). Any infant with acholic stools and elevated serum conjugated bilirubin should raise suspicion for BA (17). Due to the relative accessibility and low cost, the use of serum conjugated bilirubin has been helpful in screening for BA (14). It has been speculated that all patients with BA have an elevated conjugated bilirubin level in the immediate postnatal period (17). A retrospective study of patients with BA, showed that all 34 BA infants in the study, that also had serum conjugated bilirubin levels measured within 96 hours of birth, were abnormally elevated (18).

In a study by Harpavat *et al.*, they investigated a large newborn population for the diagnostic yield of newborn screening for BA with conjugated and direct bilirubin. In this study, they found that conjugated/direct bilirubin testing in newborn screening for BA had a sensitivity of 100%, a specificity of 99.9%, a PPV of 5.9%, and a NPV of 100.0%. However, due to the small number of true positive screening results, the 95% confidence interval for the sensitivity of conjugated/direct bilirubin as a screening test for BA was significantly wide, with the lower limits potentially being unacceptable for screening tests (19). Notable, this study also looked at patient age at time of diagnosis and KPE before and after implementing newborn screening with conjugated/direct bilirubin and found that after initiation of screening the mean age at diagnosis decreased to only 36 days from 56 days prior to starting screening practices (19). Although serum bilirubin levels have shown promise as a screening tool for early

diagnosis of BA, its utilization in differentiating BA from other non-BA cholestatic liver diseases is less clear. In a large, prospective, multicenter study, looking for clinical factors that could expedite the diagnosis of BA, they found no statistically significant difference regarding conjugated baseline bilirubin and total baseline bilirubin concentrations between the BA and non-BA cholestasis groups (20). Additionally, a small study following conjugated bilirubin trends in the BA infants found that 89% of BA infants had an initial decrease in conjugated bilirubin concentrations in the first 3 weeks of life, therefore a declining trend of conjugated bilirubin is not an indication for exclusion of BA diagnosis (21).

Another serum value commonly utilized in the identification of cholestatic liver diseases including BA, is ALP. Serum ALP is commonly elevated in cholestatic liver diseases secondary to increasing synthesis and release of ALP from the biliary canalicular membrane. Buildup of bile salts also increases release of ALP from the surface of the bile duct epithelia (22). While also found elsewhere in the body, such as bone, kidney and leukocytes, increased levels of ALP along with elevated TB, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and GGT indicate a hepatic etiology. Although an elevated ALP can indicate cholestasis, its role in differentiating BA is poor. In a large study of Chinese patients with BA and non-BA cholestasis, concentrations of ALP were statistically lower in the BA cohort, however another study out of Iran, showed significantly higher concentrations of ALP, in BA patients compared to non-BA infants (23,24).

While LFT such as AST and ALT are frequently measured in patients being evaluated for liver diseases, these values are non-specific for BA and cholestatic liver diseases, in general. AST and ALT are indicators of hepatocyte injury, elevated with loss of hepatocyte integrity (22). Significant LFT elevation and poor synthetic function, such as coagulopathy, can be seen in advanced liver disease and should be evaluated prior to KPE or when considering need for liver transplant.

New directions in laboratory screening and diagnosis of BA

Gamma-glutamyl transferase

Serum GGT is more recently being regarded as another potential diagnostic tool for BA. GGT is a marker for proliferation of bile ducts, which is pathologically increased in BA. It has been shown that an elevated GGT level of >300 U/L, or an increase in its serum concentration of ≥6 U/L per day, had an accuracy of 85% and 88% for differentiating BA from neonatal hepatitis (NH), respectively (25). However, in another study looking to differentiate BA from NH, a GGT level greater than 300 IU/L had a sensitivity of only 39.7%, but a specificity of 98.1% in the diagnosis of BA. In this study they found that GGT could potentially be analyzed with AST and ALT values to better discern BA from NH. GGT/AST ratio values were over 2 in 55/68 BA and only 15/54 NH (OR =11.0; 95% CI, 4.7–25.7; P<0.001) and were 80.9% sensitive and 72.2% specific in the diagnosis of BA. GGT/ALT ratio values were over 2 in 54/65 BA and only 19/50, NH respectively (OR =8.0; 95% CI, 3.4–19.0; P<0.001) with 83.1% sensitive and 62.0% specific in the diagnosis of BA versus NH. The investigators concluded a GGT/ALT ratio >2 should prompt further workup for BA (26).

In a study by Shen *et al.*, retrospective analysis of 282 infants with cholestasis (135 BA and 147 non-BA) demonstrated significantly higher concentrations of GGT in BA infants. Of note, although GGT alone was found to be significant, this study also evaluated liver stiffness measurements and found that in infants <90-day old, diagnostic sensitivity was improved when cutoffs for both GGT and liver stiffness were met (27).

However, patient age may play a role in the utility of GGT for diagnosis of BA. In healthy infants, GGT levels have been shown to be highest in infants younger than 1 month of age, reduced at 1 to 3 months of age, and lowest after 5 months to 7 months of age (26). Rendón-Macías *et al.* found improvement in BA diagnosis by analyzing GGT values while simultaneously correcting for infant age. By using different cut-off levels of serum GGT for BA diagnosis in different age groups, sensitivity and specificity values increased (28). Additionally, Chen *et al.* found that GGT values were useful to diagnose and discern BA from other causes of neonatal cholestasis up until the patients were 120 days old. After 120 days, the GGT levels decreased in both BA and non-BA study groups, possibly due to damaged biliary endothelial cells that could no longer produce GGT (29).

Matrix metallopeptidase-7 (MMP-7)

MMP-7 is a protease expressed by cholangiocytes that is released upon epithelial injury and plays a role in ECM remodeling. MMP-7 also has documented pro-fibrotic

functions in the liver, lung, and kidney (30). Recent studies have implicated MMP7 as a potentially invaluable tool in the diagnosis of BA. Elevated expression of MMP7 in liver tissue of BA infants was first described by Huang *et al.* in 2005, and authors concluded that it likely plays a major role in liver tissue remodeling and progressive liver fibrosis in BA (31). Then, in a large-scale proteomics study in 2017 by Lertudomphonwanit *et al.*, a discovery cohort identified MMP7 to be the lead serum biomarker differentiating BA patients *vs.* non-BA cholestasis patients and was confirmed in two validation cohorts (32). Additionally, coupling serum MMP-7 and GGT together increased the sensitivity and specificity (97% and 94% respectively) of BA diagnosis greater than either lab value alone. They concluded that MMP7 alone or in conjunction with GGT is a valuable marker for BA diagnosis and also a potential target for future therapies (32). Since then, additional studies have validated the accuracy of MMP7 as a diagnostic tool in BA, including Yang *et al.*, in which they found with a serum MMP7 concentration cutoff value of ≥52.85 ng/mL, the diagnostic sensitivity and specificity were 98.67% and 95%, respectively (33). Additionally, MMP-7 performed better than GGT, with a higher area under the curve (AUC), sensitivity, and specificity for diagnosis (33). Jiang *et al.* also demonstrated good diagnostic value of MMP-7 for BA, additionally noting a statistically significant correlation of serum MMP-7 measurements with the degree of liver fibrosis in BA patients (34).

IL-33

IL-33 is a cytokine related to IL-1 expressed widely throughout the body and released upon cell death. It is an inflammatory marker thought to play a role in multiple autoimmune and inflammatory diseases and is indicative of cell damage. IL-33 forms a complex with its phosphatase receptor ST2 and IL-1 receptor accessory protein to activate T helper cell 2 differentiation, thus promoting an immune response (35). Liu *et al.* demonstrated increased IL-33/ST2 levels in surgical samples associated with liver fibrosis and inflammation. Increased positive immunostaining and increased expression of IL-33 mRNA and protein in BA liver biopsy tissues compared to tissue from patients with choledochal cysts with normal liver function also show the possible role of IL-33 in BA tissue diagnosis (36). Additionally, these levels were increased especially in poor-prognosis BA patients compared to good-prognosis patients. This also correlated with serum levels, Dong *et al.* demonstrated a positive correlation between elevated IL-33 levels and elevated GGT levels in BA patient serum, however lacked significant healthy control cohorts (37). More recently, Behairy *et al.* conducted a larger study with BA, non-BA neonatal cholestasis and healthy controls, in which they found a significant increase in serum IL-33 levels in the BA cohort (BA group median 48.0 pg/mL and non-BA group median 17.3 pg/mL), suggesting a role for serum IL-33 in BA diagnosis (38).

Stool bile acids

One of the hallmarks of BA is acholic stools and therefore stool markers are an intriguing area of interest in the diagnosis of BA. Nguyen *et al.* published an initial pilot study using liquid chromatography-mass spectroscopy (LC-MS) of 13 patients showing the total bile acid content of stools was highest in healthy neonates when compared to total parenteral nutrition cholestasis and BA patients (3,354.01±2,102.56, 1,476.27±1,361.07, and 34.29±10.30 μM/mg, respectively). This was in stark comparison to their respective serum TB levels. There was an over 40-fold difference between TPN cholestasis and BA stool bile acid content, whereas a less than 1.4-fold difference was observed between the groups based on serum TB (39).

Cholic acid (CA) and chenodeoxycholic acid (CDCA) are the primary bile acids synthesized de novo in the liver. In samples from control patients, CA was greater than half the total bile acid content. The ratio of CA to CDCA was reversed in cholestatic stool samples compared to normal patients' stool, which had a higher proportion of CDCA. Although it did not reach statistical significance, the trend of CA not being the dominant bile acid was also seen in BA samples. This study was limited by relatively small sample sizes (39).

In a follow up study from the same group, experimental mouse models of BA were similarly evaluated with LC-MS, alongside human samples from non-cholestatic and cholestatic infants. Two experimental mouse models were tested: 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) chow which induces cholangiopathy and bile duct ligation (BDL) in 6-week-old male wildtype C57 BL/6 mice compared to standard chow and sham operation, respectively. Human samples were again collected from hyperbilirubinemia and age match control infants (40). Serum TB concentrations were significantly elevated in BDL and DDC animals compared to controls, as expected. Serum bile acids were predominantly conjugated and

primary in the experimental cholestatic models. The stool secretion of total bile acids as well as concentration of bile acids were also disrupted compared to controls. Both BDL and DDC mice had significantly lower stool total bile acids, with predominantly conjugated and secondary bile acid metabolites, suggesting the utility of stool and serum LC-MS in its ability to detect differences in bile acid levels and composition (40).

In the human component of this subsequent study, cholestatic non-BA (n=15) and BA (n=16) patients were compared to age-matched healthy infants (n=10). In a sub analysis of primary versus secondary and conjugated versus unconjugated bile acids, stool bile acids were predominantly unconjugated, though no significant difference was seen between non-BA cholestasis and BA groups. However, primary bile acids and primary conjugated bile acids [glycochenodeoxycholic (GCDCA) and taurochenodeoxycholic acid (TCDC)] were significantly higher in BA than non-BA cholestatic infants. Wherein, a GCDCA concentration of greater than 30 mM had 100% sensitivity, 83.3% specificity, and concentration less than 30 mM had 100% NPV for the diagnosis of BA (40).

Although, the above molecular studies show significant diagnostic benefits, there are potential limitations to their use. GGT is a mainstream lab value that is available in most institutions without a significant cost burden or delayed time to results, therefore can be implemented into BA diagnosis management and decision making quite easily. However, MMP7, IL33 and stool bile acids are not available as in-house studies at most institutions, requiring these tests to be sent to specific laboratories for processing, resulting in increased costs and duration to results. Because early diagnosis and treatment is paramount in BA, delay in laboratory results may not be feasible in some situations. Hopefully, with continued utilization and literature supporting the use of these laboratory tests, they will become more mainstream, increasing availability and decreasing cost and time to results.

Novel diagnostics models in BA

As there is not yet a singular non-invasive test that accurately diagnoses BA and many features of BA overlap with other neonatal cholestasis pathologies, diagnostic models have been established for improved accuracy in the diagnosis of BA. Dong *et al*., sought to develop a novel nomogram utilizing GGT and other BA-associated risk factors. In this large, single center, retrospective study out of China, 1,512 BA and 216 non-BA cholestasis infants were enrolled over a 6-year period. They found five predictors, including gender, weight, conjugated bilirubin, ALP, and GGT that were significantly different between BA and non-BA cholestasis infants. With these factors, the investigators developed a decision tree (DT), random forest (RF), and multivariate logistic regression-based nomograms. The performance comparison of the three models showed that the nomogram demonstrated greater discriminative ability with sensitivity 85.7%, specificity 80.3%, and PPV 0.969. Validation of the nomogram showed high stability and reproducibility, leading the authors to conclude that the nomogram has more potential in clinical application, than GGT alone, in the preoperative diagnosis of BA (23).

To validate a bile acid profile-based scoring system for rapid identification of BA, cholestatic and non-cholestatic patients were prospectively enrolled to develop a three-variable scoring system by Zhao *et al*. Sixty-six patients were enrolled in the derivation cohort, 75 in the validation cohort, and 37 were age-matched controls. BA patients had higher frequency of reported clay stools and hepatomegaly, abnormal ultrasound gallbladder findings, and positive hepatobiliary scintigraphy, as well as LFT including elevated TB and GGT. The serum bile acid concentrations of CDCA were significantly decreased in BA patients, whereas glycocholic acid (GCA) and GCDCA were significantly higher. The ratio of GCDCA/CDCA was significantly higher in BA infants, with the median ratio of 685 in BA, while non-BA infants was 266 ($P<0.05$). They used statistically significant variables (GGT, GCDCA/CDCA ratio, and clay stool) from the univariate analysis to create a multivariable model for the derivation cohort, and a composite score system was established. This BA Score system form the multivariable model linearly corresponded to the risk estimate; using a ROC (receiver operating characteristic) curve analysis, the diagnostic efficacy of the score system was evaluated, and a cutoff point was selected to stratify BA risk. The AUC of the scoring system was 0.87, with 85.3% sensitivity and 81% specificity with a score cutoff of 15 points. Validation of this system with 40 BA and 35 non-BA cholestatic patients yielded a diagnostic sensitivity of 90.0% and specificity of 80.0% (41).

Prognostic markers in BA

Due to the significant variability in clinical course, studies now focus on pinpointing markers at time of KPE that will help stratify patients into risk categories. Outside of time to

surgical intervention, it is still difficult to provide prognostic information to families of BA patients after KPE. Here, we review laboratory and liver histology markers that may aid in this endeavor.

Laboratory markers

As described previously, Bilirubin is a simple and common laboratory marker followed for cholestasis. Post-KPE, the decrease of TB has been found to positively correlate to better outcomes. Shneider *et al*., prospectively enrolled 137 patients with BA undergoing KPE as part of the Childhood Liver Disease Research Network (ChiLDReN) multicenter study. Fifty percent of the infants had TB <2.0 mg/dL in the first 3 months after KPE, and those patients had a significantly higher transplant free survival (86% *vs.* 20%, P<0.0001). Patients with TB >2.0 at 3 months post-KPE had diminished weight gain, greater probability of ascites development (OR =6.4), hypoalbuminemia (OR =7.6), coagulopathy (OR =10.8), liver transplant (OR =12.4), or death (OR =16.8) (42).

Other studies have also investigated bilirubin as an early biomarker that may be predictive of native liver survival. In a retrospective cohort study of 217 patients with BA who underwent KPE, a backwards-stepwise elimination of univariate and multivariate logistic regression identified variables associated with improved 2-year native liver survival, this included center of care, BA type, age at KPE, degree of fibrosis at diagnosis, development of ascites within 3 months, TB, albumin (Alb), ALT, and change in length *z* score at 3 months post-KPE. A TB <4.3 mL/dL and Alb >3.5 mg/dL at 3 months post-KPE were found to be the optimal cut-off to predict 2-year native liver survival. In multivariate analysis, patients were assigned to 1 of 3 prognostic groups based on parameters: group 1 (TB <4.3; Alb >3.5), group 2 (TB <4.3; Alb <3.5), group 3 (TB >4.3; Alb <3.5). Group 3 had the worst 2-year native liver survival rate of 20.2% and group 1 with native liver survival rate of 92.6%. They conclude that serum TB and Alb levels at 3 months post-KPE were independently associated with native liver survival at 2 years of age, and identification of BA infants who are likely to require early transplant will allow for more aggressive and earlier nutrition management (43). Infants who have better nutritional status undergoing liver transplant have decreased pre- and post-transplant morbidity and mortality, and better neurodevelopmental outcomes (44-46).

The ChiLDReN group also completed a multicenter phase I/IIA trial administering intravenous immunoglobulin to BA patients after KPE but did not find statistically significant improvements in TB at 90 days post-KPE or native liver survival at 1 year. However, in looking at the flow cytometry of peripheral blood cell markers at 60, 90, 180, and 360 days after KPE, it was found that increased TB was correlated with increased percentage of HLA-DR + CD38+ natural killer cells, expression of natural killer cell activation markers CD69 and HLA-DR, decreased percentage of regulatory T cells, and increased interleukin-8 and associated neutrophil products. Using Cox modeling, increased percentage of HLA-DR + CD38+ NK cells and plasma IL-8 was associated with increased risk of poor outcomes as designated by liver transplant or death by 1 year (47).

The clinical course, character of complications, and prognosis of patients who survive with their native liver past the first 2 years differs from those who fail early. Approximately 50% of BA infants who undergo KPE will receive a liver transplant by age 2 years, and an additional 20–30% undergo liver transplant between 2–18 years (48). In the United Kingdom, 5- and 10-year native liver survival have been documented as 46% and 40%, respectively (49). Twenty-year survival with the native liver ranges from 14–44% around the globe (50,51).

As these patients approach adolescence and adulthood, transitional care must be established. Furthermore, as BA patients tend to retain the synthetic liver function when compared to other etiologies of liver failure requiring transplantation, understanding the types of complications affecting BA patients is paramount. Understanding that adolescent BA patients are a unique cohort with disease process that may not be as familiar to the adult hepatologist. Jain *et al*. sought to identify laboratory, clinical, and radiological parameters in BA patients with their native liver at 16 years of age that were associated with future need of liver transplant. In this retrospective observational study, 89 patients with BA who underwent KPE at their institution between 1980 and 1996 and still followed with their native liver at the age of 16 were included. Within this cohort, 67 patients still had their native liver, whereas 21 had undergone or were awaiting transplant. Overall, median waiting time for transplant was 267 (8–1,664) days, with the following indications: cholangitis [9], gastroesophageal variceal bleed [4], portal hypertension related synthetic dysfunction [4], portal hypertension related lethargy [2], jaundice [1], and liver nodule [1] . The overall liver survival showed 75% of patients had their native liver at 6.9 years

after their 16th birthday, and 80% and 60% were alive with their native liver at 5 and 10 years after their 16th birthday, respectively (52).

Increased risk of liver transplant was associated on univariate analysis with higher TB, INR, AST, ALT, GGT, and spleen size, and lower creatinine, Alb, and platelet levels. In the transitional adolescent period as defined after their 16th birthday, cholangitis was more common (31.8% *vs.* 3.03%) in patients who ultimately underwent a liver transplant. The presence of portal hypertension or gastroesophageal varices also increased the risk of liver transplant by 7- and 8.6-fold, respectively. On multivariate analysis, elevated TB and decreased creatinine were independent predictors for liver transplant in this adolescent cohort (52).

Liver Biopsy Markers

The National Institutes of Health-sponsored BA Research Consortium (BARC) has a repository of liver biopsies from infants with cholestasis. Ten pathologists participating in BARC evaluated 97 anonymous liver biopsy samples, developed a semiquantitative scoring system based on 16 histologic features, and validated their findings. The pathologists were blinded to clinical history, imaging results, and laboratory data. They found that diagnosis of biliary obstruction on histopathology alone was highly sensitive, however even with experienced histopathologists, making the diagnosis of BA on histology alone was not possible, and the best predictors of BA diagnosis were bile duct proliferation, portal fibrosis, and absence of sinusoidal fibrosis (53).

Expanding on this study, Russo *et al.* reviewed 316 liver biopsies from clinically proven BA cases enrolled in the prospective ChiLDReN. Patients were followed for up to 6 years post KPE to assess whether histologic features correlated with clinical outcomes. They found that there was extensive variability in the severity of the histologic features in the biopsies taken at time of KPE. Using a significance threshold of P<0.01, the authors found that an older age at KPE was the only feature significantly associated with poorer bile drainage (TB <1.5) at 6 months post-KPE. Lobular sinusoidal fibrosis (P=0.03) and interlobular bile duct injury (P=0.05) were found to be marginally associated with bile drainage at 6 months post-KPE. On univariate analysis, degree of fibrosis, interlobular bile duct injury, ductal plate malformation (DPM), age at KPE, and INR were associated with shorter time to transplant or death (P<0.01). Ductular reaction, macrosteatosis, BA subtype, and ALP were marginally associated with transplant-free survival and overall survival (P<0.05). The hazard of transplant was 1.73 times greater for patients with stage 3 or 4 fibrosis; 1.55 for patients with moderate or marked interlobular bile duct injury; and 1.53 for presence of DPM. With every unit increase in baseline INR, there was 1.26 factor increase of transplant or death. In multivariate analyses, DPM, INR, and age at KPE were significant predictors of transplant-free survival. Although postoperative outcome was not the primary aim of the study, Russo *et al.* found extensive variability in the severity of histologic changes around the time of KPE, and that none of the histologic features correlated significantly with drainage at six months post-KPE. However, severity of portal fibrosis, DPM, bile duct injury, and baseline INR appear to be associated with decreased transplant-free survival (54).

Further analysis of liver samples was conducted by Luo *et al.*, who reviewed liver biopsies and clinical data from patients enrolled at their institution as well as ChiLDReN. By RNA sequencing analysis of liver samples, they identified 14 genes associated with survival in the discovery cohort, which was reproduced in the validation cohort. Utilizing this 14-gene signature in conjunction with TB at 3mo post KPE, they developed a prognostic index that stratifies patients into high and low survival groups and found that 24–33% of the low survival group had their native liver at 9 months, whereas 60–70% survived with their native liver at 23 months in the high survival group. mRNA expression encoding proteins that regulate fibrosis were found to be increased in liver samples of infants that did not survive with their native liver at 2 years of age, whereas genes known to regulate glutathione metabolism were increased in infants who survived at the same time point. This prognostic information not only helps predict outcomes for clinicians, patients and families but may also help tailor postoperative treatment to improve outcomes in BA (55).

Summary

BA remains a very complex and time sensitive diagnosis with definitive diagnosis requiring an invasive procedure with significant morbidity and mortality. However, many recent studies have shown promising diagnostic accuracy with non-invasive modalities utilizing novel serum, stool, and liver biomarkers. Further development of early, non-invasive, and accurate screening and diagnostic methods

in BA are necessary for the continued improvement in the early diagnosis and management of infants with BA.

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