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Review

Recent studies on non-invasive biomarkers useful in biliary atresia – a literature review

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The aim of this review is to specify new potential reliable and non-invasive methods for the diagnosis of biliary atresia (BA) that could shorten the way to diagnose BA, and finally the surgical treatment. Apart from the biomarkers that have been proven helpful and are used nowadays in neonatal wards, there are several new potential biomarkers that researchers have found to be helpful in the diagnosis of biliary atresia. Circulating microRNAs, matrix metalloproteinase-7, stool proteins, interleukin-33, Th17-associated cytokines, urinary metabolomics, anti-smooth muscle antibodies, heat shock proteins 90 and positive biliary epithelial cells CD56 are among those presented in this summary. These markers may play a new significant role in BA diagnosis. The described methods include Nomogram, Circulating microRNAs (miRNAs), Matrix metalloproteinase-7 (MMP-7), Stool proteins, Interleukin-33 (IL-33), Th17-associated cytokines, Alpha-aminoadipic acid and N-acetyl-d-mannosamine in urine, Anti-smooth muscle antibodies (ASMA), Heat shock proteins 90 (HSP90), Positive biliary epithelial cells CD56.

Key words: biliary atresia, neonatal cholestasis, jaundice

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Abbreviations: ALP, alkaline phosphatase; ALT, Alanine transaminase; ASMA, Anti-smooth muscle antibodies; AST, aminotransferase; AUC-ROC, the area under the receiver operating characteristic curve; BA, biliary atresia; DB, direct bilirubin; GC–MS, gas chromatography–mass spectrometry metabolomics; GGT, gamma-glutamyl transpeptidase; HSP90, Heat shock proteins; IHS, infantile hepatitis syndrome; IIF, indirect immunofluorescence; IL-33, Interleukin-33; MIP3a, macrophage inflammatory protein-3alpha ; MMP-7, Matrix metallopro-teinase-7; MRCP, magnetic resonance cholangiopancreatography; OPLS-DA, orthogonal partial least squares discriminant analysis; TB, total bilirubin

INTRODUCTION

Biliary atresia (BA) (ORPHA:244283; OMIM: 210500) is an uncommon infancy fibroinflammatory obliterative cholangiopathy that affects extrahepatic and intrahepatic biliary ducts. It is associated with obstructive jaundice, pale stools and hepatomegaly, and leads to severe cholestasis and fatal biliary cirrhosis. The etiology of BA remains elusive. If not treated in time with hepatoportoenterostomy which restores bile flow (Kasai procedure KPE – the primary treatment of BA), it leads to liver failure within 2 years (Basset *et al.*, 2008; Chardot, 2006; Sokol *et al.*, 2007; Feldman *et al.*, 2015). BA remains the most common cause of π liver transplant in young children. It affects approximately 1 in 5000 to 25000 live births. It is more common in the Asia-Pacific region, especially in South China where the prevalence is about 1/5000 (Chen *et al.*, 2019).

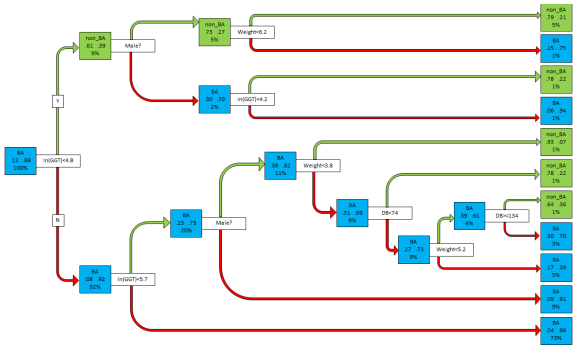
An early diagnosis is highly problematic due to unspecific symptoms which can mimic other cholestatic diseases (non-BA). There are no reliable and specific biomarkers that are recommended for routine clinical use. Currently, the gold standard for BA diagnosis is an intraoperative cholangiogram which is highly invasive. Liver biopsy with 96.6% accuracy is the most reliable method (Chen *et al.*, 2016). The longer it takes to diagnose chronic cholestasis, the higher the risk of liver fibrosis. Because the success of Kasai portoenterostomy depends, among others, on age at the time of surgery, due to delayed diagnosis many infants with BA lose the chance for proper treatment. Also, a late diagnosis worsens the outcomes of other cholestasis-associated diseases like hypopituitarism, galactosaemia and tyrosinaemia.

The investigation of biliary atresia requires taking the following blood tests: gamma-glutamyl transpeptidase (GGT), also combined with total bilirubin, direct bilirubin, alkaline phosphatase, and aminotransferase (AST). The diagnosis is also based on clinical evaluation, hepatomegaly, stool colour, duodenal juice colour, bile acid in duodenal juice, ultrasonography (the gallbladder triad, the triangular cord sign or strip-apparent hyperechoic foci), hepatobiliary scintigraphy, MRCP and liver biopsy (Dong *et al.*, 2018).

NOMOGRAM

In the Dong and others research, bile acid level in duodenal juice and hepatobiliary scintigraphy showed the highest (100%) sensitivity for diagnosing BA (Dong Ch *et al.*, 2018). Ultrasonography (triangular cord sign or presence of strip-apparent hyperechoic foci) showed the highest specificity (99.5%). The colour of the stool is also pathognomic. High levels of bilirubin alter the hue of the stool and turn it into light yellow. The diagnostic accuracy by feces color was 84.3% with a sensitivity of 96.1% and specificity of 74.8% in Dong's and others research (Dong Ch *et al.*, 2018). All of the known differential diagnosis methods are useful. Gamma-glutamyl transpeptidase (GGT) is used in combination with other biomarkers of BA cholestasis (Chen *et al.*, 2016; Liu *et al.*, 1998; Cabrera-Abreu *et al.*, 2002; Rendon-Macias *et al.*, 2008; Tang *et al.*, 2007; Maggiore *et al.*, 1991). Younger infants with BA (age <30 days) proved to have significantly higher GGT levels than older patients (Dong Ch *et al.*, 2018). The researchers demonstrated that in a 2-year treatment, 53.7% of patients survived with their native liver, while the remaining patients needed liver

transplantation. Sira and others reported that the serum activity of GGT had a sensitivity of 76.7% and specificity of 80% for the diagnosis of BA (Sira *et al.*, 2012). Nonetheless, the GGT level was also increased in the serum of infants on the day of birth or varied depending on age (Dong Ch *et al.*, 2018). In the Dong's and others research, carried out in a large Chinese children's hospital on 1728 patients, to confirm BA intraoperative cholangiography and liver biopsy for histological assessment were used (Dong R *et al.*, 2018). 1512 patients were diagnosed with BA and 216 patients were confirmed to have non-BA cholestasis. 80% of non-BA patients were male, however, there was no statistical difference in gender in the BA group (51% male, 49% female). The research also included weight, age and the levels of: total bilirubin (TB), direct bilirubin (DB), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT), AST and ALT (Alanine transaminase). There was no significant difference in TB, AST and ALT levels. Patients' age also did not show any notable influence on results. The non-BA group presented higher alkaline phosphatase levels (ALP). Gender, weight, DB, ALP, and GGT showed a good prediction with the AUC-ROC curve (the area under the receiver operating characteristic curve) >0.8 (a value close to 1 is considered perfect discrimination). Rui Dong and others (Dong R *et al.*, 2018) created a nomogram based on those factors (gender, weight, DB, ALP, and GGT) allowing to predict BA.



Nomogram based on various factors (gender, weight, DB, ALP, and GGT) proved helpful in diagnosing BA.

The nomogram demonstrated a good discriminative ability with a sensitivity of 85.7% and specificity of 80.3%. It proveduseful in diagnosing BA and, additionally, to have the potential for clinical application (Dong R *et al.*, 2018).

CIRCULATING MICRORNAS (miRNAS)

Zahm and others (Zahm *et al.*, 2011) hypothesized that unique miRNAs may serve as a new class of biomarkers that could help to distinguish BA from other forms of neonatal cholestasis and therefore become a new diagnostic biomarker of BA. Goldschmidt's analysis says that circulating MicroRNA as the miR-21 and miR-29a cluster may be used as markers of liver diseases in children (Goldschmidt *et al.*, 2016). MicroRNAs are short non-coding parts of RNAs which are necessary for organ development (Thai *et al.*, 2007; Lynn *et al.*, 2007; Hand *et al.*, 2009). MiRNAs are dysregulated in many pathologies including BA. Increased levels of circulating miRNAs can suggest specific tissue damage (Morimura *et al.*, 2011; Laterza *et al.*, 2009; Zahm *et al.*, 2011). Zahm et al. proved that a cluster of miRNAs (miR-200b/429) is elevated in BA patients' sera compared to cholestatic control samples. The researchers used serum samples obtained from patients with confirmed BA (by cholangiography, operative exploration, and/or histology) and extrahepatic bile duct tissue from mouse liver. 11 miRNAs were selected for further analysis out of which the miR-200b showed significant alterations. To prove the importance of miR-200b, they measured levels of miR-200a and miR-429 (which are co-transcribed with miR-200b from the miR-200b/429 locus (Zahm *et al.*, 2012)). They were both altered in the sera of BA patients. The AUC values 0.8 for the miR-200b/429 cluster with a sensitivity of 71% and specificity of 92%. Adam M. Zahm et al. also discovered that cholangiocytes serve as the source of miR-200b/429 cluster in biliary atresia. Detecting elevated levels of miR-200b/429 in serum can be useful not only in distinguishing BA from non-BA patients, but also in predicting survival after KPE. The miR-200b/429 cluster classifies correctly 85% of patients (Zahm *et al.*, 2012). Because not all samples are correctly classified, further research is needed. Nevertheless, elevated circulating miR-200b/429 levels may prove useful in BA diagnostics. Another microRNA analysis done by Zahm and others (Zahm *et al.*, 2011) showed that circulating miR-4429 and miR-4689 can play a certain role in diagnosing BA. The AUC of miR-4429 was 0.789 with a sensitivity of 83.3% and a specificity of 80.0%, and the AUC of miR-4689 was 0.722 with a sensitivity of 66.7% and specificity of 80.0% for the prediction of BA (Zahm *et al.*, 2012).

MATRIX METALLOPRO-TEINASE-7 (MMP-7)

MMP-7 (also known as matrilysin (Hugh, 2017)) is a zinc-dependent endopeptidase that breaks down the extracellular matrix and its expression can correlate with BA-related liver fibrosis (Yang *et al.*, 2018; Jiang *et al.*, 2019). The researchers have recently discovered that an altered level of MMP-7 in the serum of a cholestatic patient can be associated with biliary atresia (Yang *et al.*, 2018; Jiang *et al.*, 2019). To prove the diagnostic value of MMP-7, Yang and others (Yang *et al.*, 2018) tested 135 infants with cholestasis (75 with BA and 60 non-BA) and a 54 control group at three pediatric centers. The results showed that MMP-7 levels in BA patients sera were significantly higher than in non-BA cases. Serum levels of MMP-7 in a non-cholestatic group had a median of 2.86 ng/mL, the non-BA group was circa 11.47 ng/mL and BA patients had an average with a median of 121.1 ng/mL, that is 10-times higher than in others. Yang and others (Yang *et al.*, 2018) have also compared GGT and MMP-7 levels in the sera of BA- neonates. The AUC of MMP-7 was 0.9900 and the AUC for GGT was 0.7186. For GGT, the sensitivity was 64.00% and the specificity was 71.67%. In comparison, the sensitivity and the specificity of MMP-7 were respectively 98.67% and 95.00%. The negative predictive value of MMP-7 was 98.28% (Yang *et al.*, 2018). The number needed to misdiagnose (NNM) was also calculated and for MMP-7 it was 25 (which means that 1 out of 25 patients was misdiagnosed). For GGT, the NNM was 3 (so 1 out of 3 was misdiagnosed). Combining MMP-7 and GGT with an AUC of 0.9898 does not increase diagnostic accuracy (Hugh, 2017; Yang *et al.*, 2018). Lertudomphonwanit et al. investigated samples of 35 BA patients and 35 cases with intrahepatic cholestasis. Serum levels of MMP7 and

GGT were different in these two groups and the researchers estimated that 19.1 infants could be diagnosed with BA with the MMP7 plus GGT as a biomarker (Lertudomphonwanit *et al.*, 2017; Hugh, 2017). Jiang and others (Jiang *et al.*, 2019) diagnosed 288 patients with jaundice. The median of MMP-7 in BA group sera was 38.89 ng/mL and in non-BA: 4,4 ng/mL. The sensitivity, specificity, positive predictive value and negative predictive value were 95.19%, 93.07%, 97.27%, and 91.43%, respectively. The accuracy was improved when MMP-7 was combined with GGTP. Based on this data, alterations in the serum MMP-7 levels may be helpful in the diagnosis of biliary atresia. It also correlates with liver fibrosis, therefore it can be potentially used as a therapeutic target or a prognostic biomarker (Hugh, 2017; Yang *et al.*, 2018; Jiang *et al.*, 2019). Another study by Wu et al. was based on a group of 100 neonates including 36 with BA. The serum level of MMP-7>1.43 ng/mL for predicting biliary atresia has a median of 10.26 ng/mL for BA (Yang *et al.*, 2018; Harpavat, 2019). They also observed that serum MMP-7 levels may rise with age, as liver damage proceeds, and that MMP-7 is a less useful serum marker in neonates due to its correlation with other liver diseases. There is pressure to include the MMP-7 level in the prognostic algorithm. However, the usefulness of this marker has limitations: the values of the MMP-7 level can vary depending on the type of immunosorbent assay kits used (Yang *et al.*, 2018; Harpavat, 2019). In comparison, the median serum MMP-7 levels were 38.89 ng/mL for the BA group and 4.4 ng/mL for the non-BA group in the Yang *et al.* research. Hence, the role of MMP-7 as a reliable biomarker remains controversial. The confirmation of the diagnostic utility of serum MMP-7 levels may be an objective for therapeutics.

STOOL PROTEINS

There are two kinds of stool tests: Sudan III staining of stool fat and measurement of duodenal bile acid. Steatorrhea in a stool is patognomic for BA patients as well as acholic feces (Okajima *et al.*, 2016). Detection of steatorrhea in Sudan III straining is an easy-to-obtain and rapid examination. Gu et al. have published their research where they claim that the fat content of a stool may be helpful especially when feces are non-acholic (Gu *et al.,* 2015). They have examined 313 230 infants, 34 with biliary atresia. The stool colour card screening showed 76.5% sensitivity and 99.9% specificity in this research. Lien et al.'s analysis also shows that stool colour card is useful in detecting BA patients. The research includes babies born before 2002 – the beginning of using the stool colour card in Taiwan, and after 2002. The analysis conclusion was that the stool colour card screening program allows to implement earlier Kasai operation (Lien *et al.,* 2010).

Another research concerned an analysis of proteins using data-independent acquisition mass spectrometry (DIA–MS) (Watanabe *et al.*, 2020). The assumption was that because of the obstruction of the normal flow of bile juice, BA patients' stool contains fewer proteins that are produced in the biliary tract. Also, some specific proteins that originate from the biliary tract are possibly absent or reduced compared to the non-BA patients (Watanabe *et al.*, 2020). The researchers examined four patients with BA and three others with non-BA cholestasis. They proved that 49 proteins were higher and 54 proteins lower in the stool of BA patients. The analysis showed that proteins RBP4, SHMT2, HMGCS1, ADH6, ALDH1A1, ACADS, ADK, KHK, ACAA2, PSAT1, AMACR, and PTGR1 are lower in stool samples of BA-patients (Watanabe *et al.*, 2020). And conversely: the CEACAM1, CEACAM5, and CEACAM8 were significantly higher in patients with BA (Watanabe *et al.*, 2020). CHI3L1 is correlated with worse BA outcome (Watanabe *et al.*, 2020). The measurement of those dominant proteins in the stool of BA patients could be of assistance in the early diagnosis of the disease. Watanabe et al. emphasized that the research needs evaluation on a larger cohort.

INTERLEUKIN-33 (IL-33)

A specific cytokine involved in the pathogenesis of liver fibrosis and severe inflammation (Tan *et al.*, 2018). It is released in the course of cell death (Rinella, 2015; Zhang *et al.*, 2015). It is assumed that IL-33 assists as an "alarm signal" released by stressed hepatocytes (Heymann *et al.*, 2016). In the following research, Ola G. Behairy et al. observed 60 infants with cholestasis (BA and non-BA) and 30 healthy ones. BA diagnosis was based on surgical cholangiography. Cholestasis was recognized when conjugated bilirubin was 20% of the total bilirubin or when total bilirubin exceeded 17 mg/dL. The level of IL-33 in the serum was measured and the results showed that the IL-33 gene expression in the BA group was markedly upregulated. The median in the BA group was 48.0 pg/mL and in the non-BA group with cholestasis it was 17.3 pg/mL (Behairy *et al.*, 2020), while at the same time, healthy infants presented a 7.3 pg/mL average. There was also a gradual increase in the serum IL-33 level in BA and non-BA group with fibrosis stage (Behairy *et al.*, 2020) with the cut-off level determined at 20.8 pg/mL (specificity of 95% and sensitivity of 96.7%) (Behairy *et al.*, 2020). Still, jaundice, ascites and stool colour were the most visible symptoms in both cholestatic patient groups. Significantly, the aminotransferases and total leucocytic count levels were higher in the non-BA group, and gamma-glutamyl transferase, total protein, alkaline phosphatase and total and direct bilirubin levels were higher in the BA cohort (Behairy *et al.*, 2020). The total protein showed the highest level in the group of healthy infants, whereas the total leucocytic count was the lowest. The role of IL-33 in the diagnostics of biliary atresia is indisputable. Its serum concentration correlates with the levels of aminotransferases, as well as total and direct bilirubin levels (Behairy *et al.*, 2020). Another analysis made by Liu and others (Liu *et al.*, 2019) consists of 36 BA patients and 8 patients as a control group. The expression level of IL-33 was increased in BA patients compared to the control group (3.9 ± 0.5 *vs.* 1.0 ± 0.3). The result of the analysis was that the IL-33/ST2 receptor signaling axis is activated in BA patients. IL-33 exacerbates inflammatory reactions and drives liver fibrosis (Li *et al.*, 2014). Measuring serum interleukin-33 levels may have a diagnostic value in infants with biliary atresia as a reliable, non-invasive and fast tool.

Th17-ASSOCIATED CYTOKINES

Those cytokines are the ones that play an important role in the immune-mediated injury against intrahepatic bile duct epithelial cells (Chen *et al.*, 2019). Chen and colleagues conducted a study of 31 BA-infants and 45 non-BA infants with cholestasis. They tested 25 Th17-associated cytokines in those groups. They observed a

different gene expression of cytokines: IL-17F, IL-10, macrophage inflammatory protein-3alpha (MIP3a), IL-22, IL-13, IL-33, IL-6, IL-17E, IL-27, IL-31, TNF-a and TNF-b in BA patients (Chen *et al*., 2019). The highest AUC showed MIP3a in comparison to the others (Chen *et al*., 2019). MIP3a – cysteine motif chemokine ligand 20 (CCL20) used alone or in combination with other biomarkers may become useful in BA diagnosis. MIP3a overexpression is related to inflammatory damage in bile ducts and to the development of BA (Chen *et al*., 2019). For validation, Chen and others studied another group of 68 cholestatic patients (30 BA and 38 non-BA). BA was diagnosed by intraoperative cholangiography. In both studies, BA samples showed clay stool and higher GGT levels. 12 following cytokines showed a significant difference in concentrations between BA and non-BA infants: IL-17F, IL-10, MIP3a, IL-22, IL-13, IL-33, IL-6, IL-17E, IL-27, IL-31, TNF-a and TNF-b (Chen *et al*., 2019). MIP3a showed better results in the diagnosis of BA than the other cytokines. Furthermore, the AUCs of MIP3a, IL-13 and TNFa were higher than 0.8. The sensitivity and the specificity of MIP3a were 90.40% and 80.0%. The AUC of GGT was 0.802 and clay stool showed an AUC of 0.792. The researchers went further and investigated if combining the marker with clay stool and GGT would increase the diagnostic value compared to the marker alone. Their research showed that the AUC of clay stool + GGT, clay stool + MIP3a, GGT + MIP3a and clay stool + GGT + MIP3a was 0.824, 0.918, 0.880 and 0.892 respectively (Chen *et al*., 2019). A combined test would increase the diagnostic specificity. This finding indicates that a single MIP3a plus a single biomarker increases the diagnostic accuracy of BA (Chen *et al*., 2019).

ALPHA-AMINOADIPIC ACID AND N-ACETYL-D-MANNOSAMINE IN URINE

Metabolomics can aid in understanding the disease mechanisms and identifying biomarkers for the diagnosis and, furthermore, monitoring the disease (Mamas *et al*., 2011; Vinayavekhin *et al*., 2010). By analyzing the urine we can observe the underway metabolic processes. Urine is an excellent material for discovering new biomarkers because it can be obtained non-invasively. Li and others (Li *et al*., 2014) characterized urinary metabolomic profiles in infants with BA and infantile hepatitis syndrome (IHS). The levels of identified metabolites were further measured using the human metabolome database to determine if they were endogenous or exogenous. A urinalysis from an infant is simple because of its restricted diet. The research was based on 25 BA patients, 38 IHS patients, and on 38 healthy ones. The researchers used gas chromatography-mass spectrometry metabolomics (GC–MS) and orthogonal partial least squares discriminant analysis (OPLS-DA) for testing. In the analysis, total bilirubin, GGT and alkaline phosphatase were significantly higher in BA patients compared to IHS. 41 urine metabolites demonstrated a different expression between BA *vs*. NC, IHS vs. NC, and BA *vs*. IHS (Li *et al*., 2018). The most notable differences showed N-acetyl-d-mannosamine and alpha-aminoadipic acid obtained from the urine of children with BA. The alpha-aminoadipic acid level was significantly increased in IHS (Li *et al*., 2018). Alpha-aminoadipic acid's AUC was 0.95 in distinguishing BA from IHS in the training set, and 0.88 in distinguishing BA from IHS in the test set. N-acetyl-D-mannosamine had an AUC of 0.91 for distinguishing BA from IHS in the training set, and 0.94 in the test set (Li *et al*., 2018). After using the permutation test, the researchers presumed that alpha-aminoadipic acid and D-man-nosamine may be useful in BA diagnosis. On the other hand, 19 metabolites showed the same trend in BA *vs*. NC and IHS *vs*. NC (ex. lysine, leucine and tyrosine, tyrosine metabolite-hydroxyphenyllactic acid, ornithine, glutamine and glycocyamine), which suggests that those two diseases may have the same metabolic mechanism (Chen *et al*., 2019). Li *et al*. study showed that the level of adenine was also increased, and the xanthine level was reduced in BA and IHS infants. They also found that Myo-inositol and glucuronic acid were elevated in BA and IHS patients. According to the study, urinary metabolites may be useful as potential biomarkers for a differential diagnosis of BA and IHS, but it still needs to be validated in further studies.

ANTI-SMOOTH MUSCLE ANTIBODIES (ASMA)

The study of Rafeey and others (Rafeey *et al*., 2021) included 18 BA patients and 12 patients with neonatal hepatitis. The research showed a higher expression of anti-smooth muscle antibodies (ASMA) in the sera of BA patients. ASMA is detected by indirect immunofluorescence (IIF), fibroblasts, or HEp-2 cells. The conclusion was that ASMA levels were higher in neonatal hepatitis samples but not statistically significant in BA (the sensitivity and specificity were respectively 66.7% and 75%) (Rafeey *et al*., 2021), whereas GGT and ALP levels were significantly increased in the sera of BA patients (for GGT, the sensitivity and specificity were 88.9%, 66.7% and for ALP the sensitivity and specificity were 77.8% and 75%). The study showed that ASMA may be useful for the differentiation of BA from neonatal hepatitis, however, it still needs to be further evaluated.

HEAT SHOCK PROTEINS 90 (HSP90)

HSP 90 are proteins involved in the folding and unfolding of other proteins (Dong R *et al*., 2013). Their level is higher in response to stress (De Maio, 1999). Another HSP protein – HSP 47 has been linked to fibrosis in BA (Deng *et al*., 2011). Cholangiocytes or hepatocytes secrete proteins in response to cholestatic diseases such as BA (De Maio, 1999). Cholangiocytes affect the bile composition (Dong R *et al*., 2013). Following that thought, Dong *et al*. have looked for a protein that plays a crucial role in biliary atresia pathogenesis. They used liver tissue obtained by biopsy of 20 BA patients and 12 non-BA cholestatic infants (Dong R *et al*., 2013). In the study, two-dimensional electrophoresis was used to reveal 15 proteins highly upregulated in BA. From among that group, nine were elevated and six were downregulated in the liver tissues of BA patients. Among 19 proteins, the HSP90 level was significantly higher. The mean level of HSP90 in BA patients was significantly lower than in non-BA cholestatic patients (Dong R *et al*., 2013). To confirm those results, mass spectrometric identification and immunoblotting analysis of HSP90 were used. Dong and others (Dong *et al*., ????) suggest that HSP 90 could play a protective role during cholestasis and may serve as a prognostic marker for NC compared to BA. Yet, further analyses need to be conducted on a larger cohort of patients.

POSITIVE BILIARY EPITHELIAL CELLS CD56

cells that play an important role in morphogenesis, remodeling, and migration in several organs through cell–cell and cell–matrix interactions (Sira *et al.*, 2012). In Sira et al. research, 30 infants with BA and 30 infants with non-BA cholestasis were included. CD56-positive cells were found in the epithelium of bile ducts and ductules where their level was significantly higher in the BA group (83.3%) than in the non-BA group (6.7%) (Sira *et al.*, 2012). BA patients with biliary causes compared to the non-BA group had a significantly higher CD56 staining in the biliary epithelium than in the BA group. The results showed that the CD56 cells were highly elevated in the sera of BA patients. Simultaneously, CD56 natural killer cells were significantly elevated in the non-BA group. The research concluded that CD56 immunostaining could be helpful in differentiating patients with BA.

SUMMARY

Biliary atresia is a cause of chronic cholestasis in neonates and if untreated leads to liver failure. BA has to be managed operatively as soon as it is possible. Because of a lack of non-invasive markers, the worth of a reliable and easy-to-obtain biliary atresia marker is invaluable. New discoveries allow us to have a new picture of diagnosing BA. Plenty of studies showed the diagnostic validity of laboratory investigations in the differentiation of BA from other cases of cholestasis. Circulating microRNAs with their 92% specificity (Zahm *et al.*, 2012) have a great value in diagnosing BA. Levels of the miR-200b/429 cluster were elevated in BA patients in comparison to cholestatic controls. Nevertheless, future work on a larger cohort is needed. Matrix metallopro-teinase-7 (MMP-7), as noted in several reports, still needs more evaluation because its level depends on the patient's age and the type of laboratory kit used. Stool proteins and alpha-aminoadipic acid, and *N*-acetyl-D-mannosamine obtained from urine may be the future of BA diagnostics. The sample is easy to obtain and there is no need to keep it sterile. It is especially worth it when it comes to developing countries. The research shows that Interleukin-33 (IL-33) along with gamma-glutamyl transferase, total protein, alkaline phosphatase and total and direct bilirubin can be useful in diagnosing BA (Behairy *et al.*, 2020). In the study diagnostic accuracy of Anti-smooth muscle auto-antibody (ASMA) was evaluated. Compared with the accuracy rates of invasive procedures ASMA may be a useful marker for the differentiation of BA from non-BA patients. Heat shock protein 90 (HSP90) needs tissue from a liver biopsy to evaluate the result which makes it hard to determine. The nomogram presented in Chen Dong and others research (Dong Ch *et al.*, 2018) is a good way to deduce the probability of biliary atresia using gender, weight, GGT, ALP and direct bilirubin levels. Bile acid level in duodenal juice and hepatobiliary scintigraphy showed the highest (100%) sensitivity for diagnosing BA (Dong Ch *et al.*, 2018). On the basis of all the above-mentioned findings, we may conclude that an easier diagnostics of biliary atresia in newborns is within range of our hospital capabilities. Clearly, some medical research will still require more comprehensive verification. Rapid and accurate diagnostics with possible avoidance of liver biopsy is crucial in the case of cholestasis of an unknown nature, therefore every additional parameter achieved in a non-invasive way, straight from a laboratory, would be of life importance. In the opinion of the authors, in clinical practice, the Stool Proteins method seems to be the most promising and practical thanks to its simplicity and fully non-invasive character.

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