ORIGINAL ARTICLE

Efficacy of the seven feature, fifteen point histological scoring system and CD56 in interpretation of liver biopsies in persistent neonatal cholestasis: A five-year study

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ABSTRACT

Context: Neonatal cholestasis (NC) lasting more than 2 weeks affects one in 2500 live births. Extrahepatic biliary atresia (EHBA) and idiopathic neonatal hepatitis account for about 70% of all cases of NC. Differentiating these two conditions is important as patient management is very different for both the conditions. **Aims:** To assess the usefulness of the seven-feature, 15-point histological scoring system in the interpretation of liver biopsy in NC and usefulness of immunostaining with CD56 (N-CAM) in EHBA. **Settings and Design:** Retrospective study of 5 years' duration at a pediatric referral institute, where the case load of NC is high and definitive surgery for EHBA is undertaken after histological confirmation. **Materials and Methods:** The study is of a 5-year duration conducted between June 2007 and May 2012. A total of 210 cases of NC were clinically diagnosed during this period. All the slides were reviewed with reference to a seven-feature, 15-point histological scoring system assessing its usefulness in the interpretation of liver biopsy in NC and utility of the immunohistochemical marker CD56 was also assessed as an aid in the characterization of bile ductular proliferation in EHBA. **Statistical Analysis:** Statistical analysis was performed and sensitivity and specificity of the histological scoring system for EHBA was analyzed. **Results:** Of the 210 liver biopsies reviewed using the scoring system, 122 cases were diagnosed as EHBA and 88 cases were diagnosed as other causes of NC. The overall sensitivity of this scoring system was 95.5%, specificity was 93.1% and diagnostic accuracy was 94.6%. **Conclusions:** The seven-feature, 15-point histological scoring system has good diagnostic accuracy in the interpretation of liver histology in NC as advanced histopathological findings even at younger age require immediate surgery. CD-56 is a useful marker in the assessment of bile ductular proliferation in EHBA.

KEY WORDS: CD56, EHBA, histological scoring system, neonatal cholestasis

INTRODUCTION

Neonatal cholestasis (NC) is defined as prolonged conjugated hyperbilirubinemia that occurs in the newborn period. Extrahepatic biliary atresia (EHBA) and idiopathic neonatal hepatitis (INH) account for 50-70% of all cases of NC. The treatment of the former is early surgical intervention, while the latter requires non-surgical supportive care. Failure to differentiate the two conditions may result in avoidable surgery in neonatal hepatitis.[1]

The lack of differentiating clinical features, biochemical markers and other specific investigations to distinguish the two conditions is still a major problem. In the last few decades, there has been a remarkable progress in imaging methods and molecular biological techniques, challenging the concept of the traditionally considered gold standard of liver disease, liver biopsy. Despite this, liver biopsy is still one of the most important diagnostic modalities in the evaluation of EHBA.[2]

We used an objective, seven-feature, 15-point histological scoring system for the interpretation of liver histology in order to differentiate EHBA from other causes of NC[3] as diagnosis of EHBA can be challenging and the histological features can overlap with other neonatal cholestasic liver disease.[4]

Bile ducts and ductules are CD56 positive in most cases of EHBA and CD56 immunostaining can be a useful tool for diagnosing EHBA in the early, ductular proliferative phase.[5]

MATERIALS AND METHODS

The present study includes all the liver biopsies of cases of NC received between June 2007 and May 2012 for a period of 5 years at our institute. We reviewed all the slides with reference to a seven-feature, 15-point histological scoring system proposed by Lee WS and Looi LM[3] and assessed its usefulness in the diagnosis of EHBA. The seven features are portal ductal proliferation, bile plugs in portal ductules, porto–portal bridging, lymphocytic infiltration in the portal region, multinucleated hepatocytes, neutrophilic infiltration and hepatocellular swelling, scored with a 15-point (0 to 15) scoring system [Table 1].

A total of 210 cases were clinically diagnosed as NC during this period. The definition of NC is taken as the onset of clinically apparent jaundice within the first 4 months of life, with the conjugated bilirubin being greater than 17 μmol/L (1 mg/dL) if the total bilirubin is less than 85 μmol/L (5 mg/dL) or conjugated bilirubin being more than 20% of the total bilirubin if the total bilirubin is more than 85 μmol/L (5 mg/dL). All cases of EHBA were confirmed with an operative cholangiogram or demonstration of atretic gall bladder and extrahepatic biliary tree intraoperatively.

All the liver biopsies of NC sent for histopathology were included in this study. The biopsy materials were screened for adequacy in size and the number of portal tracts present. All the portal tracts were observed for the number, size and shape of the portal bile ductules. The average number of bile ductules present in all the visible portal tracts was noted.

Table 1: Seven-feature, 15-point histological scoring system devised for the interpretation of liver biopsy materials in neonatal cholestasis (Lee WS and Looi LM, 2009)[3]

Parameter (7 features)	*Histological characterization*	*Histological grade*
Portal ductal proliferation	None	0
	Mild	1
	Moderate	2
	Marked	3
Bile plug in portal ductules	Absent	0
	Present	2
Porto–portal bridging	None	0
	<50% of portal tracts	1
	>50% of portal tracts	2
Lymphocytic infiltrate in portal region	None	2
	Mild	1
	Moderate/severe	0
Multinucleated hepatocytes	None	2
	Only around central vein	1
	Diffuse	0
Neutrophils in the infiltrate	Absent or mild	2
	Moderate or marked	0
Hepatocellular swelling	None	2
	Mild/focal	1
	Periportal/diffuse	0

Standard histological features indicative of EHBA are bile ductular proliferation, [Figure 1] bile plugs in bile ductules [Figure 2], and porto–portal bridging [Figure 3], and those indicative of neonatal hepatitis include giant cell transformation [Figure 4] lymphocytic and neutrophilic infiltration [Figure 5], and hepatocellular swelling.

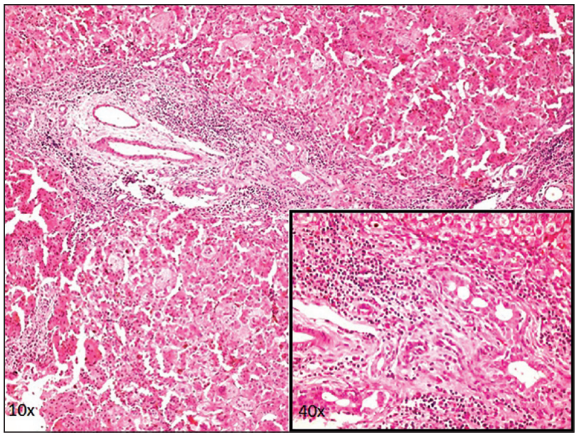

Figure 1: Showing marked portal duct proliferation (materials and methods)

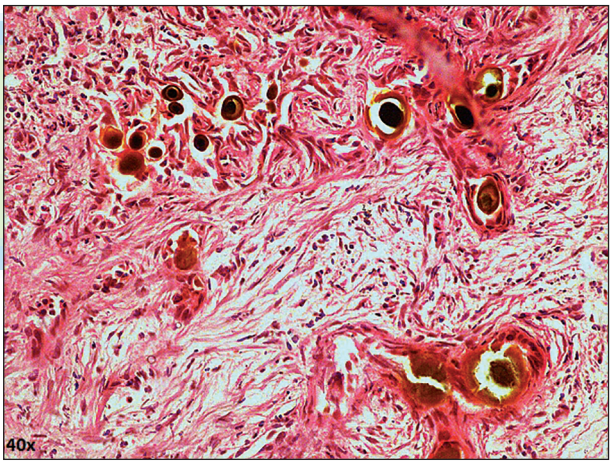

Figure 2: Bile plugs in portal ductules (materials and methods)

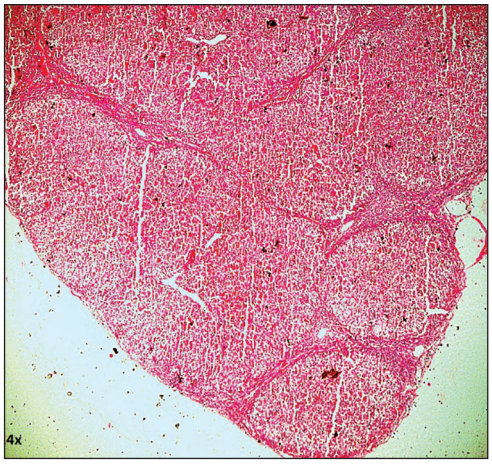

Figure 3: Showing porto–portal bridging (materialsand methods)

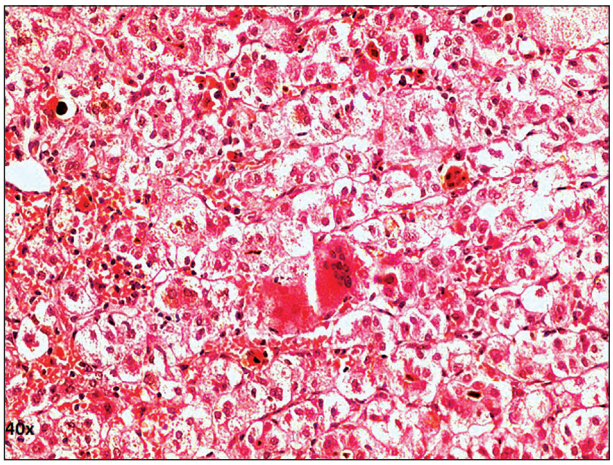

Figure 4: Multinucleate hepatocyte (materials and methods)

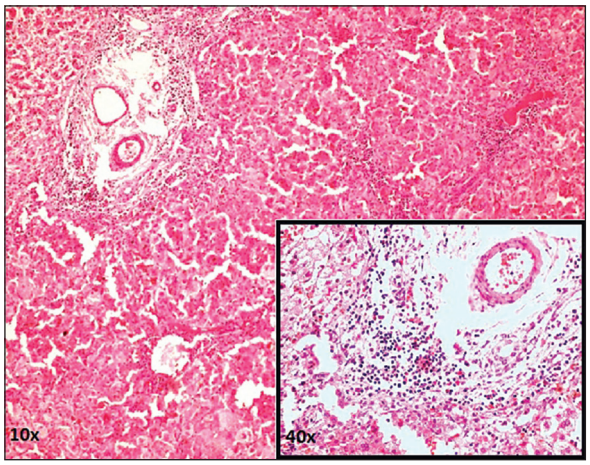

Figure 5: Lymphocytic infiltrate in portal region (materials and methods)

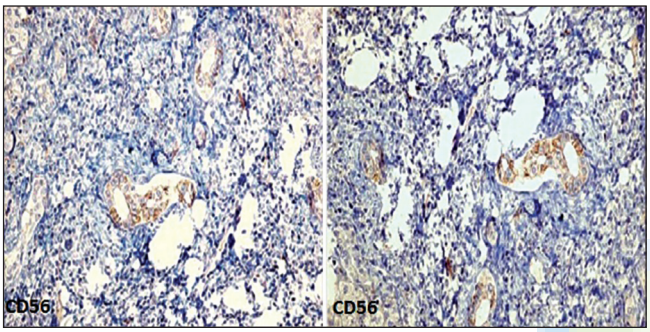

Figure 6: Showing CD56 positivity in proliferative bile ductules

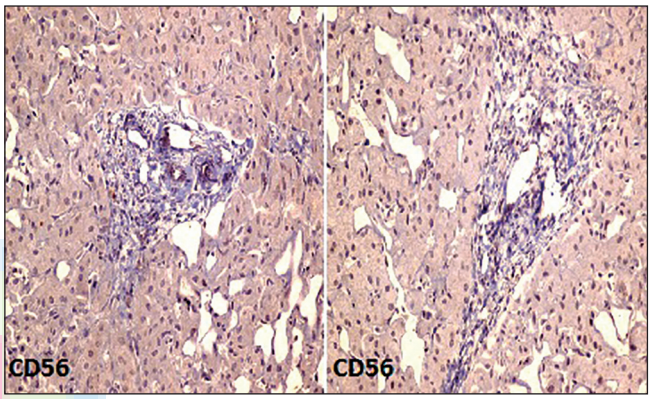

Figure 7: CD56 negative in non-proliferative ductules

The histological diagnosis was designated as either EHBA or non-EHBA. The 15-point scoring system based on histology of liver biopsy, consisting of seven histological criteria regarded as sufficiently specific to differentiate EHBA from non-EHBA, was used.

Each histological feature was scored according to the seven-feature, 15-point histological scoring system and sum of the scores is calculated out of 15 points. The score ranges from 0 to 15 points. Features against EHBA are negatively scored.

Bile ductular proliferation was considered to be present if the average number of ductules in the portal tract was more than five. The following criteria were used to grade the degree of bile ductular proliferation: No proliferation - average number of bile ductules per portal tract <5; mild - average number of bile ductules per portal tract between 5 and 9; moderate - average number of bile ductules per portal tract ≥10; marked proliferation - elongated, attenuated, angulated bile ductules in addition to proliferation (average number of bile ductules per portal tract ≥10) [Figure 1].

Specimen collection

Two hundred and six open wedge and four percutaneous liver biopsies of size range 1.5-2 cm (median of 1.7 cm) were routinely fixed and processed. Sections of 5-micron-thickness were cut and slides prepared and stained with hematoxylin and eosin (H&E). All the biopsies were adequate and contained — seven to eight portal triads each. Inadequate biopsies were not included in the study.

Immunohistochemistry (IHC) of the CD56-stained liver biopsy

IHC was performed on the paraffin-embedded tissue. Thin-sliced sections of 3-micron thickness were immunostained with antibody against CD56 (clone 123C3, ready to use; Dako, Glostrup, Denmark. and secondary antibody k5007 envision using an auto immunostainer after heat-induced antigen retrieval using the pressure-cooking method. CD56 immunostaining was performed in 29 cases: 22 cases of EHBA and seven cases of non-EHBA.

Non-EHBA cases were used as controls only for the purpose of comparison between the epithelial cells of normal and proliferating bile ducts. Because of financial constraints, only 22 cases in which bile ductular proliferation had a lower score although other scores for EHBA were high and were chosen for CD56 staining.

RESULTS

Our study included 141 male and 69 female children. The age range of the children was 3 days to 5 months. Ninety-two cases

aged between 1 and 2 months, 51 cases between 2 and 3 months and the remaining cases were in 2-5 month age group.

Of 210 liver biopsy cases, 122 cases (58%) were diagnosed as EHBA and 88 cases (42%) were diagnosed as other causes of NC (non-EHBA) on routine histopathology [Table 2].

With the seven-feature, 15-point histological scoring system, a score of ≥7 had the best diagnostic utility to differentiate EHBA from other intrahepatic cholestasis histologically.

Using the scoring system, of the 210 cases, EHBA was diagnosed in 128 cases and non-EHBA was diagnosed in 82 cases. However, among 6 clinically suspected cases of EHBA, 4 were labeled as neonatal hepatitis and 2 as storage disease on routine histology. These 6 cases were scored as EHBA by the scoring system correlating with the clinical diagnosis.

Sensitivity, specificity, positive and negative predictive values and diagnostic accuracy of the various histological features in differentiating EHBA from non-EHBA were calculated using the seven-feature, 15-point histological scoring system [Table 3].

We got a sensitivity of 95.5%, specificity of 93.1%, positive predictive value of 95.5%, negative predictive value of 93.1% and diagnostic accuracy of 94.6%.

In our study, the presence of moderate to severe bile ductular proliferation was the most consistent histological feature noted in EHBA, and was present in 98% of EHBA cases, bile plugs were present in 80% and porto–portal bridging was present in 85% of the EHBA cases [Table 4].

In our experience, routine histopathology of EHBA is bound to have many pitfalls as there are many overlapping findings of neonatal hepatitis, which is the closest differential diagnosis. The scoring system that we have used is helpful in answering this dilemma by placing positive scores on findings specific to EHBA and negative scores on findings specific to neonatal hepatitis.

Hence, the scoring system could differentiate EHBA and neonatal hepatitis. Apart from this, the intensity of finding is graded semi-quantitatively and higher scores indicate more advanced disease and worse prognosis.

The individual scoring of fibrosis has a prognostic utility as higher degree of fibrosis associated with cirrhosis is an irreversible finding and requires liver transplantation.

IHC with CD56 was strongly positive in all the bile ductules of the 22 cases of EHBA on which it was performed. Cases were limited due to financial constraints. Seven cases of non-EHBA, used as controls, were negative for CD56.

Of the 22 cases, the score increased in 10 cases after using CD-56 IHC staining as CD56 stained the immature bile ductules that were not visualized on routine histopathology.

Table 2: Different cases diagnosed by histopathology

Histopathology diagnosis	*EHBA*	*Non-EHBA*
Extrahepatic biliary atresia	122	
Choledochal cyst		69
Neonatal hepatitis		10
Storage disorder		6
Alagille syndrome		1
Alfa 1 antitrypsin deficiency		1
Wilsons disease		1
Total cases	122	88

Table 3: Comparison of histopathological diagnosis with final diagnosis by the histological scoring system

Cases of neonatal cholestasis	*Cases diagnosed by histopathology*	*Cases diagnosed by scoring system*
EHBA	122	128
Non-EHBA	88	82
Total	210	210

Table 4: Final results of our study

Results	*Percentage*
Sensitivity	95.5
Specificity	93.1
Positive predictive value	95.5
Negative predictive value	93.1
Diagnostic accuracy	94.6

Hence, we advocate the use of CD56 as a useful adjunct to aid scoring of EHBA in which proliferation of bile ducts is an important component of the scoring system.

DISCUSSION

NC lasting more than 2 weeks affects one child in 2500 live births. Of these, idiopathic neonatal hepatitis represents as many as 50% of the cases, biliary atresia represents another 20% and α_1-antitrypsin deficiency represents 15%. In the first 3-4 months of life, infants have some degree of physiologic cholestasis because of the inefficient uptake of bile acids and other organic anions by the hepatocytes and the presence of immature hepatocellular pathways for bile acid conjugation and biliary secretion. Under these circumstances, the immediate priority is to differentiate pathologic cholestasis from the usually benign physiologic forms of this condition.[7]

Biliary atresia is defined as a complete or partial obstruction of the lumen of the extrahepatic biliary tree within the first 3 months of life. It is characterized by progressive inflammation and fibrosis of intrahepatic or extrahepatic bile ducts.[8] Liver biopsy is one of the most important diagnostic steps in the evaluation of EHBA.[2] The Cholestasis Guideline Committee of NASPGN recommends that a liver biopsy should be performed in most infants with undiagnosed cholestasis and should be interpreted by a pathologist with expertise in pediatric liver disease.[1,9] Because of the non-specific nature of the histology in cholestatic neonatal liver disease, many authors have attempted to devise various histological scoring systems.[3,10,11]

The present study attempts to validate the scoring system proposed by Lee WS and Looi LM to grade and prognosticate the histological diagnosis of EHBA.

In our study, using conventional H&E staining in 210 liver biopsies obtained from 210 patients with NC, EHBA was correctly diagnosed by the histological scoring system in 128 cases while non-EHBA was correctly diagnosed in 82 cases; the overall diagnostic accuracy was 94.6%, which correlated with the study by Way Seah lee and Lai Meng Looi who first advocated the use of this scoring system in their Asian study. They had a sensitivity of 88%, specificity of 94% and diagnostic accuracy of 92% in their study [Table 5].

In our study, we got a sensitivity of 95.5%, specificity of 93.1% and diagnostic accuracy of 94.6%.

A seven-feature, 15-point histological scoring system had good diagnostic accuracy in the interpretation of liver histology in NC.

The advantage of a histological scoring system in addition to its higher diagnostic accuracy is that it provides a more objective and systematic way of assessing liver biopsy. As more than one criterion is used, it is less influenced by the adequacy of the biopsy material. Another advantage of the scoring system used in this study was its simplicity and non-complicated nature.[3]

CD56 (N-CAM) is a cell surface adhesion molecule that plays an important role in morphogenesis, remodeling and migration in several organs through cell–cell and cell–matrix interactions. Normal ducts do not express CD56 (N-CAM) on the membranes of the biliary epithelial cells.[6] Several previous studies have noted that the biliary epithelium is positive for CD56 in the setting of EHBA.[5] CD56 immunostaining can be a useful supplemental stain for diagnosing EHBA in its early, ductular proliferative phase when used in conjunction with traditional H&E morphology and clinical information.[5] N-CAM may have a function in the development of the intrahepatic bile ducts, and N-CAM-positive immature biliary cells can contribute to the repair of damaged bile ducts in chronic liver diseases.[6] Based on our findings of strong N-CAM expression in the bile ducts of EHBA specimens, we suggest that the CD56 immunostaining should be routinely used in the diagnosis of EHBA as a supplement to the scoring system. As CD56 was positive in all our EHBA cases, we got a specificity of 100%. Our study is comparable to the study performed by Sira and Guindi, in which they got a specificity of 100%.[17]

Because biliary atresia is an important cause of NC, early diagnosis and surgery for atresia is important to ensure a higher success rate for surgery and better long-term outcome.[12,13] Kasai's portoenterostomy procedure remains the only form of therapy that can be offered to these patients beside liver transplantation in advanced cases.[2,14,15] Prognosis depends on the treatment used and on the post-operative outcomeHence, accurate diagnosis using the scoring system is a useful aid.

Table 5: Comparison of our study with the study conducted by Lee WS and Looi LM at Kuala Lumpur

Parameters	*Our study (2012) (%)*	*Study by Way and Meng (2009) (%)*
Sensitivity	95.5	88
Specificity	93.1	94
Diagnostic accuracy	94.6	92

REFERENCES

1. Mowat AP, Psacharopoulos HT, Williams R. Extrahepatic biliary atresia versus neonatal hepatitis. Review of 137 prospectively investigated infants. Arch Dis Child 1976;51:763-70.
2. de Carvalho E, Ivantes CA, Bezerra JA. Extrahepatic biliary atresia: Current concepts and future directions. J Pediatr (Rio J) 2007;83:105-20.
3. Lee WS, Looi LM. Usefulness of a scoring system in the interpretation of histology in neonatal cholestasis. World J Gastroenterol 2009;15:5326-33.
4. Tagge DU, Tagge EP, Drongowski RA, Oldham KT, Coran AG. A long-term experience with biliary atresia. Reassessment of prognostic factors. Ann Surg 1991;214:590-8.
5. Torbenson M, Wang J, Abraham S, Maitra A, Boitnott J. Bile ducts and ductules are positive for CD56 (N-CAM) in most cases of extrahepatic biliary atresia. Am J Surg Pathol 2003;27:1454-7.
6. Okada T, Itoh T, Sasaki F, Honda S, Naito S, Todo S. CD56-immunostaining of the extrahepatic biliary tree as an indicator of clinical outcome in biliary atresia: A preliminary report. Turk J Pediatr 2008;50:542-8.
7. Lee H, Kang J, Kim KM, Jang JY, Jang S, Yu E. The Clinicopathological Parameters for Making the Differential Diagnosis of Neonatal Cholestasis. Korean J Pathology 2009;43:43-7.
8. Cocjin J, Rosenthal P, Buslon V, Luk L Jr, Barajas L, Geller SA, *et al*. Bile ductule formation in fetal, neonatal, and infant livers compared with extrahepatic biliary atresia. Hepatology 1996;24:568-74.
9. Balistreri WF, Grand R, Hoofnagle JH, Suchy FJ, Ryckman FC, Perlmutter DH, *et al*. Biliary atresia: Current concepts and research directions. Summary of a symposium. Hepatology 1996;23:1682-92.
10. Lee WS. Neonatal cholestasis and biliary Atresia: Perspective from Malaysia. Jummec 2010:1372-9.
11. Zerbini MC, Gallucci SD, Maezono R, Ueno CM, Porta G, Maksoud JG, *et al*. Liver biopsy in neonatal cholestasis: A review on statistical grounds. Mod Pathol 1997;10:793-9.
12. Lee WS, Chai PF. Clinical features differentiating biliary atresia from other causes of neonatal cholestasis. Ann Acad Med Singapore 2010;39:648-54.
13. Lai MW, Chang MH, Hsu SC, Hsu HC, Su CT, Kao CL, *et al*. Differential diagnosis of extrahepatic biliary atresia from neonatal hepatitis: A prospective study. J Pediatr Gastroenterol Nutr 1994;18:121-7.
14. Moreira RK, Cabral R, Cowles RA, Lobritto SJ. Biliary atresia: A multidisciplinary approach to diagnosis and management. Arch Pathol Lab Med 2012;136:746-60.
15. Dessanti A, Ohi R, Hanamatsu M, Mochizuchi I, Chiba T, Kasai M. Short term histological liver changes in extrahepatic biliary atresia with good postoperative bile drainage. Arch Dis Child 1985;60:739-42.
16. Sharma S, Das P, Dattagupta S, Kumar L, Gupta DK. Liver and portal histopathological correlation with age and survival in extra hepatic biliary atresia. Pediatr Surg Int 2011;27:451-61.
17. Sira MM, EL-Guindi MA, Saber MA, Ehsan NA, Rizk MS. Differential hepatic expression of CD56 can discriminate biliary atresia from other neonatal cholestatic disorders. Eur J Gastroenterol Hepatol 2012;24:1227-33.

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