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ORIGINAL ARTICLE

The morphological and histopathological assessment of Alagille syndrome with extrahepatic bile duct obstruction: the importance of the differential diagnosis with subgroup "*o*" biliary atresia

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Abstract

Purpose The differential diagnosis between Alagille syndrome (AGS) with extrahepatic bile duct obstruction (EHBDO) and biliary atresia (BA) is difficult. We report a case series of AGS with EHBDO with detailed validation of the morphological and histopathological features for the differential diagnosis of BA.

Methods Six liver transplantations (LTs) were performed for AGS with EHBDO. All patients were diagnosed with BA at the referring institution and the diagnosis of AGS was then confirmed based on a genetic analysis before LT. We verified the morphological and histopathological findings of the porta hepatis and liver at the diagnosis of BA and at LT.

Results All patients had acholic stool in the neonatal period and were diagnosed with BA by cholangiography. The gross liver findings included a smooth and soft surface, without any cirrhosis. The gross findings of the porta hepatis included aplasia of the proximal hepatic duct, or subgroup "*o*", in five patients. The histopathological examination of the EHBD also revealed obstruction/absence of the hepatic duct. There were no patients with aplasia of the common bile duct.

Conclusions Aplasia of the hepatic duct and the macroscopic liver findings may help in to differentiate between AGS with EHBDO and BA.

Keywords Alagille syndrome · Biliary atresia · Subgroup "*o*" · Extrahepatic bile duct obstruction

Introduction

Alagille syndrome (AGS) is an inherited autosomal-dominant multisystem disorder involving the liver, that is mostly caused by mutations in the *Jagged1* (*JAG1*) gene [1]. An early differential diagnosis between biliary atresia (BA) and AGS remains difficult, because the clinical features for the diagnosis of AGS are obscure in the neonatal or infantile period and a mutational analysis of *JAG1* takes a long time in comparison to timely Kasai portoenterostomy (KPE) [2]. Lin et al. recently showed that a case genetically diagnosed with AGS was associated with histopathologically proven complete extrahepatic bile duct obstruction (EHBDO) [3]. This is histopathological evidence of the combination of AGS and EHBDO, and demonstrates the importance of further validation of morphological and histopathological findings in the porta hepatis, including the EHBD and gallbladder, in AGS with EHBDO. There have been many reports of AGS diagnosed with BA in which KPE was performed [4, 5]. The dilemma of the early diagnosis of AGS and timely KPE is an issue that still bothers clinicians [3].

We examined the detailed clinical and morphological characteristics of a series of AGS with EHBDO who underwent liver transplantation at our institution, in which the patients were initially diagnosed with BA by the referring institution.

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Methods

Between November 2005 and April 2020, six living donor liver transplantation (LDLT) operations for patients with AGS, who had diagnosed with BA at the referring institution, were performed at the National Center for Child Health and Development, Tokyo, Japan. At the referring institution, these patients were initially diagnosed with BA based on gross findings of the bile duct and intraoperative cholangiography. At our institution, a mutation analysis for the diagnosis of AGS is usually performed for patients with any of the five features of AGS at the time of the pre-transplant assessment. All six patients eventually showed the *JAG1* null mutation and met the classical diagnostic criteria for AGS before LDLT. LDLT was performed by a single team of skilled transplant surgeons, MK, SS, AF.

We retrospectively verified the clinical characteristics, and the morphological and histopathological findings of the EHBD, gall bladder and liver at the diagnosis of BA and AGS. Two experienced pediatric pathologists, blinded to clinical data, reviewed the slides together until reaching a consensus. The morphological findings of the porta hepatis were classified according to the classification proposed by the Japanese Biliary Atresia Society (Fig. 1) [6].

In addition, of the six AGS with EHBDO cases, five had aplasia of the proximal hepatic duct, corresponding to subgroup "*o* (omicron)" (AGS with "*o*" group). The clinical characteristics and gross and pathological findings of the liver at KPE were compared between the AGS with "*o*" group and the five cases of BA in subgroup "*o*" without AGS who received LT at our institution (BA with "*o*" group).

This study was approved by the ethics committee of the National Center for Child Health and Development (NCCHD #404) and was conducted in accordance with the Declaration of Helsinki (2008).

Results

AGS with EHBDO cases

None of these patients were diagnosed with AGS based on satisfying three of the five major features in the AGS classical diagnostic criteria before KPE (Table 1). All six patients were diagnosed with BA by exploratory laparotomy, the gross findings of the porta hepatis, and intraoperative cholangiography.

The gross liver findings included a smooth and soft surface, without any cirrhosis (Table 2) (Fig. 2). The histopathology of the excised EHBD confirmed the obstruction or absence of the hepatic duct in all cases (Table 3). According to the gross findings of the porta hepatis, five patients (83.3%) who showed aplasia of the proximal hepatic duct were classified into subgroup "*o*". Three patients with

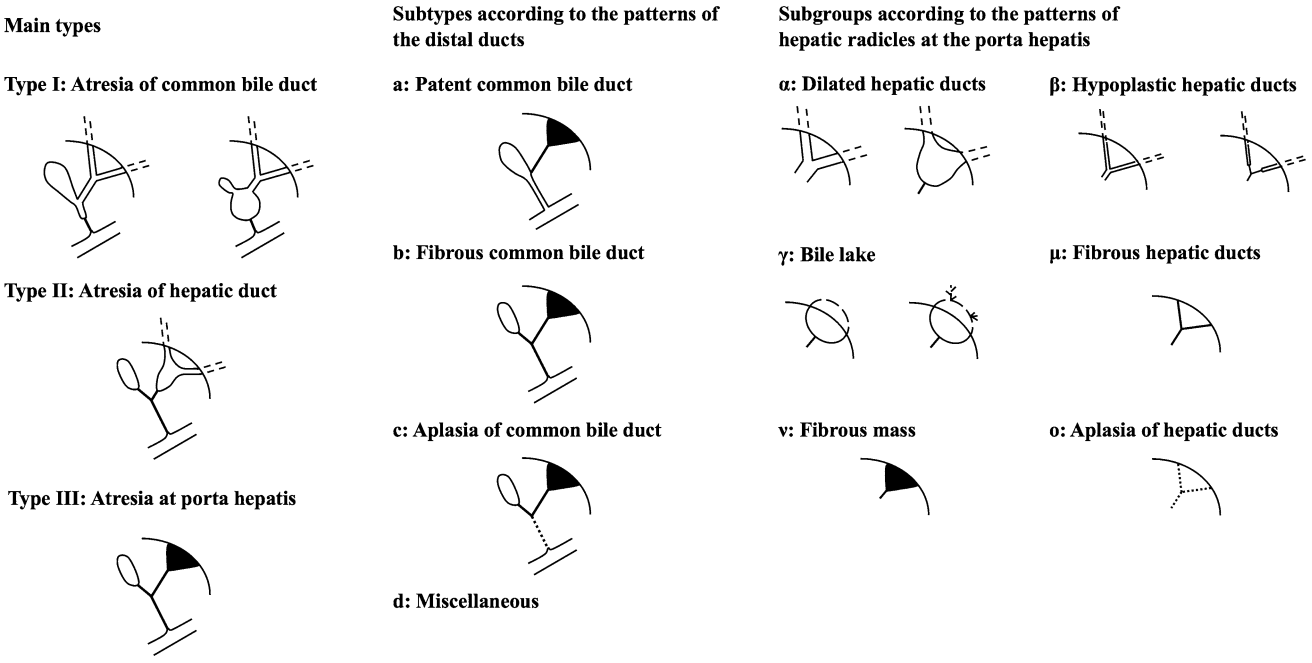

Fig. 1 Morphological classification of biliary atresia. The main types are classified according to the site of obstruction. The subtypes are classified according to the patterns of the distal duct (common bile duct). The subgroups are classified according to the patterns of the proximal duct (hepatic radicles)

Table 1 Clinical features of the cases of Alagille syndrome with EHBDO

Case no	Characteristic facies		Posterior embryotoxon		Cardiovascular abnormalities		Butterfly vertebrae	
	At diagnosis	At LT	At diagnosis	At LT	At diagnosis	At LT	At diagnosis	At LT
1	n/a	+	–	–	PPS	PPS	+	+
2	+	+	–	+	–	PPS	+	+
3	–	+	–	+	–	PPS	+	+
4	+	+	–	–	PPS	PPS	–	–
5	n/a	+	–	–	PPS	PPS	–	–
6	–	+	+	+	VSD	VSD	–	–
Total (*n*, %)	2 (50.0)	6 (100)	1 (16.7)	3 (50.0)	4 (66.7)	6 (100)	3 (50.0)	3 (50.0)

EHBDO extrahepatic bile duct obstruction, *LT* liver transplantation, *n/a* not available, *PPS* peripheral pulmonary stenosis, *VSD* ventricular septal defect

subtype "a", or a patent common bile duct, had a histopathologically normal gallbladder wall structure without inflammation, and no inflammation around the location of the absent hepatic duct (cases 2–4) (Fig. 3). The other three patients with subtype "b", or fibrous common bile duct, showed chronic gallbladder wall inflammation with deprivation of the wall structure, and chronic inflammation around the obstruction/absence of the hepatic duct (cases 1, 5 and 6). There were no patients with subtype "c", or aplasia of the common bile duct.

KPE was performed in cases 1–5. KPE was also first attempted in case 6, but was discontinued, because KPE was judged to be ineffective due to the proximal hepatic duct aplasia. Eventually, all patients required LT due to an exacerbation of Jaundice and failure to thrive, and were diagnosed with AGS before LDLT, as described above.

Comparison of the AGS with "*o*" group and BA with "*o*" group

Three of the five patients (60.0%) in the AGS with "*o*" group had premature birth or were small for gestational age (Table 4). All five patients in the BA with "*o*" group had term births following a normal pregnancy, and their birth weights were within the normal range (Table 5). In the neonatal period, Jaundice or acholic stool was noted in all patients in the AGS with "*o*" group (100 vs. 20.0%). The age at cholangiography or KPE tended to be earlier in the AGS with "*o*" group than in the BA with "*o*" group (median 48.0 vs. 73.0 days). Macroscopic liver findings showed cirrhotic liver in four of five patients in the BA with "*o*" group (0 vs. 80.0%). In contrast to the AGS with "*o*" group, four of five patients in the BA with "*o*" group had subtype "c", or aplasia of the common bile duct (Table 5). In other words, four cases in the BA with "*o*" group had aplasia of the entire EHBD. An intraoperative wedge liver biopsy at KPE showed portal-to-portal bridging fibrosis and biliary duct proliferation in all five patients in the BA with "*o*" group (20.0 vs. 100% and 20.0 vs. 100%, respectively). A paucity of interlobular bile ducts was shown in only one patient in the AGS with "*o*" group (case 3).

Discussion

This is the first case series to evaluate the detailed morphological and histopathological findings of the porta hepatis in AGS with EHBDO. This report highlights that hepatic ductal aplasia is common in AGS with EHBDO and that the macroscopic liver findings may be useful for differentiating between BA and AGS with EHBDO.

One of the characteristics of AGS with EHBDO in this case series was aplasia of the proximal hepatic ducts, which was observed in 5/6 patients (83.3%), or subgroup "*o*" in the classification proposed by the Japanese Biliary Atresia Society [6], similarly to the previous case report [3]. Gunadi et al. reported that 4/7 cases (57.1%) of AGS who underwent KPE for the diagnosis of BA were classified into subgroup "*o*" [7]. This is a special subgroup that has been discussed in Japan, including the effectiveness of KPE [8]. However, according to a report from the Japanese Biliary Atresia Society in 2019, subgroup "*o*" was extremely rare, accounting for just 119 (3.5%) of 3356 registered patients with BA [9]. Thus, hepatic ductal aplasia, or subgroup "*o*", may suggest

Table 2 Morphological findings of the cases of Alagille syndrome with EHBDO

Case no	Gestational week	Birth weight (g)	Age at cholangiography or KPE (days)	Main type	Subtype	Subgroup	Macroscopic findings of the liver	Macroscopic findings of gallbladder	Clinical course after KPE
1	33	1110	79	III	b	*ν*	Smooth surface, cholestasis, not cirrhotic liver	Atrophy, white bile	Three more operations were performed for redo-KPE, jejunal perforation, and hemostasis before LT. LT was performed due to an exacerbation of Jaundice and failure to thrive after KPE
2	34	1696	51	III	a	*o*	Smooth surface, cholestasis, not cirrhotic liver	Mild atrophy	LT was performed due to an exacerbation of Jaundice and failure to thrive after KPE
3	35	2064	58	III	a	*o*	Smooth surface, dark red, not cirrhotic liver	Mild atrophy	LT was performed due to an exacerbation of Jaundice and failure to thrive after KPE
4	38	2242	34	III	a	*o*	Smooth surface, not cirrhotic liver	Mild atrophy, white bile	LT was performed due to an exacerbation of Jaundice and failure to thrive after KPE
5	39	2684	37	III	b	*o*	Smooth surface, not cirrhotic liver	Atrophy, some inflammatory findings	LT was performed due to an exacerbation of Jaundice and failure to thrive after KPE
6	40	2798	48	III	b	*o*	Smooth surface, dark red, not cirrhotic liver	Atrophy, white bile	KPE was not performed. LT was performed due to an exacerbation of Jaundice and failure to thrive

EHBDO extrahepatic bile duct obstruction, *KPE* Kasai portoenterostomy, *LT* liver transplantation

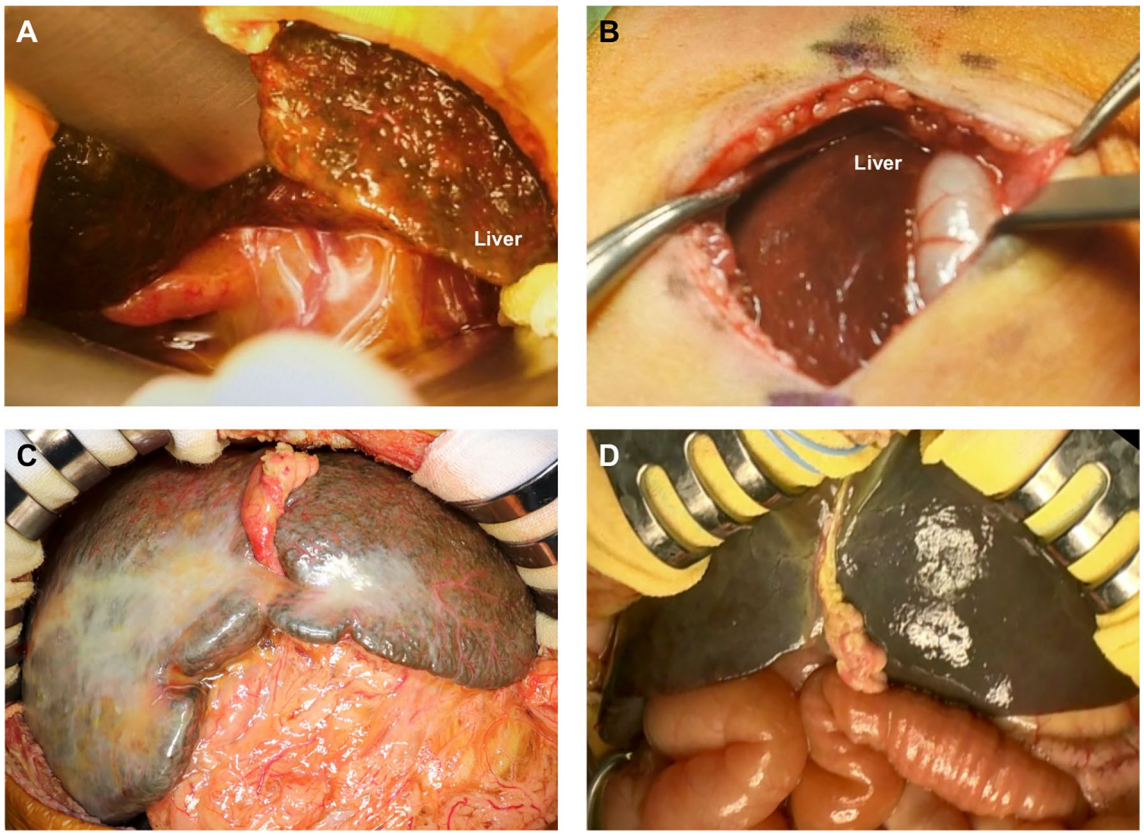

Fig. 2 Macroscopic liver findings in BA and the AGS with EHBDO at KPE and LT. Liver cirrhosis was grossly observed in BA at KPE (**A**) and LT (**C**). In AGS with EHBDO, the macroscopic findings showed cholestasis, but no cirrhosis at KPE (**B**) or LT (**D**). *BA* biliary atresia, *AGS* Alagille syndrome, *EHBDO* extrahepatic bile duct obstruction, *KPE* Kasai portoenterostomy, *LT* liver transplantation

AGS with EHBDO. Pediatric surgeons should strongly suspect AGS with EHBDO if any signs of AGS are combined with hepatic ductal aplasia.

In Japan, where needle liver biopsies in infants are uncommon as a screening procedure, in contrast to Western countries, the differential diagnosis of conjugated hyperbilirubinemia by early cholangiography and a wedge liver biopsy simultaneously is commonly performed to ensure the timely conduct of KPE for BA [10]. However, it should be reaffirmed that cholangiography alone does not clearly distinguish between AGS with EHBDO and BA, as was the case in the present study. A previous report showed that on cholangiography for AGS suspected BA, the proximal extrahepatic tree was normal in 26% of cases, small or hypoplastic in 37%, and not visualized in 37% [11]. Furthermore, AGS with EHBDO patients tended to have acholic stool in the neonatal period, which led to early cholangiography and a wedge liver biopsy. It has already been shown that the pathological findings do not clearly show the characteristic findings of BA and AGS in early infancy [11, 12]. On the other hand, as recently reported by Okazaki et al. the macroscopic findings of the liver may be useful for the differential diagnosis of BA and AGS [10]. Patients with AGS rarely progress to cirrhosis and exhibit less fibrosis than other chronic liver diseases due to impaired Notch signaling, which plays an important role in the liver fibrotic response [13]. In fact, at exploratory laparotomy, none of the patients had cirrhosis and the liver surface was smooth and soft in all cases. Thus, the combination of the classic clinical findings of AGS, the EHBD morphology, and the gross findings of the liver may allow for the early diagnosis of AGS with EHBDO. Although the macroscopic findings of the liver are still completely inadequate as an objective assessment

Table 3 Pathological findings of the cases of Alagille syndrome with EHBDO

Case no	Pathological findings of the extrahepatic bile duct	Pathological findings of the gallbladder	Liver biopsy at the diagnosis				Explanted liver at liver transplantation			
			Inflammation at portal area	P–P bridging fibrosis	Bile duct proliferation	Paucity of interlobular bile ducts	Inflammation at portal area	P–P bridging fibrosis	Bile duct proliferation	Paucity of interlobular bile ducts
1	Obstruction of HD/chronic inflammation	Extensive deprivation of epithelium/chronic inflammation	+	+	+	–	+	+	+	+
2	Absence of HD/no inflammation	Normal wall structure/no inflammation	–	–	–	–	+	–	–	+
3	Absence of HD/chronic inflammation	Normal wall structure/no inflammation	–	–	–	+	+	–	–	+
4	Absence of HD/no inflammation	Normal wall structure/no inflammation	–	–	–	–	+	–	–	+
5	Absence of HD/chronic inflammation	Extensive deprivation of epithelium/chronic inflammation	+	–	–	–	+	+	–	+
6	Absence of HD/chronic inflammation[a]	Extensive deprivation of epithelium/chronic inflammation	+	+	+	–	+	+	+	+

EHBDO extrahepatic bile duct obstruction, *HD* hepatic duct, *P–P* bridging fibrosis, portal-to-portal bridging fibrosis

[a]A specimen of extrahepatic bile duct resected at the time of liver transplantation was evaluated because of the unperformed Kasai portoenterostomy

method, these findings are worthy of further validation and may become a new adjunct diagnostic indicator for differentiation between BA and other cholestatic diseases.

In this case series, the morphological and histopathological findings of the porta hepatis suggested that the pathogenesis of EHBDO in BA and AGS might differ. Our cohort showed that the morphological findings of the common bile duct differed obviously between AGS with EHBDO and BA with "*o*" group. The patients in BA with "*o*" group, including biliary atresia splenic malformation (BASM) syndrome, were more likely to develop aplasia of the entire extrahepatic bile duct (subtype "c" with subgroup "*o*") (Table 5) [14], whereas in patients with AGS and EHBDO, only the proximal bile duct showed aplasia, and common bile duct formation was observed (subtype "a" or "b", with subgroup "*o*"). This morphological difference of EHBD is suggested to result from differences in embryological pathogenesis.

Although the developmental junctional process of the intrahepatic and extrahepatic ducts, which have different origins, is not yet clear, it is considered to share a common gene expression background [15]. One possible cause of the development of EHBDO in AGS is a developmental abnormality of this junctional process. Particularly in cases 2–4, where there was no inflammation in the porta hepatis or gallbladder and no gross abnormality in the common bile duct (subtype "a"), only a congenital abnormality in the junctional process was likely to have caused EHBDO. On the other hand, cases 1, 5 and 6 had fibrous common bile duct obstruction (subtype "b") and histopathologically proven chronic inflammation in the gallbladder wall and EHBD. The severe hypoplasia of proximal hepatic duct might have been originally present from the fetal stage, which just happened to be combined with some unexplained inflammation to cause atrophy of the gall bladder and obstruction of the common bile duct, because these patients also had prolonged acholic stools from the early postnatal period. Importantly, these cases with inflammation in the porta hepatis (cases 1, 5 and 6) are clinically more difficult to distinguish from BA, because even liver biopsy showed portal cellular infiltration and portal-to-portal bridging fibrosis, as well as BA, and the paucity of the interlobular bile ducts of AGS was not noticeable in the early period (Table 3). A detailed examination of the pathogenesis and new indicators may be needed for the early differential diagnosis of AGS with EHBDO and BA.

This case series showed that AGS was associated with morphologically and histopathologically proven EHBDO. There is still no effective way to differentiate AGS with EHBDO from BA at an early stage, and the only way to make an appropriate differential diagnosis is to synthesize existing diagnostic techniques and macroscopic findings, including those of the porta hepatis and liver. Since liver

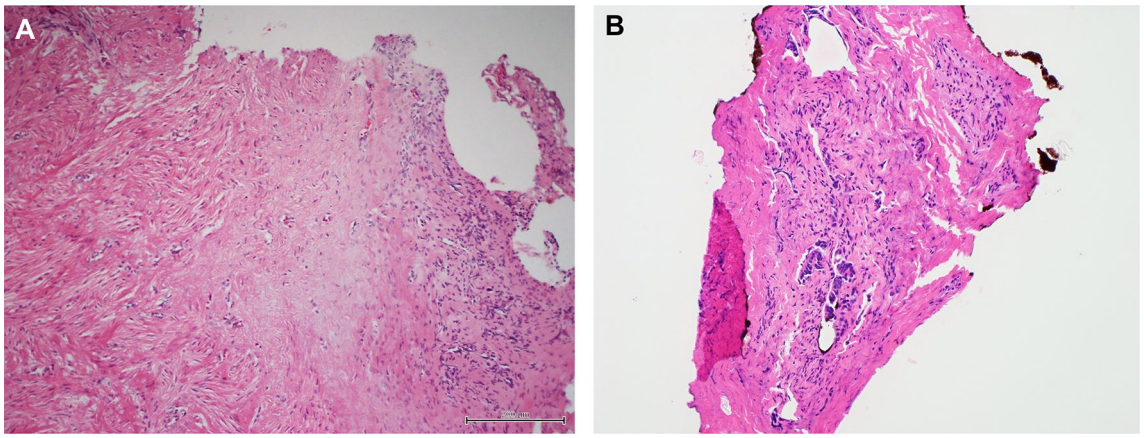

Fig. 3 Pathological findings of the porta hepatis at Kasai portoenterostomy in AGS with EHBDO cases. The pathological findings of the resected tissue of the porta hepatis showed no inflammation around the location of the absent hepatic duct at Kasai portoenterostomy (cases 2–4) (**A**). In contrast, the other three patients had chronic inflammation around the obstruction/absence of the hepatic duct (case 1, 5 and 6) (**B**). *AGS* Alagille syndrome, *EHBDO* extrahepatic bile duct obstruction

Table 4 Characteristics of the AGS with "*o*" group and BA with "*o*" group before KPE

	AGS with "*o*" group (*n* = 5)	BA with "*o*" group (*n* = 5)
Premature birth or small for gestational age (*n*, %)	3 (60.0)	0 (0)
Male:female	3:2	2:3
Jaundice or acholic stool in the neonatal period (*n*, %)	5 (100)	1 (20.0)
Gallbladder atrophy on ultrasonography (*n*, %)	5 (100)	5 (100)
No biliary excretion in biliary scintigraphy (*n*, %)	3/3 (100)	2/2 (100)
The age at cholangiography or KPE (days)	48.0 (37.0–51.0)	73.0 (47–102)
Macroscopic cirrhotic liver (*n*, %)	0 (0)	4 (80.0)
Liver biopsy		
P–P bridging fibrosis (*n*, %)	1 (20.0)	5 (100)
Bile duct proliferation (*n*, %)	1 (20.0)	5 (100)
Paucity of interlobular bile ducts (*n*, %)	1 (20.0)	0 (0)

Values are presented as the median; values in brackets represent the interquartile range (IQR)

AGS Alagille syndrome, *KPE* Kasai portoenterostomy, *P–P* bridging fibrosis, portal-to-portal bridging fibrosis

Table 5 Characteristics and morphological findings in BA with subgroup "*o*" cases

Case no	Gestational week	Birth weight (g)	Age at cholangiography or KPE (days)	Main type	Subtype	Subgroup	Clinical course
7	37	2912	31	III	a	*o*	Jaundice improved temporarily, but LT was performed due to repeated cholangitis
8	38	3210	47	III	c	*o*	BASM syndrome Redo-KPE was performed and failed. LT was performed due to exacerbation of Jaundice and failure to thrive after KPE
9	39	3456	73	III	c	*o*	BASM syndrome Redo-KPE was performed and failed. LT was performed due to exacerbation of Jaundice and failure to thrive after KPE
10	39	2766	102	III	c	*o*	KPE was not performed. LT was performed due to exacerbation of Jaundice and failure to thrive
11	39	2586	145	III	c	*o*	LT was performed due to acute exacerbation of liver failure with hyperammonemia

BA biliary atresia, *KPE* Kasai portoenterostomy, *BD* bile duct, *CBD* common bile duct, *LT* liver transplantation, *BASM syndrome* biliary atresia splenic malformation syndrome

transplantation is likely to be necessary in the future, it is important for a comprehensive team of pediatric hepatologists, pediatric surgeons, and transplant surgeons to monitor the clinical course and decide on a treatment plan.

Author contributions MT: designed the study and wrote the draft of the manuscript; SS: helped design the study and critically revised the article for clinical content; HU, SS, YY, AF, TY: collected the data; MK: contributed to the study design and critical revision of the article for clinical content.

Declarations

Conflict of interest All authors declare no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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