CD56-immunostaining of the extrahepatic biliary tree as an indicator of clinical outcome in biliary atresia: a preliminary report

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The aim of this study was to evaluate the usefulness of CD56-immunostaining using extrahepatic biliary tree (EHBT) specimens as a predictive factor in biliary atresia (BA). The staining intensity of CD56-stained specimens was scored in 13 children with BA as follows: 0 = no staining, 1 = weak staining, 2 = moderate staining, and 3 = strong staining. There was strong (score 3) staining of CD56 in the EHBT specimens of 4 patients with more than 1.5 mg/dl of serum total bilirubin in accordance with a decrease of jaundice. Weak staining of CD56 was seen in the EHBT specimens of patients with no or only one episode of postoperative cholangitis. Strong staining of CD56 was noted in the EHBT specimens of 4 patients who underwent liver transplantation. Our present findings support the possibility of discriminating between good and poor prognoses of clinical outcome after Kasai portoenterostomy.

Key words: CD56, biliary atresia, extrahepatic biliary tree, liver biopsy.

The Kasai operation is an accepted method of achieving bile drainage in biliary atresia (BA)[1]. Reports from several institutions in Japan show that more than 80% of BA patients become jaundice-free after the Kasai operation[1]. A favorable course depends essentially on early surgical therapy[2]. However, progressive liver disease develops in a few patients with successful Kasai operations, and liver transplantation is needed for patients with frequent postoperative cholangitis and liver cirrhosis. There are presently no clear clinical markers or laboratory findings that can predict the future need for liver transplantation at the time of the Kasai operation.

CD56 (a neural cell adhesion molecule, N-CAM) is a marker of immature cells committed to the biliary lineage and is expressed by reactive bile ductules in human liver disease[3]. Davenport[4] and Azar[5] reported that liver biopsy findings using CD56-immunostaining were important in diagnosing BA. Currently, little information is available regarding the role of CD56-immunostaining as a predictive factor regarding BA pathophysiology[6,7]. The aim of this study was to characterize the immunohistologic features of CD56 in both the liver and resected extrahepatic biliary trees (EHBTs), and to investigate whether there is an association between CD56-immunostaining in the liver and resected EHBTs and the clinical outcome after Kasai portoenterostomy, as a preliminary study.

Material and Methods

Materials and Tissue Specimens

Thirteen children with BA were identified via surgical cholangiographic findings obtained by exploratory surgery between 1999 and 2005. A retrospective review of case notes and pathological reports was carried out. As the definition of BA, the surgical cholangiogram obtained by means of gallbladder puncture or intraoperative surgical exploration showed an

obliteration or discontinuity of the biliary ducts. Furthermore, contrast medium was extravasated into the periportal space without filling of the intrahepatic bile trees within the liver in BA[8].

There were 10 girls and 3 boys, and thus a female predominance. The age of the patients at the time of the operation for BA ranged from 30 to 182 days after birth (mean: 72 days after birth). Two patients had type I (the Japanese Association of Pediatric Surgeons classification)[9] cystic BA and 11 had type III BA.

The extrahepatic biliary structures are totally excised en bloc, and the fibrous cone transected at the liver hilus is anastomosed to a Roux-en-Y jejunal limb according to hepatic portoenterostomy (the Kasai procedure)[10]. The microscopic biliary structures contained within transected fibrous tissue drain bile into the intestinal conduit. Over time, auto-anastomosis occurs between the intestinal and ductal epithelial elements and provides biliary drainage.

Specimens measuring approximately 1 cm^3 were taken from the surface of the right lobe of the liver at the same time as the radical operation in all patients, and the pathology (including histology) of these surgically excised specimens was examined.

Immunohistochemistry of the CD56-Stained Liver Biopsy and EHBTs

The selected specimens were fixed in 10% buffered formalin and processed for histology in the usual manner. Immunohistochemistry was performed on the paraffin-embedded tissue. The thin-sliced materials were immunostained with antibody against CD56 (clone 123C3, diluted 1:100, Zymed, San Francisco, CA) using an autoimmunostainer (Ventana Medical Systems, Tucson, AZ, USA) after heat-induced antigen retrieval using the pressure-cooking method. Immunohistological findings of the CD56-stained liver biopsy specimens, especially with regard to the staining in the interlobular bile ducts and ductules, were classified into four categories according to the staining intensity[6]. Sections were counterstained with hematoxylin. The staining intensity was scored as follows: 0 = no staining, 1 = weak staining, 2 = moderate staining, and 3 = strong staining. CD56 expression as assessed by multiple organ sausage block, including the central nervous system, served as a positive control[11].

Clinical Methods

Our standard postoperative regimen consists of oral antibiotics for three months, fat-soluble vitamin supplementation, and ursodeoxycholic acid. All infants underwent routine follow-up at Hokkaido University Hospital by clinicians blinded to the results of the immunohistochemical study. An assessment of biochemical liver function was made six months after the operation. For this study, clearance of jaundice was defined as a serum total bilirubin level of less than 1.5 mg/dl.

We studied the liver specimens and resected EHBT specimens with BA, and classified them immunohistologically according to the CD56-staining intensity. We statistically evaluated parameters such as the age of the patient at the time of Kasai operation, number of postoperative cholangitis attacks, number of patients who cleared hyperbilirubinemia, and number of patients with a need for liver transplantation between the No-Weak staining and Moderate-Severe staining groups using Mann-Whitney U test and Fisher's exact probability test. A p value of less than 0.05 was considered significant.

Results

The results of the immunohistological examination of CD56 staining in both the liver and resected EHBTs according to the operative days after birth are shown in Figure 1. In all liver biopsy specimens, the adequacy of CD56 staining was confirmed by the positivity of peripheral nerve fibers as an internal positive control. The staining intensity in the interlobular bile ducts and ductules varied irrespective of the age at operation.

There was strong (score 3) staining of CD56 in the resected EHBT specimens of four patients with more than 1.5 mg/dl of serum total bilirubin in accordance with a decrease of jaundice (Fig. 2). Weak (score 0) staining of CD56 was seen in resected EHBT specimens of patients with no or only one incidence of postoperative cholangitis (Fig. 3). In contrast, strong (score 3) CD56 staining was observed in resected EHBT specimens of four patients who underwent liver transplantation (Fig. 4).

In this study, there were a significant number of patients who cleared icterus ($p=0.0291$) and a number of patients who needed liver

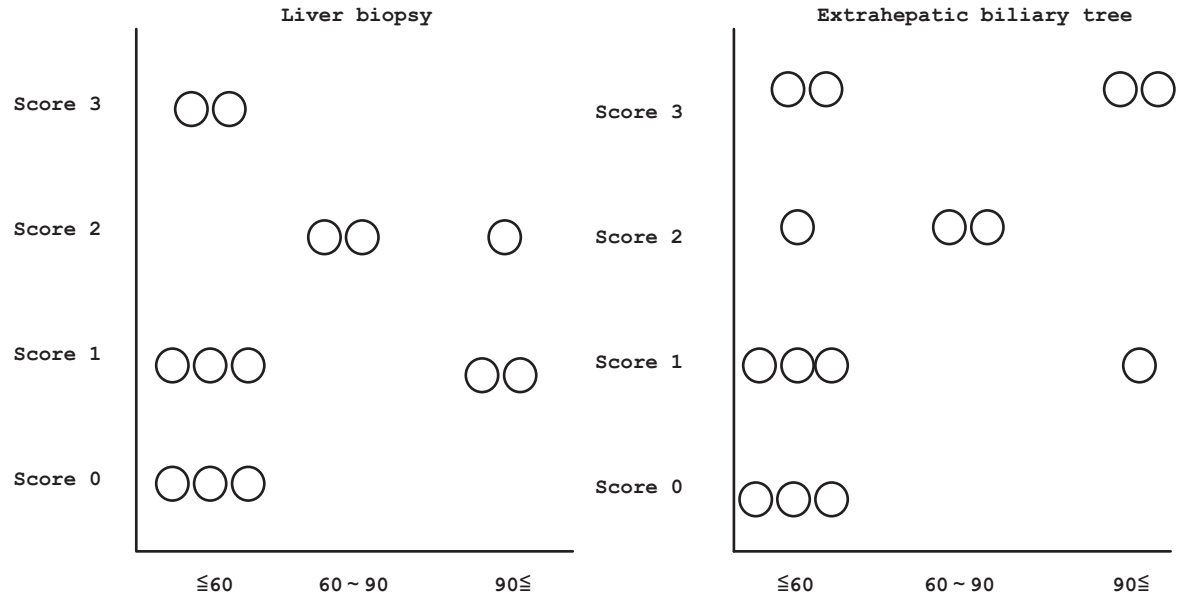

Fig. 1. Age at Kasai operation.

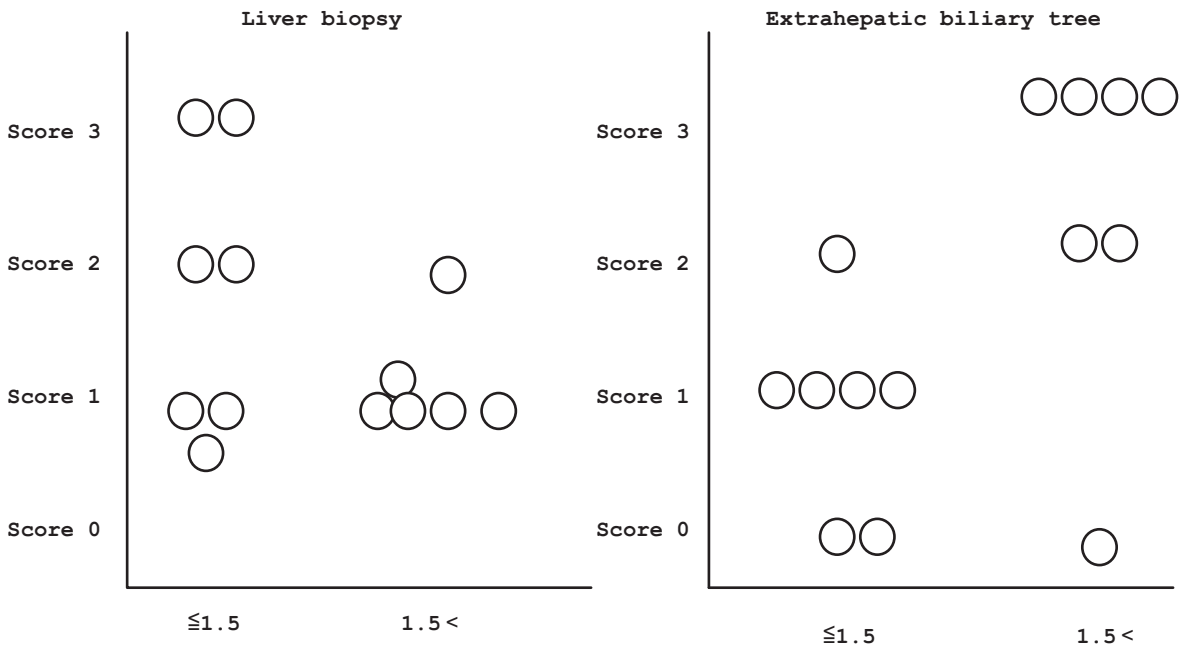

Fig. 2. T-bil (mg/dl)

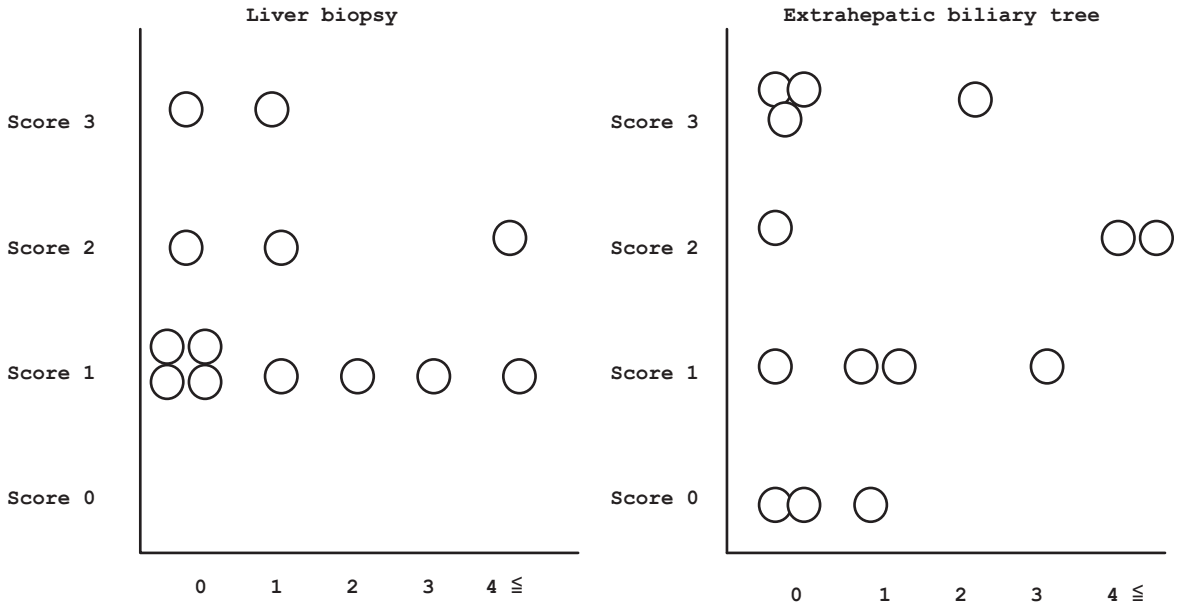

Fig. 3. Frequency of postoperative cholangitis.

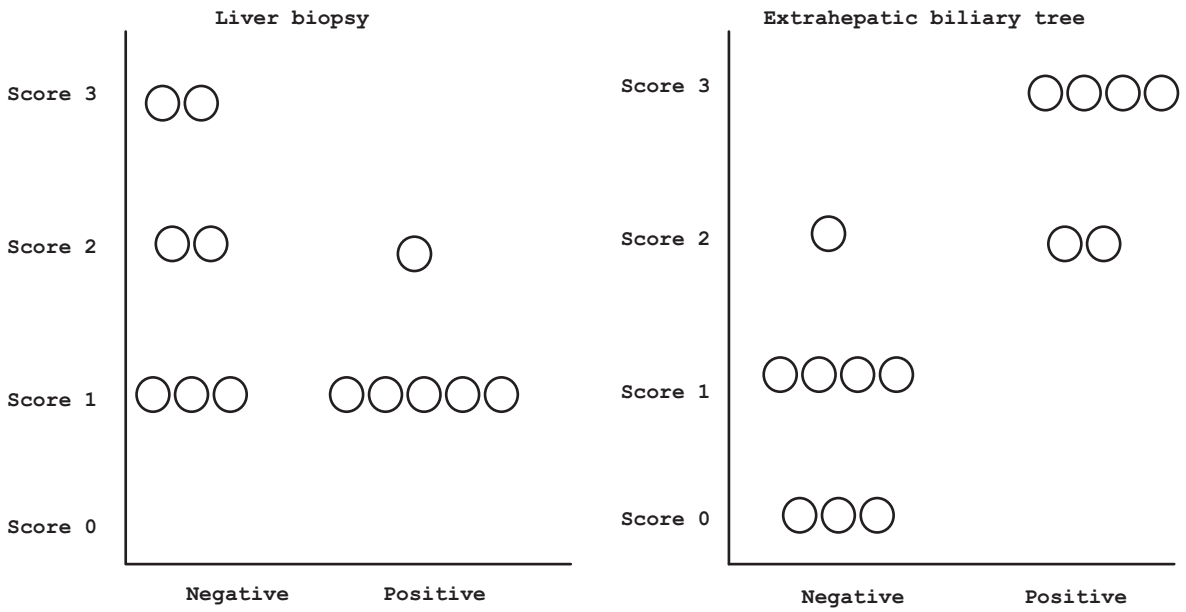

Fig. 4. Liver transplantation.

transplantation (p=0.0047) according to the CD56-immunostaining intensity of EHBT specimens. No immunoreactivity for CD56 was observed in the hepatic parenchyma of this group. These data are summarized in Tables I and II.

Table I. Clinical Data and CD56-Immunostaining Intensity of Liver Biopsy Specimens

	No-Weak staining (score 0-1) (n=8)	Moderate-Strong staining (score 2-3) (n=5)	p value
Operation day (mean)	48-182 (75)	30-99 (64)	0.5485
No. of postop cholangitis attacks (mean)	0-6 (2)	0-6 (2)	0.9417
No. of patients who cleared icterus	3	4	0.2657
No. of patients who needed liver transplantation	4	1	0.2929
No. of patients with cirrhosis at operation	2	2	>0.9999

Table II. Clinical Data and CD56-Immunostaining Intensity of EHBT Specimens

	No-Weak staining (score 0-1) (n=7)	Moderate-Strong staining (score 2-3) (n=7)	p value
Operation day (mean)	30-99 (57)	42-182 (87)	0.1252
No. of postop cholangitis attacks (mean)	0-3 (1)	0-6 (2)	0.8480
No. of patients who cleared icterus	6	1	0.0291
No. of patients who needed liver transplantation	0	6	0.0047
No. of patients with cirrhosis at operation	2	2	>0.9999

We statistically evaluated the number of patients who were operated on earlier than 60 days of age according to a greater expression of N-CAM in liver biopsy and EHBT specimens between the No-Weak staining and Moderate-Severe staining groups by Fisher's exact probability test. P values were 0.2929 for liver biopsy specimens and 0.1434 for EHBT specimens. From these findings, there was no significant difference regarding N-CAM expression of liver biopsy and EHBT specimens according to age at the time of Kasai operation.

Discussion

Biliary atresia occurs in 1/8,000 to 1/14,000 births, accounting for half of all cases of neonatal cholestasis[12], and is a disease that results in progressive sclerosing fibrous obliteration of the extrahepatic bile ducts[13]. If the obstruction is unrelieved, portal and periportal fibrosis will progress, and cirrhosis becomes inevitable[13]. However, surgery is not entirely effective because, even after a successful Kasai operation, a number of patients still progress to liver fibrosis. In patients with BA, early surgery may be required to restore liver function and prevent hepatic fibrosis. The two major milestones toward improved treatment of BA have been the Kasai portoenterostomy and liver transplantation[1].

Clinical outcomes after the Kasai operation can be divided into three categories: patients who continue in a jaundice-free state and reach adulthood with few manifestations of liver disease and portal hypertension; patients who continue in a jaundice-free state but whose quality of life is impaired because of some manifestations of liver disease owing to ongoing cirrhosis, and who thus need follow-up in planning liver transplantation; and patients whose disease process continues unrelentingly, leading to death from cholestatic liver failure within the first two years of life unless successful liver transplantation is achieved[1].

Our hypothesis in this study was that there was an association between CD56- immunostaining using the liver and resected EHBTs and the clinical outcome after Kasai portoenterostomy. Our results showed that the degree of CD56-positivity tended to be characteristically elevated in the resected EHBT specimens of patients with a serum total bilirubin level higher than 1.5 mg/dl, in accordance with a decrease of jaundice and whether the patients

underwent liver transplantation. Furthermore, weak CD56 staining was seen in the resected EHBT specimens of patients with no or only one episode of postoperative cholangitis. Our present findings support the possibility of predicting whether the clinical outcome will be good or poor after Kasai portoenterostomy based on the pathological findings of CD56-immunostained resected EHBT specimens.

Many researchers have focused on immune and inflammatory responses as a trigger of BA pathogenesis, including the aberrant expression of MHC class 2 antigens (e.g., HLA-DR) and intercellular adhesion molecules (ICAM) on bile ductules or hepatocytes[4,14]. Inflammatory cell recruitment to sites of inflammation is dependent upon a complex series of events involving a number of adhesion molecule receptors. This process is dominated by ICAM-1, which serves as the cell surface ligand for the CD-18 integrin receptors lymphocyte-associated antigen-1 (LEA-1) and macrophage-1 (MAC-1)[14]. According to another immunohistopathological study, extensive CD-14 expression was found in Kupffer cells and sinusoidal endothelial cells in nine patients with early-stage BA, presenting as an inflammatory reaction. In six late-stage cases, CD-14 expression at liver transplantation was surprisingly downregulated and no explanation could be found for this observation[15].

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[3,7]. The role of CD56 (N-CAM) in BA pathophysiology remains unclear, as the significance of its expression is clouded by the fact that the etiology of the disease process in BA remains unknown. Davenport et al.[4] reported that the cellular infiltrate in the portal tracts of BA contained a high proportion of natural killer cells staining for CD56. Normal ducts do not express CD56 (N-CAM) on the membranes of the biliary epithelial cells. N-CAM binds in vitro to collagen 4[16], which is an extracellular matrix component expressed around the ductal plate and incorporating bile ducts. Libbrecht[3] reported that diseases characterized by ductal plate malformations in the development of intrahepatic bile ducts were positive for CD56 (N-CAM) and that bile ducts showing complete or patchy immunoreactivity for CD56 (N-CAM) were found in cirrhotic livers[3]. In transgenic mice, overexpression of N-CAM by myofibers leads to the formation of neuromuscular junctions that show excessive sprouting of the nerve terminals compared with those in normal mice[17]. 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[3]. Libbrecht[3] suggested that N-CAM might have a function in the normal development of intrahepatic bile ducts, probably by regulating cell-cell and cell-intercellular matrix interactions during ductal plate remodeling. Based on our findings of strong N-CAM expression on EHBT specimens in groups with elevated postoperative total bilirubin and liver transplantation, we might speculate that the CD56-immunostaining intensity of EHBT specimens could be used as a predictive factor of clinical prognosis at the time of the Kasai operation.

Larger longitudinal studies are required to determine the accuracy of discrimination between BA with good and poor clinical outcomes based on the CD56-immunostaining findings obtained using extrahepatic biliary remnant specimens.

In conclusion, we suggest that immunohistologic examination by CD56-immunostaining using extrahepatic biliary remnant specimens is a reliable test for predicting the outcome of BA after Kasai portoenterostomy.

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