Quantification of Serum Matrix Metallopeptide 7 Levels May Assist in the Diagnosis and Predict the Outcome for Patients with Biliary Atresia

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Objective To assess the diagnostic and prognostic usefulness of the serum matrix metallopeptidase-7 (MMP-7) level for biliary atresia in infants with cholestasis after hepatoportoenterostomy.

Study design We enrolled 100 infants with cholestasis (age, 43.56 ± 1.97 days; 62 males) with a direct bilirubin level of >1 mg/dL, of whom 36 (36%) were diagnosed with biliary atresisa. The MMP-7 levels in serum samples collected during the cholestasis workup and 6 months after hepatoportoenterostomy were assessed by enzyme-linked immunosorbent assay. We quantified liver fibrosis by Picro Sirius red staining of collagen in specimens from the 81 infants with cholestasis.

Results Infants with biliary atresisa had a significantly higher serum MMP-7 level than that of non–biliary atresisa infants with cholestasis of equivalent age ($P < .0001$). Receiver operating characteristic analysis showed that a serum MMP-7 level of >1.43 ng/mL was predictive of biliary atresisa in infants with cholestasis (diagnostic accuracy, 88%). There was a positive correlation between the serum MMP-7 level and the severity of liver fibrosis ($P = .0002$). Survival analysis showed that the frequency of liver transplantation was significantly higher in infants with biliary atresisa with a serum MMP-7 level of >10.30 ng/mL compared with a serum MMP-7 level of ≤10.30 ng/mL after hepatoportoenterostomy (hazard ratio, 4.22; $P = .02$).

Conclusions The serum MMP-7 level, which reflects the severity of liver fibrosis and can be determined noninvasively, may facilitate the diagnosis of biliary atresisa among infants with cholestasis. Moreover, the serum MMP-7 level after hepatoportoenterostomy is associated with a need for liver transplantation in infants with biliary atresisa. *(J Pediatr 2019;208:30-7)*.

See editorial, p 8

Biliary atresia, a progressive fibroinflammatory disease of the intrahepatic and extrahepatic biliary tree, is the most common clinical indication for pediatric liver transplantation globally.[1-4] The clinical presentations of biliary atresisa (persistent jaundice, hepatomegaly, and acholic stools) are similar to those of other cholestatic liver diseases in early infancy.[3,4] This similarity hampers early clinical diagnosis and surgical intervention in infants with biliary atresisa.

Early hepatoportoenterostomy establishes good bile flow and improves the long-term survival of patients with biliary atresisa.[5-7] Early hepatoportoenterostomy is reported to associate with a jaundice-free status 3 months after surgery, a higher rate of native liver survival, fewer cholestatic complications, and better clinical outcomes.[8] However, early diagnosis of biliary atresisa in infants with cholestasis is challenging.[9] Abdominal ultrasound examination, magnetic resonance cholangiopancreatography, and biliary scintigraphy are widely used for the differential diagnosis of biliary atresisa in infants with cholestasis, but their diagnostic accuracies for biliary atresisa are unsatisfactory.[3,10-16] Intraoperative cholangiography, the gold standard for biliary atresisa diagnosis, is an invasive procedure and not suitable for routine use in infants with cholestasis.[1-3]

The assessment of liver stiffness in infants with cholestasis may assist the diagnosis of biliary atresisa,[17,18] suggesting that the progression of liver fibrosis is faster in infants with biliary atresisa than in those with other cholestatic liver diseases.[18] The

AST	Aspartate aminotransferase
ELISA	Enzyme-linked immunosorbent assay
GGT	Gamma-glutamyl transferase
MMP-7	Matrix metallopeptidase-7
NPV	Negative predictive value
PFIC1	Type I progressive familial intrahepatic cholestasis
PPV	Positive predictive value
ROC	Receiver operating characteristic
TPN	Total parenteral nutrition

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intrahepatic matrix metalloproteinase-7 (MMP-7) expression level is reportedly associated with biliary atresisa-related liver fibrosis.[19-22] Indeed, infants with biliary atresisa have higher serum MMP-7 levels than those of other infants with cholestasis.[23] The clinical implications of the serum MMP-7 level, and its relationship with liver fibrosis, are unclear.

In this study, we evaluated the relationship between the serum MMP-7 level and liver fibrosis in infants with cholestasis, and the value of using the serum MMP-7 level to diagnose biliary atresisa. The prognostic power of the serum MMP-7 level in infants with biliary atresisa in terms of cholestatic complications and the need for liver transplantation after hepatoportoenterostomy was also assessed.

Methods

We recruited 100 consecutive infants with cholestasis (62 males) undergoing a workup for cholestasis from January 2008 to April 2018 at the Department of Pediatrics of National Taiwan University Hospital who had available serum samples obtained before the final diagnosis. The serum samples were stored at −80°C immediately after extraction. All of the subjects presented with cholestasis with a serum direct bilirubin level of >1 mg/dL, and all underwent blood tests, urine tests, metabolic workup, abdominal ultrasound examination, and magnetic resonance imaging. Liver biopsy was performed in 81 infants for diagnostic purposes, and intraoperative cholangiography was performed in 51 infants; 36 infants with cholestasis were diagnosed with biliary atresisa by intraoperative cholangiography, all of whom underwent hepatoportoenterostomy. All patients in our institution undergo a standard clinical follow-up schedule after a cholestatic workup. The serum total and direct bilirubin levels and the aspartate aminotransferase (AST), alanine aminotransferase, gamma-glutamyl transferase (GGT), and alkaline phosphatase levels were assessed during the clinical follow-up. The study protocol was approved by the Institutional Review Board of National Taiwan University Hospital.

Calculation of the AST to Platelet Ratio Index

The AST to platelet ratio index is a simple method of assessing liver fibrosis in patients with biliary atresisa.[24,25] In this study, the first AST and platelet data available during the cholestatic workup were used to calculate the AST to platelet ratio index score.

Serum MMP-7 Level Measurement

The serum MMP-7 level was determined in the 100 infants with biliary atresisa using a sensitive sandwich enzyme-linked immunosorbent assay (ELISA; DuoSet, R&D Systems, Inc, Minneapolis, Minnesota). The serum MMP-7 levels of 32 infants with biliary atresisa (88.89%) at 6.74 ± 1.24 months after hepatoportoenterostomy were also evaluated. All samples were assayed in triplicate, and the mean serum MMP-7 level was analyzed.

Picro Sirius Red Staining in Liver Specimens

Liver fibrosis was assessed histologically by Picro Sirius red (Abcam, Cambridge, Massachusetts) staining of collagen in the liver specimens of 81 infants (81%) obtained by needle biopsy during the cholestatic workup or by wedge biopsy during surgery. Deparaffinized sections were incubated for 60 minutes with Picro Sirius red solution (Abcam) followed by a brief rinse with acetic acid (0.05%). Sections were dehydrated by washing with absolute alcohol and observed under a light microscope (Axioplan2; Carl Zeiss, Hallbergmoos, Germany). The intensity of Picro Sirius red staining was quantified in 10 randomly selected high-power fields per section using ImageJ software (Bethesda, Maryland); the data are expressed as the proportion of the total tissue area stained with Picro Sirius red.

Statistical Analyses

STATA (version 14; StataCorp, College Station, Texas) and MedCalc (version 18.6; MedCalc Software, Ostend, Belgium) were used for the statistical analyses. For continuous variables, the Student *t* test was used to assess differences in the means and 95% CIs between the 2 groups. The Fisher exact test or χ^2 test was performed to assess differences in incidence between groups. Receiver operating characteristic (ROC) analyses were performed to determine the optimal cutoff value and area under the curve. The positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of these cutoff levels were assessed. ANOVA was performed to evaluate the significance of the differences among multiple groups.

Univariate and multivariate logistic regression was used to assess the OR and 95% CI for predicting biliary atresisa. The linear regression analysis was also used for data analysis. A Cox proportional analysis, Kaplan-Meier plot, and log-rank test were applied to assess survival and the need for liver transplantation. The primary outcome was a diagnosis of biliary atresisa, and the secondary outcome was the cholestatic complications (thrombocytopenia, splenomegaly, esophageal varices 6 months after hepatoportoenterostomy, and liver transplantation in biliary atresisa subjects). A *P* value of <.05 was regarded as statistically significant.

Results

General Characteristics of the Subjects

Of the 100 subjects, biliary atresisa was diagnosed in 36 infants, neonatal hepatitis in 44, Alagille syndrome in 6, total parenteral nutrition-related cholestasis in 3, neonatal intrahepatic cholestasis caused by citrin deficiency in 2, urinary tract infection in 2, choledochal cyst in 2, hepatic congestion related cholestasis in 2, type I progressive familial intrahepatic cholestasis [PFIC1] in 1, cystic fibrosis in 1, and inborn error of bile acid synthesis in 1 infant.

The 36 infants with biliary atresisa had higher serum GGT and MMP-7 levels than did those without biliary atresisa of similar age ($P < .0001$; **Table I**). The serum MMP-7 levels of the infants with biliary atresisa are shown in **Figure 1**, A. The serum MMP-7 level was significantly higher in

Table I. General baseline characters of the study population between biliary atresia and non–biliary atresisa infants with cholestasis groups analyzed in this study

	Nonbiliary atresisa (n = 64)	Biliary atresisa (n = 36)	*P* value
Age, d	44.23 ± 2.36 (39.53-48.94)	42.36 ± 3.56 (35.13-49.59)	.65
MMP-7, ng/mL	1.09 ± 0.18 (0.74-1.45)	10.36 ± 1.12 (8.08-12.63)	<.0001
T-bil, mg/dL	7.65 ± 0.35 (6.95-8.35)	8.07 ± 0.56 (6.94-9.21)	.50
D-bil, mg/dL	3.74 ± 0.21 (3.32-4.15)	4.33 ± 0.26 (3.80-4.87)	.08
GGT, U/L	170.33 ± 21.76 (126.85-213.80)	690.67 ± 105.54 (476.41-904.92)	<.0001
ALP, U/L	578.88 ± 43.85 (491.24-666.51)	486.64 ± 51.34 (382.40-590.87)	.19
AST, U/L	124.05 ± 11.93 (100.22-147.88)	159.67 ± 21.41 (116.21-203.13)	.12
ALT, U/L	84.06 ± 9.78 (64.52-103.61)	108.67 ± 17.22 (73.71-143.63)	.18
APRI	1.03 ± 0.16 (0.71-1.36)	0.92 ± 0.16 (0.60-1.24)	.66
Collagen in liver specimens stain by Picro Sirius red, %*	6.56 ± 0.66 (5.22-7.89)	13.35 ± 1.26 (10.79-15.90)	<.0001
Male sex	48 (75%)	14 (38.89%)	.0004
Liver biopsy performed	45 (70.31%)	36 (100%)	.0001

ALP, Alkaline phosphatase; *ALT*, alanine aminotransferase; *APRI*, AST to platelet ratio index; *D-bil*, direct bilirubin; *T-bil*, total bilirubin.
Values are mean ± SE (95% CI) or n (%).
*There are 45 infants with cholestasis without biliary atresia and 36 infants with biliary atresisa who underwent a liver biopsy in this study cohort.

the infants with biliary atresisa (median, 10.26 ng/mL; IQR, 3.38-15.24 ng/mL) than in those with Alagille syndrome (median, 0.47 ng/mL; IQR, 0.42-1.15 ng/mL), choledochal cyst (median, 0.83 ng/mL; IQR, 0.75-0.92 ng/mL), cystic fibrosis (median, 0.64 ng/mL), congestive heart disease (median, 1.13 ng/mL; IQR, 1.09-1.18 ng/mL), inborn error of bile acid synthesis (median, 1.11 ng/mL), neonatal hepatitis (median, 0.76 ng/mL; IQR, 0.51-1.21 ng/mL), neonatal intrahepatic cholestasis caused by citrin deficiency (median, 0.89 ng/mL; IQR, 0.14-1.63 ng/mL), total parenteral nutrition cholestasis (median, 0.62 ng/mL; IQR, 0.44-1.49 ng/mL), and urinary tract infection–related cholestasis (median, 0.64 ng/mL; IQR, 0.37-0.90 ng/mL). The 1 infant with PFIC1 had a serum MMP-7 level of 11.09 ng/mL in this cohort. The serum MMP-7 level did not differ in samples collected before vs after 2013 from the infants with cholestasis (n = 25 vs n = 75; MMP-7 level, 4.65 ± 1.23 ng/mL vs 4.35 ± 0.71 ng/mL; $P = .84$). The serum MMP-7 level also did not differ in samples collected before vs after 2013 from the infants with biliary atresisa (n = 12 vs n = 24; MMP-7 level, 8.60 ± 2.03 ng/mL vs 11.23 ± 1.33 ng/mL; $P = .27$).

The serum bilirubin, AST, alanine aminotransferase, AST to platelet ratio index, and alkaline phosphatase levels were not different between the biliary atresisa and non–biliary atresisa groups ($P > .05$). The prevalence rate of female sex was higher in those with biliary atresisa than in those without biliary atresisa ($P = .0004$).

Diagnostic Role of the Serum MMP-7 Level for Biliary Atresisa

The ROC analysis showed that a serum MMP-7 level of >1.43 ng/mL was optimal for predicting biliary atresisa (sensitivity, 97.30%; specificity, 83.20%; $P < .001$; Figure 1, B; Table I). The PPV, NPV, and diagnostic accuracy of a serum MMP-7 level of >1.43 ng/mL for biliary atresisa were 76.07%, 98.15%, and 88%, respectively. The serum MMP-7 level was significantly higher in samples obtained from infants with biliary atresisa at >30 days of age (n = 22) compared with ≤30 days of age (n = 14; 12.33 ± 1.31 ng/mL vs 7.25 ± 1.77 ng/mL; 95% CI, 9.61-15.05 ng/mL vs 3.43-11.07 ng/mL; $P = .02$; Figure 1, C).

The ROC analysis showed that a GGT level of >216 IU/mL is optimal for predicting biliary atresisa (sensitivity, 83.33%; specificity, 84.37%; Figure 1, D). The PPV, NPV, and diagnostic accuracy of a GGT level of >216 IU/L for biliary atresisa were 69.77%, 89.47%, and 81%, respectively.

A serum MMP-7 level of >1.43 ng/mL (OR, 168.64; 95% CI, 20.83-1364.98; $P < .0001$) and GGT level of >216 IU/L (OR, 19.61; 95% CI, 6.75-57.03; $P < .0001$) were predictive of biliary atresisa in univariate logistic regression analyses (Table II). The power of a serum MMP-7 level of >1.43 ng/mL and a GGT level of >216 IU/L to predict biliary atresisa remained significant in a multivariate logistic regression analysis (OR, 178.03 and 23.59; $P < .001$ and $P < .001$, respectively; Table II).

The PPV, NPV, and diagnostic accuracy of a serum MMP-7 level of >1.43 ng/mL or a GGT level of >216 IU/L for biliary atresisa were 62.07%, 100%, and 78%, respectively. The PPV, NPV, and diagnostic accuracy of a serum MMP-7 level of >1.43 ng/mL combined with a GGT level of >216 IU/L for biliary atresisa were 93.55%, 89.86%, and 91%, respectively.

Correlation Between the Serum MMP-7 Level and Liver Fibrosis

Picro Sirius red staining showed that the abundance of collagen in liver specimens from 81 infants with cholestasis (81%) was significantly higher in the infants with biliary atresisa than in those without biliary atresisa of similar age (13.35 ± 1.26% vs 6.56 ± 0.66%; $P < .0001$; Table I). There was a positive correlation between the serum MMP-7 level and percentage of collagen in liver specimens ($P = .0002$; Figure 2, A).

Serum MMP-7 Level in Infants with Biliary Atresisa before and after Hepatoportoenterostomy

Among the 36 infants with biliary atresisa, 12 subsequently received liver transplantation at 1.52 ± 0.46 years of age (95% CI, 0.51-2.53 years). The serum MMP-7 level before hepatoportoenterostomy was not different between the infants

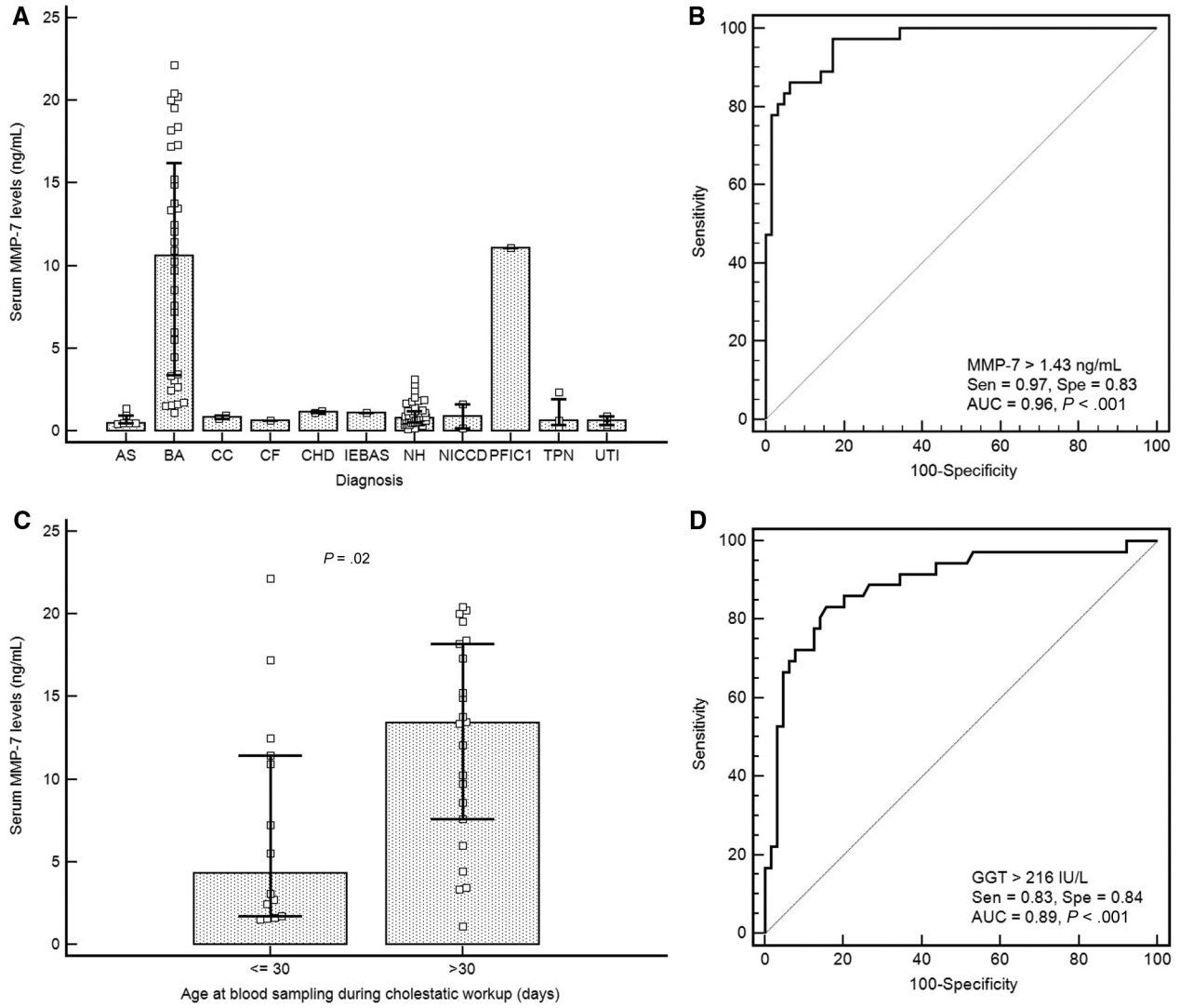

Figure 1. **A**, The serum MMP-7 level was significantly higher in the infants with biliary atresisa than in those without biliary atresisa ($P < .001$, by ANOVA). **B**, ROC analysis showed that a cutoff serum MMP-7 level of >1.43 ng/mL was optimal for predicting biliary atresisa (sensitivity [Sen], 97.30%; specificity [Spe], 83.20%, diagnostic accuracy, 88%; $P < .001$). **C**, The serum MMP-7 levels is significantly higher in infants with biliary atresisa who received a workup at >30 days of age (n = 22) than others at <30 days of age (n = 14; $P = .02$). **D**, The ROC curve analysis showed that a GGT level cutoff of >216 IU/mL is optimal for predicting biliary atresisa among infants with cholestasis in this cohort (sensitivity, 83.33%; specificity, 84.37%; $P < .001$). *AS*, Alagille syndrome; *AUC*, area under the curve; *CC*, choledochal cyst; *CF*, cystic fibrosis; *CHD*, congestive heart disease; *IEBAS*, inborn error of bile acid synthesis; *NICCD*, neonatal intrahepatic cholestasis caused by citrin deficiency; *TPN*, total parenteral nutrition-related cholestasis; *UTI*, urinary tract infection.

with biliary atresisa who did and those who did not undergo liver transplantation (7.84 ± 1.90 ng/mL vs 11.61 ± 1.34 ng/mL; $P = .12$). There was also no significant difference in pre-hepatoportoenterostomy serum MMP-7 level between infants with biliary atresia with and without thrombocytopenia, splenomegaly, or esophageal varices 6 months after hepatoportoenterostomy in this study ($P > .05$).

Thirty-two infants with biliary atresisa had available follow-up serum samples at 6.74 ± 1.24 months (range, 5-7 months) after hepatoportoenterostomy. The frequency of liver transplantation did not differ significantly between the infants with (n = 32) and those without (n = 4) available follow-up serum samples (34.38% vs 25%; $P > .05$). The mean serum MMP-7 levels of the 32 infants with biliary atresisa did not differ before and after hepatoportoenterostomy (9.64 ± 1.17 ng/mL vs 10.77 ± 1.19 ng/mL; 95% CI, 7.24-12.03 ng/mL vs 8.24-13.20 ng/mL, $P > .05$). The serum MMP-7 level of 22 (68.75%) and 10 (31.25%) infants with biliary atresisa decreased and increased, respectively, after hepatoportoenterostomy. There was no significant difference in sex, age at hepatoportoenterostomy, preoperative GGT level, or jaundice-free time postoperatively between the subjects with a decreased and those with an increased serum MMP-7 level after hepatoportoenterostomy ($P > .05$).

Table II. Clinical predictors of biliary atresia among infants with cholestasis

	Univariate		Multivariate	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Female (n = 62) vs male (n = 38)	4.71 (1.96-11.33)	.001	1.29 (0.25-6.70)	.76
GGT >216 IU/L (n = 43) vs ≤216 IU/L (n = 57)	19.61 (6.75-57.03)	<.001	23.59 (4.18-133.14)	<.001
MMP-7 >1.43 ng/mL (n = 46) vs ≤1.43 ng/mL (n = 54)	168.64 (20.83-1364.98)	<.001	178.03 (14.99-2115.13)	<.001

The serum GGT and MMP-7 levels were assessed at the mean age of 43.56 ± 1.97 days of age in this cholestatic cohort. Data were analyzed by logistic regression statistic models.

The Power of the Serum MMP-7 Level to Predict the Need for Liver Transplantation

Among these 32 infants with biliary atresisa with available serum samples 6 months after hepatoportoenterostomy, 11 (34.38%) underwent liver transplantation at 1.61 ± 0.49 years of age (95% CI, 0.51-2.71 years of age) and 21 survived with their native liver until 3.17 ± 0.53 years of age (95% CI, 2.05-4.28 years of age). The infants with biliary atresisa who underwent liver transplantation during the follow-up period (n = 11) had higher serum MMP-7 levels after hepatoportoenterostomy than did those who were not transplanted (n = 21; 13.43 ± 2.48 ng/mL vs 7.65 ± 1.03 ng/mL; 95% CI, 7.91-18.96 ng/mL vs 5.50-9.79 ng/mL; *P* = .01). The infants with biliary atresisa who had an elevated serum MMP-7 level after hepatoportoenterostomy (n = 10) had a higher frequency of liver transplantation than that of those whose MMP-7 level decreased (n = 22; OR, 5.1; 95% CI, 1.02-25.54, *P* = .047).

The ROC analysis showed that a serum MMP-7 level 6 months after hepatoportoenterostomy of >10.30 ng/mL was optimal for predicting the need for liver transplantation in infants with biliary atresisa during the first 3-4 years after hepatoportoenterostomy (PPV, 70%; NPV, 81.82%; diagnostic accuracy, 78.13%; *P* = .03; Figure 2, B). The ROC

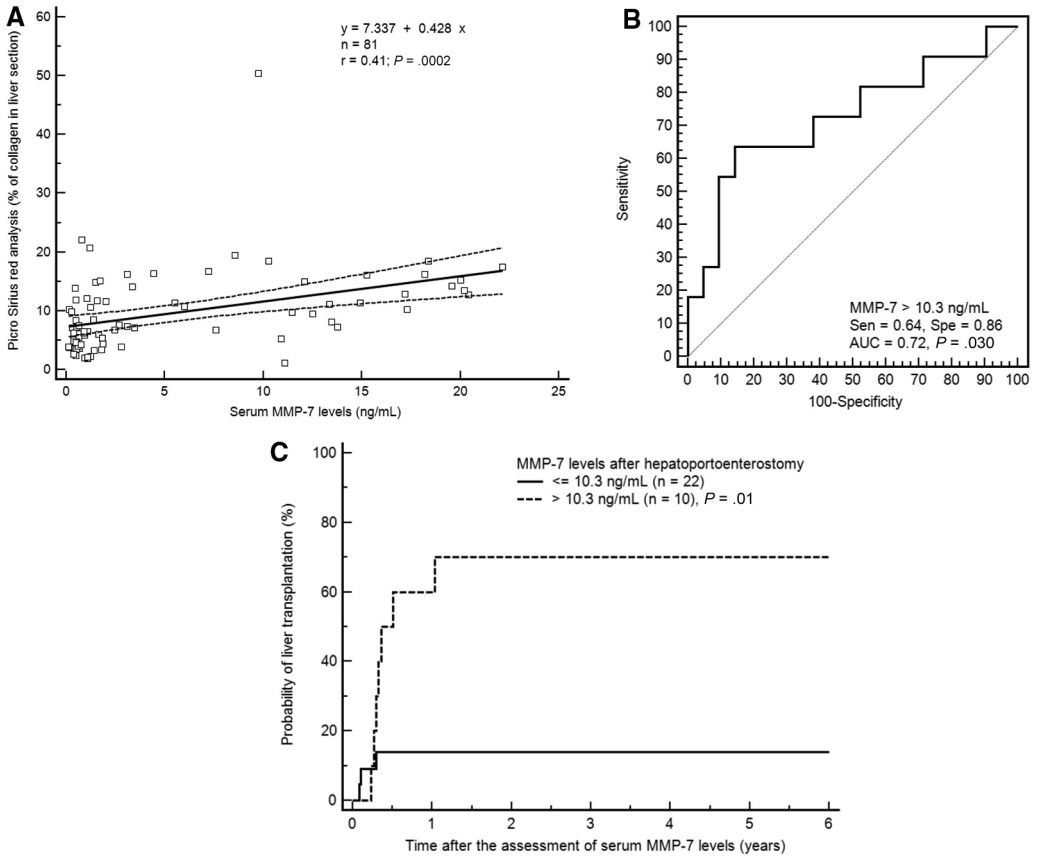

Figure 2. **A**, Positive correlation between serum MMP-7 levels and the percentage of collagen in the liver specimens stained by Picro Sirius red (*P* = .0002). **B**, A ROC curve analysis showed that an MMP-7 value cutoff of >10.30 ng/mL after hepatoportoenterostomy was optimal for predicting liver transplantation in infants with biliary atresisa (sensitivity [Sen], 64.0%; specificity [Spe], 86.0%; diagnostic accuracy, 78.13%; *P* = .03). **C**, A Kaplan-Meier plot showed that the need for liver transplantation was significantly higher in subjects with biliary atresisa with serum MMP-7 levels of >10.3 ng/mL (n = 10) than in those with serum MMP-7 levels of ≤10.3 ng/mL (n = 22) after hepatoportoenterostomy (log-rank test; *P* = .01).

analysis also showed a serum total bilirubin level 6 months after hepatoportoenterostomy of >1.12 mg/dL was predictive of liver transplantation in infants with biliary atresisa during the first 3-4 years after hepatoportoenterostomy (PPV, 75%; NPV, 90%; diagnostic accuracy, 84.38%; $P = .03$).

The univariate Cox proportional survival analysis showed that the risk of liver transplantation was significantly higher in infants with biliary atresisa with a serum MMP-7 level of >10.30 ng/mL (n = 10) than in those with a serum MMP-7 level of ≤10.30 ng/mL (n = 22) after hepatoportoenterostomy (hazard ratio, 4.22; 95% CI, 1.23-14.49; $P = .02$; **Table III**; available at www.jpeds.com). The significance of a serum MMP-7 level >10.30 ng/mL in predicting liver transplantation remains significant in the multivariate Cox proportional survival analysis even adjusting with serum total bilirubin levels (hazard ratio, 4.29; 95% CI, 1.12-16.52; $P = .03$; **Table III**). The Kaplan-Meier plot and log-rank test further yielded consistent results ($P = .01$; **Figure 2**, C).

Discussion

Biliary atresisa is a progressive fibroinflammatory cholangiopathy with an immune-mediated pathogenesis that can lead to rapid progression of liver fibrosis in early infancy.[26-32] The overlapping clinical symptoms and biochemical measures between infants with biliary atresisa and other infants with cholestasis hamper the timely diagnosis of biliary atresisa. The classification and regression decision tree predictive model (based on the GGT level, acholic stools, and weight) has an 11% false-negative rate for the diagnosis of biliary atresisa among infants with cholestasis.[9] Noninvasive modalities for the differential diagnosis of biliary atresisa among infants with cholestasis are thus needed. We reported that the noninvasive assessment of liver fibrosis by transient elastography in infants with cholestasis may assist the diagnosis of biliary atresisa.[18]

However, transient elastography is not frequently used in pediatric medicine. Hence, other noninvasive biomarkers of liver fibrosis remains needed. Identification of the clinical features of neonatal cholestasis would facilitate clinical decision making in terms of performing invasive procedures (such as liver biopsy and intraoperative cholangiography) to diagnose biliary atresisa and improve outcomes.

Liver histology can assist in the diagnosis of biliary atresisa by identifying bile plugs, periportal fibrosis, and ductular proliferation in infants with cholestasis, but this method is invasive. Intraoperative cholangiography, the gold standard for the confirmation of biliary atresisa, is unsuitable as a routine modality for the differential diagnosis of biliary atresisa among infants with cholestasis. In this study, the serum MMP-7 level was positively correlated with the severity of fibrosis in infants with cholestasis at a mean age of 1.5 months and was predictive of biliary atresisa.

The intrahepatic MMP-7 expression level is reported to associate with biliary fibrosis.[19-22] A positive correlation between intrahepatic MMP-7 immunostaining and the stage of liver fibrosis in patients with biliary atresisa was reported.[19] The serum MMP-7 level was found to be higher in infants with biliary atresisa than in non–biliary atresisa infants with cholestasis by SOMAscan protein analysis (SomaLogic Inc, Boulder, Colorado) and ELISA (Milliplex Multiplex kit, Millipore, Burlington, Massachusetts).[23] However, the serum MMP-7 level was not related to the liver fibrosis stage determined using the Scheuer fibrosis staging system.[23] The staging system developed by the Biliary Atresia Research Consortium enables differential diagnosis of biliary atresisa based on bile duct proliferation, portal fibrosis, and the absence of sinusoidal fibrosis.[33]

In this study, we quantified the severity of liver fibrosis by Picro Sirius red staining for collagen in liver specimens.[34,35] In this study, the severity of liver fibrosis was greater in infants with biliary atresisa than in infants with cholestasis without biliary atresia with a similar age, serum bilirubin level, and AST/alanine aminotransferase level. Moreover, the serum MMP-7 level was positively correlated with the severity of liver fibrosis in infants with cholestasis. Thus, the MMP-7 level, correlated with the status of liver fibrosis, has potential as a noninvasive biomarker for the diagnosis of biliary atresisa among cholestaticinfants. A cutoff serum MMP-7 level of >1.43 ng/mL according to DuoSet ELISA was optimal for predicting biliary atresisa in infants with cholestasis. The exception is in 1 infant with PFIC1 in this study who had a serumMMP-7 level of 11.09 ng/mL and high percentage of collagen (12.30%) in the liver tissue, which is similar to biliary atresisa (MMP-7 median level 10.26 ng/mL; **Figure 1**, A; median percentage of collagen, 13.35%). This finding further strengthens the relationship between serum MMP-7 levels and liver fibrosis in infants with cholestasis demonstrated in this study. A larger case series is needed to assess the serum MMP-7 levels of infants with biliary atresisa and those with PFIC1.

Although both our study and previous works reported that the serum MMP-7 level differs between infants with and those without biliary atresisa, the levels in previous studies (assessed by the Milliplex Multiplex kit and ELISA [Cloud-Clone Corp, Wuhan, China]) were considerably higher than those in this work.[23,36]

The serum MMP-7 level may differ according to ethnicity, patient age, and the ELISA kit used. The subjects of our study were younger (mean age, 42.36 days; range, 7-87 days) than those in the studies of Lertudomphonwanit et al (mean age, >62 days) and Yang et al (median age, 59 days; range, 0-6 months).[23,36] We found that the serum MMP-7 level was significantly lower in infants with biliary atresisa who underwent a cholestatic workup at a younger age in our study. Hence, the difference in the subjects' ages may explain the differences in the results among the above mentioned studies.

Multiplex and traditional ELISA kits can yield different results in the same samples.[37] The Millipore multiplex ELISA kit used in a previous study offered the advantages of reduced sample volume and time.[23] However, it had a reproducibility of only approximately 60% for the assessment of cytokine/chemokine levels, likely due to a large number of variables (eg, binding affinity to beads, protein–protein interactions, and the Luminex machine).[37] Traditional

ELISA kits are the most frequently used in clinical practice owing to their high reproducibility; in this study, we performed triplicate measurements to enhance the reliability of the results (**Table IV**; available at www.jpeds.com). Hence, different institutions should apply the MMP-7 cutoff generated by different ELISA kits used for diagnosing biliary atresisa in infants with cholestasis of different age. Further works to validate the interassay variability between these ELISA kits may be needed before the application of this observation into clinical practice is possible.

The serum samples were collected from 2008 to 2018, and their degradation over time may explain the difference in our results compared with those of previous reports. However, the serum MMP-7 level did not differ in samples collected before vs after 2013 from the infants with cholestasis or infants with biliary atresisa in this study. Hence, the degradation of MMP-7 during storage of serum was unlikely to be a confounding factor.

We demonstrated that the MMP-7 level is predictive of biliary atresisa among infants with cholestasis. The prehepatoportoenterostomy serum MMP-7 level was not correlated with thrombocytopenia, splenomegaly, esophageal varices 6 months after hepatoportoenterostomy, or the need for liver transplantation in infants with biliary atresisa. The serum MMP-7 level was elevated in 31.25% of the infants with biliary atresisa after hepatoportoenterostomy. The serum MMP-7 level at 6 months after hepatoportoenterostomy was associated with the need for liver transplantation in infants with biliary atresisa, even adjusting with the serum total bilirubin levels. This result suggests the additional prognostic power of serum MMP-7 in addition to total bilirubin levels 6 months after hepatoportoenterostomy. The inhibition of MMP-7 reportedly decreases portal inflammation and hepatocellular necrosis in neonatal BALB/c mice with biliary atresisa.[23] Hence, the serum MMP-7 level is predictive of the need for liver transplantation in infants with biliary atresisa, and MMP-7 is a potential therapeutic target in infants with biliary atresisa after hepatoportoenterostomy.

In summary, the serum MMP-7 level, which reflects the severity of liver fibrosis in infants with cholestasis, has potential as a noninvasive biomarker of biliary atresisa. Moreover, the combination of the serum GGT and MMP-7 levels may have greater diagnostic accuracy for biliary atresisa compared with the MMP-7 or GGT level alone. In infants with biliary atresisa, the serum MMP-7 level at 6 months after hepatoportoenterostomy is correlated with the need for liver transplantation. ■

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50 Years Ago in *The Journal of Pediatrics*

Ampicillin in the Treatment of Meningitis due to *Haemophilus influenzae*: An Appraisal after 6 Years of Experience

Yow MD. J Pediatr 1969;74:848-52.

In this editorial, Dr Martha Yow from Baylor University College of Medicine walks the reader through 7 questions to appraise the use of ampicillin, a novel "broad-spectrum penicillin," as therapy for suspected bacterial meningitis. The focus was on treatment of *Haemophilus influenzae* b meningitis, for which penicillin was ineffective and chloramphenicol was the drug of choice at the time. (1) What percent of strains of *H influenzae* are susceptible to ampicillin in vitro? Virtually all. […at the time]. (2) Will resistance emerge? Unlikely to be an important clinical problem. […for a while]. (3) Will sufficient ampicillin penetrate the blood brain barrier? Likely, given adequate dosage and inflamed meninges. (4) Is the drug effective in vivo? "Unequivocally yes." Occasional treatment failures had been reported and reasons were thought likely to be multifactorial, some related to pathophysiologic events specific to *H influenzae*. "Isolated treatment failures should not cause the abandonment of an effective therapeutic agent" opined Dr Yow. (5) What are optimal doses, best routes, and intervals of administration? After careful exposure of several points, Dr Yow settled on 200 mg/kg/day divided in q 4- to 6-hour doses, intravenously. […current dosage for meningitis is up to 400 mg/kg/day]. (6) What is the optimal duration of therapy? Individualize, with average 10-14 days. (7) How well is the drug tolerated? We bring this step-by-step appraisal made 50 years ago to readers' attention because it is a perfect example of how infectious diseases specialists still weigh each and every antibiotic decision, and teach our trainees and pediatricians to do the same. One of us (CJB) had the privilege of training with this cogent editorialist and wise mentor at a time when this new antibiotic revolutionized the treatment of bacterial meningitis in children.

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Table III. Clinical predictors of liver transplantation in infants with biliary atresisa 6 months after hepatoportoenterostomy

	Univariate		Multivariate	
	hazard ratio (95% CI)	*P* value	hazard ratio (95% CI)	*P* value
Total bilirubin >1.12 mg/dL (n = 12) vs ≤1.12 mg/dL (n = 20) 6 months after hepatoportoenterostomy	14.23 (3.00-67.43)	.001	14.78 (2.95-74.08)	.001
MMP-7 >10.30 ng/mL (n = 10) vs ≤10.30 ng/mL (n = 22) 6 months after hepatoportoenterostomy	4.22 (1.23-14.49)	.02	4.29 (1.12-16.52)	.03

Table IV. ANOVA between biliary atresisa and non–biliary atresisa groups between 3 ELISA measurement in this study

	Sum squares	Degree of freedom	Mean square	F test	Pr > F
ELISA test 1					
Between groups	1951.18	1	1951.18	109.49	<0.0001
Within groups	1746.49	98	17.82		
ELISA test 2					
Between groups	2001.48	1	2001.48	115.65	<0.0001
Within groups	1695.96	98	17.31		
ELISA test 3					
Between groups	1976.25	1	1976.25	113.34	<0.0001
Within groups	1708.80	98	17.44		