Serum matrix metalloproteinase-7 in biliary atresia: a Japanese multicenter study

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Abbreviations: ALT, alanine aminotransferase; AUC, area under receiver operating characteristic curve; BA, biliary atresia; BA-KP, biliary atresia requiring only Kasai portoenterostomy; BA-LT, biliary atresia requiring liver transplantation after Kasai portoenterostomy; CI, confidence interval; DB, direct bilirubin; GGT, γ-glutamyltransferase; KP, Kasai portoenterostomy; LT, liver transplantation; MMP-7, matrix metalloproteinase-7; NC, normal controls; non-BA, non-biliary-

atresia cholestatic controls; NPV, negative predictive values; PPV, positive predictive values; ROC, receiver operating characteristic; TB, total bilirubin; TBA, total bile acids.

Abstract

Background: Biliary atresia (BA) is among the commonest indications for liver transplantation (LT) in children. We examined whether serum matrix metalloproteinase-7 (MMP-7) is useful for diagnosis of BA in Japanese infants, and whether serum MMP-7 concentrations before and after Kasai portoenterostomy (KP) predicted LT within a year.

Methods: Subjects under 6 months old at 8 pediatric centers in Japan were enrolled retrospectively, including patients with cholestasis and normal controls (NC) without liver disease. Patients with cholestasis were divided into groups representing BA vs. cholestasis from other causes (non-BA). Serum samples were collected from patients with BA at diagnosis and 1 and 4 weeks after KP, as well as from non-BA and NC.

Results: Serum MMP-7 concentrations were significantly higher in BA at diagnosis (median, 89.1 ng/mL) than in non-BA (11.0; $P<0.001$) or NC (10.3; $P<0.001$). Receiver operating characteristic (ROC) analysis of MMP-7 for BA vs. non-BA yielded an area under the ROC curve of 0.99 (95% confidence interval, 0.96 to 1.00). An optimal cut-off value of 18.6 ng/mL for serum MMP-7 in diagnosing BA demonstrated sensitivity and specificity of 100% and 90%, respectively. Serum MMP-7 before and 1 week and 4 weeks after KP did not differ significantly between BA requiring only KP and BA requiring LT after KP.

Conclusion: Serum MMP-7 is a useful marker for diagnosis of BA in Japanese infants, but it could not predict LT within a year.

Keywords: biomarker, Kasai portoenterostomy, liver transplantation, infant, cholestasis, γ-glutamyltransferase, bilirubin, bile acid, liver fibrosis, diagnosis.

Introduction

Biliary atresia (BA) is a destructive neonatal inflammatory obliterative cholangiopathy that affects varying lengths of both intrahepatic and extrahepatic bile ducts (1). Untreated BA progresses to liver cirrhosis and death within 2 years. Incidence varies from approximately 1:8000 newborns in Asian countries to 1:20 000 in European countries (1, 2).

Management of BA, a multifaceted liver disease with a complex pathogenesis and a rapid, devastating course, requires timely management (3). The primary treatment for BA is a Kasai portoenterostomy (KP), where bile duct is resected and replaced with a Roux-en-Y loop of the intestine, allowing drainage. However, even a surgically and therapeutically successful KP does not reliably prevent liver fibrosis in BA; patients often progress to cirrhosis requiring liver transplantation (LT), making BA the most common indication for pediatric LT (4).

Age at KP is an important predictor of outcome; patients undergoing KP within the first 60 days of life are less likely to require LT (5). However, early diagnosis of BA is challenging because of overlap of signs and symptoms with other causes of cholestasis in infants. Abdominal ultrasound examination, magnetic resonance cholangiopancreatography, and biliary scintigraphy are widely used for diagnosis of BA in infants with cholestasis, but their diagnostic accuracies for BA are unsatisfactory. Intraoperative cholangiography, the gold standard for diagnosis of BA, is an invasive procedure, unsuitable for routine use in infants with cholestasis (6-8). A non-invasive diagnostic test with high specificity for BA would be highly desirable.

Serum matrix metalloproteinase-7 (MMP-7) recently has been investigated in infants as a non-invasive diagnostic alternative to intraoperative cholangiography in BA, showing good sensitivity and specificity in studies from the US, China, and Taiwan (9-12). However, we know of no such studies in Japanese patients with BA. An additional important issue is whether serum MMP-7 concentrations at the time of BA diagnosis and after KP might predict subsequent need for LT.

The present multicenter study aimed to clarify whether serum MMP-7 is a useful diagnostic marker for BA in Japanese infants, and whether serum MMP-7 concentrations before and after KP might predict need for LT within a year.

Methods

Design and ethical matters

This retrospective multicenter observational study was designed and conducted within the framework of the "Investigation of Oxysterol and Bile Acid Analyses for Pediatric Patients with Liver Disease and Healthy Children in Japan" (13, 14), which includes 8 collaborating Japanese pediatric centers caring for patients with BA. The study protocol complied with the ethical guidelines of the Declaration of Helsinki (2013 revision) and was approved by the Ethics Committee of Kurume University and its counterparts at other participating centers. Written informed consent for secondary use of serum samples and information was obtained from all participating families. This retrospective study was carried out by an opt-out method.

Study subjects

Subjects under 6 months old, retrospectively enrolled from 8 pediatric centers in Japan between January 2017 and March 2020, included patients with cholestasis and normal controls (NC) without liver disease. Patients with cholestasis were divided into 2 groups representing BA and non-BA cholestatic controls (non-BA). Serum samples from all enrolled subjects were analyzed. BA was diagnosed based upon histopathologic evidence of fibrotic obstruction of extrahepatic biliary remnants in tissues excised after surgical cholangiography. Non-BA cholestasis was defined by cholestatic liver diseases diagnosed by clinical findings and/or genetic analysis (including cholestasis of unknown cause), plus serum direct bilirubin (DB) exceeding 1.5 mg/dL at sample collection. Laboratory samples were collected at the time of clinical diagnosis of BA or an alternative condition. Additional serum samples were collected from patients with BA 1 and 4 weeks after KP. Patient characteristics and laboratory blood test results in BA and non-BA groups were obtained from medical records and clinical interviews. Serum samples were stored at -20℃ until MMP-7 analysis.

Serum MMP-7 assays and biochemical and histopathologic studies

Serum MMP-7 concentration was determined using a sensitive sandwich enzyme-linked immunosorbent assay according to the manufacturer's instructions (ELISA; Duo Set, R&D Systems, Minneapolis, MN).

Laboratory blood tests included serum alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), total and direct bilirubin (TB and DB), and total bile acids (TBA). Histopathology of the liver was evaluated using initial liver biopsy specimens obtained from infants with BA before KP. Liver biopsy specimens were assessed according to the New Inuyama Classification for chronic hepatitis, in which rates chronic hepatic disease according to degree of fibrosis (F): F0 (no fibrosis, equivalent to Ishak stage 0), F1 (fibrosis with portal expansion, i.e. Ishak stage 1-2), F2 (bridging fibrosis, i.e. Ishak stage 3), F3 (bridging fibrosis causing lobular distortion, i.e. Ishak stage 4), or F4 (cirrhosis, i.e. regenerative nodules surrounded by fibrous bands, or Ishak stage 5-6) (15, 16). Additionally, the classification grades chronic hepatic disease activity (A) based on intensity of lymphocytic infiltration and extent of hepatocytic necrosis as follows: A0 (no necro-inflammatory reaction), A1 (mild necro-inflammatory reaction), A2 (moderate necro-inflammatory reaction), and A3 (severe necro-inflammatory reaction) (15).

Statistical analysis

Continuous variables are expressed as median, minimum, and maximum values; categorical variables are presented as the number of subjects demonstrating the variable. The Fisher exact test, Mann-Whitney U test, Kruskal-Wallis test, Dunn's multiple comparison test, and Spearman’s rank correlation test were applied as appropriate. Diagnostic accuracy of an assay was evaluated using receiver operating characteristic (ROC) analysis. Statistical analyses were carried out with GraphPad Prism

(version 9.1.0; GraphPad Software, San Diego, CA), which also was used to produce related figures. Significance tests were two-sided. P values below 0.05 were accepted as evidence of statistical significance.

Results

Enrolled subjects

We enrolled 76 infants including 27 with BA and 20 with non-BA conditions, as well as 29 NC. Diagnoses in the non-BA group included idiopathic neonatal cholestasis (n=7), citrin deficiency (n=4), Alagille syndrome (n=4), cytomegalovirus hepatitis (n=1), Dubin-Johnson syndrome (n=1), progressive familial intrahepatic cholestasis type 2 (n=1), acute myeloid leukemia (n=1), and hypothyroidism (n=1). Demographic and baseline characteristics of study subjects in the BA group at diagnosis, the non-BA group, and the NC group are summarized in Table 1.

Serum MMP-7 concentrations at diagnosis

We compared MMP-7 concentrations among BA at diagnosis, non-BA, and NC. Serum MMP-7 concentrations for BA at diagnosis (median, 89.1 ng/mL; range, 18.8 to 368.0) were significantly higher than for non-BA (11.0; 2.9 to 34.0; $P<0.001$) or NC (10.3; 5.4 to 25.3; $P<0.001$), respectively; no significant difference was noted between non-BA and NC (Figure 1).

Serum MMP-7 as a diagnostic marker for BA

We performed ROC analysis to assess diagnostic accuracy of serum MMP-7 for BA. For ROC analysis of discrimination of BA from non-BA, the area under the ROC curve (AUC) for serum MMP-7 was 0.99 (95% confidence interval or CI, 0.96 to 1.00; Figure 2A), which was superior to GGT (AUC, 0.90). The optimal cutoff value for MMP-7 was 18.6 ng/mL, with sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 100%, 90.0%, 93.1%, and 100%, respectively. ROC analysis for serum MMP-7 in distinguishing BA from non-BA and NC considered together yielded an AUC of 0.99 (95% CI: 0.98 to 1.00; Figure 2B), which again outperformed GGT (AUC, 0.93). The optimal cutoff value for MMP-7 was 27.4 ng/mL, with sensitivity, specificity, PPV, and NPV of 92.6%, 98.0%, 96.2%, and 96.0%, respectively.

Correlation of serum MMP-7 with age and laboratory blood tests

Seeking alternative factors that might affect serum MMP-7 in patients with BA, we performed Spearman's rank correlation test concerning serum MMP-7 for patient age and various blood test results. In patients with BA, a significant positive correlation was evident between MMP-7 and age ($P<0.05$). No significant correlation was found in patients with BA between MMP-7 and liver function test results such as ALT, GGT, TB, DB, or TBA (Supplementary Figure S1).

Correlation of serum MMP-7 with liver histopathology

Grading of liver biopsy specimens from all 27 patients with BA demonstrated no significant correlation between serum MMP-7 and histopathologically assessed fibrosis or inflammation (Supplementary Figure S2).

Serum MMP-7 levels before and after KP between BA-KP and BA-LT

We compared serum MMP-7 concentrations before KP as well as 1 and 4 weeks after KP between BA patients who required only KP (BA-KP) and those who required LT within a year after KP (BA-LT). Demographics and baseline characteristics of these 2 subgroups are summarized in Table 2. Among 27 patients with BA, 9 underwent LT within a year after KP. Serum MMP-7 concentrations before KP (at diagnosis of BA) were not significantly different between BA-KP and BA-LT (n=27; median, 92.6 ng/mL vs. 87.2 ng/mL, respectively; *P=0.99*; Figure 3A). Similarly, serum MMP-7 at 1 week (n=19; 45.5 vs. 30.2; *P=0.84*; Figure 3B) and at 4 weeks after KP (n=13; 48.2 vs. 63.0; *P=0.28*; Figure 3C) did not differ significantly between BA-KP and BA-LT, respectively.

Discussion

Our multicenter study demonstrated that serum MMP-7 in Japanese infants should be useful as a marker distinguishing BA from other causes of neonatal cholestasis. However, serum MMP-7 concentrations before or after KP in patients with BA did not predict need for LT within a year.

BA requires accurate and efficient early diagnosis to assure timely and proper surgical

intervention. Previous studies from the US, China, and Taiwan concluded that serum MMP-7 is superior to other noninvasive biomarkers for diagnosis of BA such as serum GGT (9-12). However, such studies of serum MMP-7 in BA have not been reported from Japan. As has been reported elsewhere, we found that MMP-7 in Japanese patients with BA also was significantly higher than in patients with other causes of neonatal cholestasis. Although the optimal cutoff value to diagnose BA shown in our Japanese study differed from those in previous studies of serum MMP-7 in distinguishing BA from non-BA, sensitivity and specificity of serum MMP-7 in our study were 100% and 90%, comparable to sensitivity and specificity in the earlier studies, where these respectively ranged from 95 to 99% and 83 to 95% (9-12). Because cutoff values vary according to ELISA kit used and racial characteristics of the population studied, cutoff values for serum MMP-7 should take such factors into account.

In this study we evaluated correlations of serum MMP-7 with age, ALT, GGT, TB, DB, and TBA in patients with BA, finding that serum MMP-7 had a significant positive correlation with age as previously reported but not with the other laboratory variables; GGT correlated with MMP-7 but not significantly; Yang et al (10) however, found significant positive correlations between MMP-7 and age and, as well as GGT. These results suggest that MMP-7 might reflect disease duration and biliary injury, raising questions about suitability for diagnosis at very early ages such as less than 30 days after birth.

We also evaluated correlation of serum MMP-7 with fibrosis or inflammation demonstrated by liver histopathology, finding no significant correlation with either. In previous studies, various

results were reported with respect to correlation of serum MMP-7 with fibrosis or inflammation shown by liver histopathology. Previous studies found varying associations between intensity of intrahepatic MMP-7 expression and degree of liver fibrosis (17-20). Jiang et al (11) reported a significant positive correlation between serum MMP-7 and stage of hepatic fibrosis, while no significant differences were found for intensity of inflammation. On the other hand, Lertudomphonwanit et al (9) reported that serum MMP-7 correlated poorly with liver fibrosis stage at the time of diagnosis. Altogether, serum MMP-7 is useful for diagnosis of BA, but is limited in predicting extent of histopathologically evident liver damage.

BA is the most common indication for LT in the pediatric population, and most patients with BA ultimately require LT even after a successful KP (21). Noninvasive predictors of eventual need for LT that are informative before and immediately after KP are needed for optimal management of BA. So far, no reported biomarker has accurately predicted eventual requirement of LT. In this study, serum MMP-7 also failed to predict this need at the time of BA diagnosis or immediately after KP. Wu et al (12) reported significantly higher serum MMP-7 concentrations 6 months after KP in patients eventually requiring LT than in patients who did not, but by that time serum total bilirubin concentrations were also significantly higher. Considering their findings and ours, MMP-7 may not be a sufficiently timely predictor of need for LT.

Even though our present study offers the strength of a multicenter study, a number of limitations are evident. First, relatively small numbers of BA and non-BA subjects were enrolled. In

particular, only 2 BA patients under 30 days of age were included. Second, our samples included only Japanese subjects, so findings should not be generalized to other Asian countries or different ethnic groups. Prospective multicenter controlled studies including larger and more diverse populations might provide further information concerning MMP-7 in BA and prediction of need for LT.

Conclusion

Serum MMP-7 is a useful marker for diagnosis of BA in Japanese infants, but assays before and after KP failed to predict need for LT within a year.

Supplemental data

Supplementary Figures S1-2.

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Declarations

Conflict of interest: All authors declare that they have no conflict of interest.

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Ethics approval: The study protocol complied with the ethical guidelines of the Declaration of Helsinki (2013 revision) and was approved by the Ethics Committee of Kurume University and its counterparts at other participating centers.

Consents to participate and for publication: Written informed consent for secondary use of serum samples and information was obtained from all participants. This retrospective study was carried out by an opt-out method.

Code availability: Not applicable.

Availability of data and material: The data leading to the findings of this study are available on request from the corresponding author.

Authors' contributions: HSak and TMiz, contributed to the concept and design of the study, analysis and interpretation of data, and to writing the manuscript; KK, RY, HSas, KY, TT, SF, YS, II, HSho, MK, TMat, JF, HU, MN, and YY contributed to acquisition of samples and data; HSas, MN, and YY contributed to supervision of the study; and all authors contributed to reviewing the final version of the manuscript. Thus, all authors contributed to the manuscript.

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after surgery for biliary atresia: a report from the Japanese Biliary Atresia Registry. Journal of pediatric surgery. 2003;38(7):997-1000.

Figure Legend

Figure 1. Serum MMP-7 concentrations among BA, non-BA, and NC.

Serum matrix metalloproteinase-7 (MMP-7) concentrations are shown for subjects with biliary atresia (BA) and for non-biliary-atresia cholestatic controls (non-BA), as well as normal controls (NC). Horizontal lines in middle of boxes indicate medians; tops and bottoms of boxes correspond to 75th and 25th percentiles, respectively. Whiskers above and below boxes give maximum and minimum values, respectively. ***, $P<0.001$.

Figure 2. ROC curves for serum MMP-7.

Receiver operating characteristic (ROC) curves for serum matrix metalloproteinase-7 (MMP-7) in distinguishing biliary atresia (BA; n=27) from non-biliary-atresia cholestatic controls (non-BA; n=20) (A), and from non-BA and normal controls (NC; n=29) (B) were obtained by plotting sensitivity against 1-specificity. Areas under ROC curves are shown with 95% confidence intervals.

Figure 3. Serum MMP-7 concentrations between BA-KP and BA-LT before and after KP.

Serum matrix metalloproteinase-7 (MMP-7) concentrations are compared between biliary atresia treated with only Kasai portoenterostomy (BA-KP) and BA treated with liver transplantation within a year after KP (BA-LT) at diagnosis (A), 1 week (B), and 4 weeks (C) after KP. Horizontal lines in the

middle of boxes give medians, while tops and bottoms of boxes show 75th and 25th percentiles, respectively. Whiskers above and below boxes correspond to maximum and minimum, respectively.

Supplementary Figure S1. Correlations between serum MMP-7 and age and laboratory blood tests.

Correlations are shown between serum matrix metalloproteinase-7 (MMP-7) and age (days after birth; A), alanine aminotransferase (ALT; B), γ-glutamyltransferase (GGT; C), total bilirubin (D), direct bilirubin (E) and total bile acids (F) in patients with biliary atresia. Rs, Spearman's rank correlation coefficient.

Supplementary Figure S2. Correlations between serum MMP-7 and fibrosis stage or inflammation grade of the liver histopathology.

Correlations are shown between serum matrix metalloproteinase-7 (MMP-7) and fibrosis stage (A) or inflammation grade (B) according to liver histopathology. F0, no fibrosis; F1, fibrosis evident as portal expansion; F2, bridging fibrosis; F3, bridging fibrosis with lobular distortion; F4, cirrhosis (regenerative nodules encircled by fibrous bands); A0, no necro-inflammatory reaction; A1, mild necro-inflammatory reaction; A2, moderate necro-inflammatory reaction; A3, severe necro-inflammatory reaction.

Figure 1

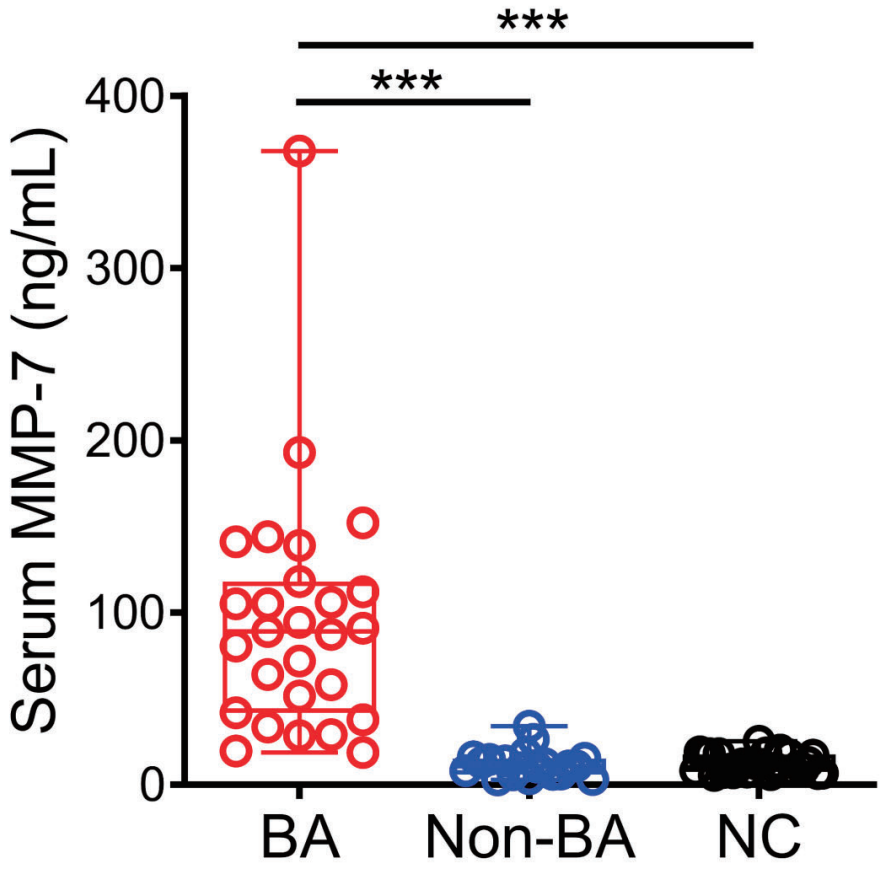


Figure 2

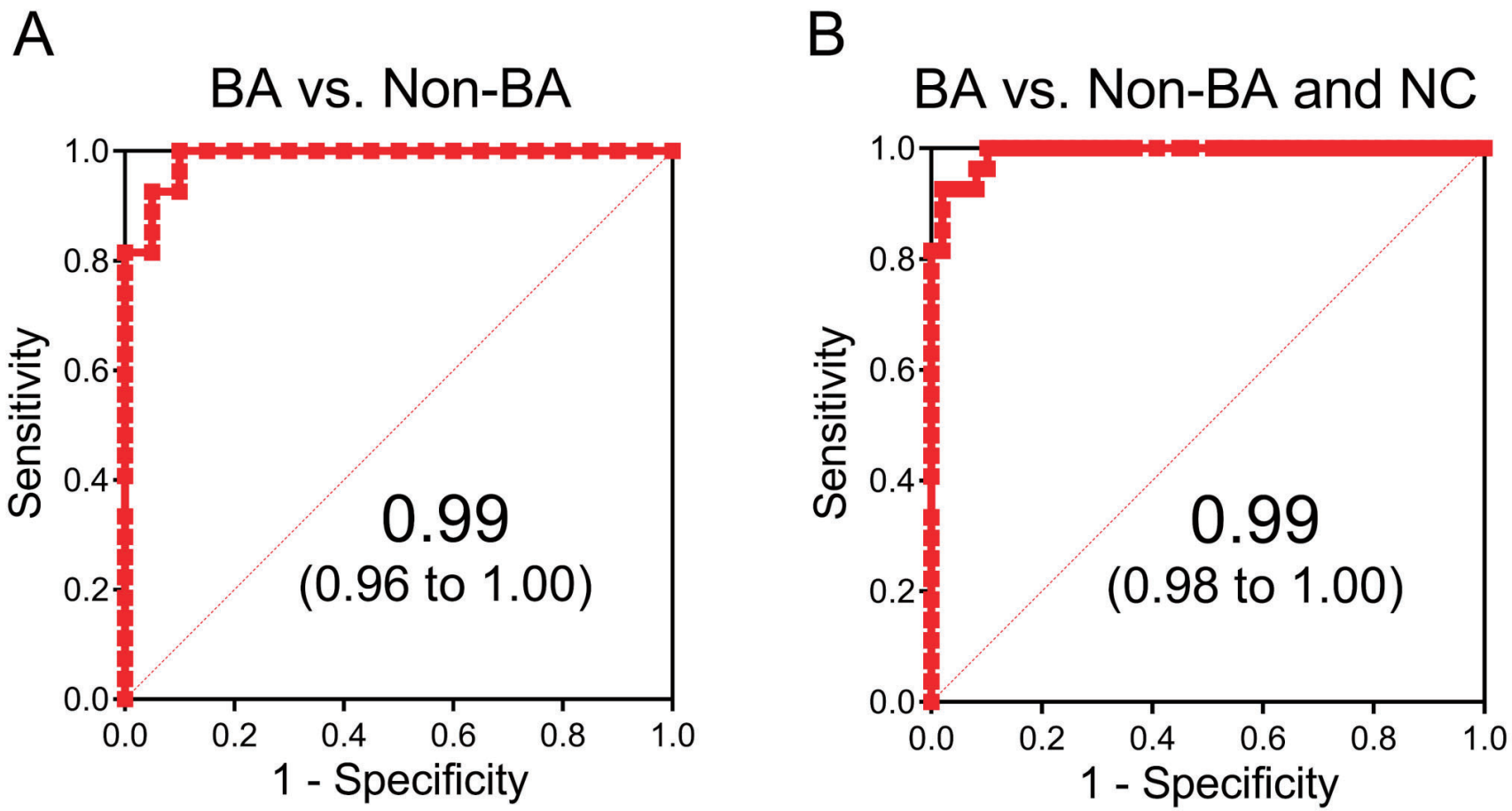
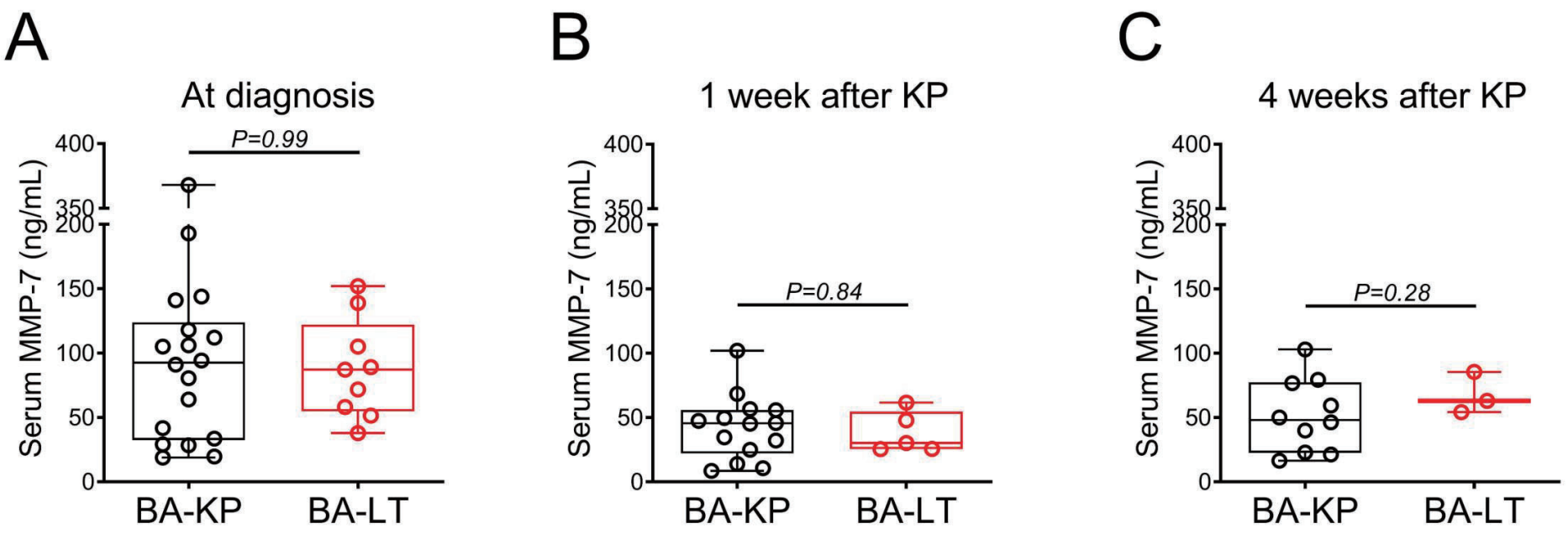
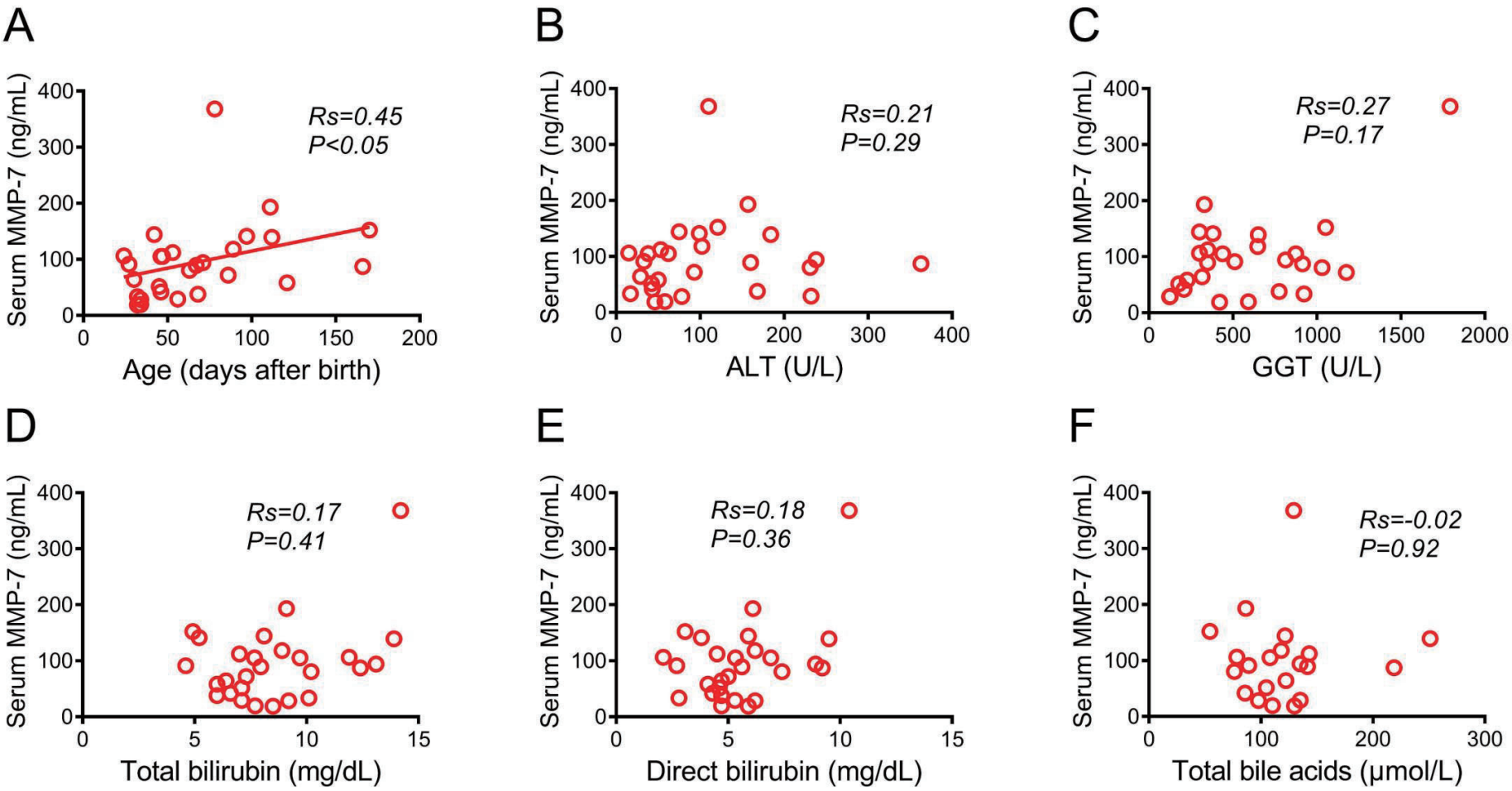

A
BA vs. Non-BA
Sensitivity
1 - Specificity
0.99
(0.96 to 1.00)
B
BA vs. Non-BA and NC
Sensitivity
1 - Specificity
0.99
(0.98 to 1.00)

Figure 3


A
At diagnosis
P=0.99
Serum MMP-7 (ng/mL)
400
350
200
150
100
50
0
BA-KP
BA-LT
B
1 week after KP
P=0.84
Serum MMP-7 (ng/mL)
400
350
200
150
100
50
0
BA-KP
BA-LT
C
4 weeks after KP
P=0.28
Serum MMP-7 (ng/mL)
400
350
200
150
100
50
0
BA-KP
BA-LT

Supplementary Figure S1



Supplementary Figure S2

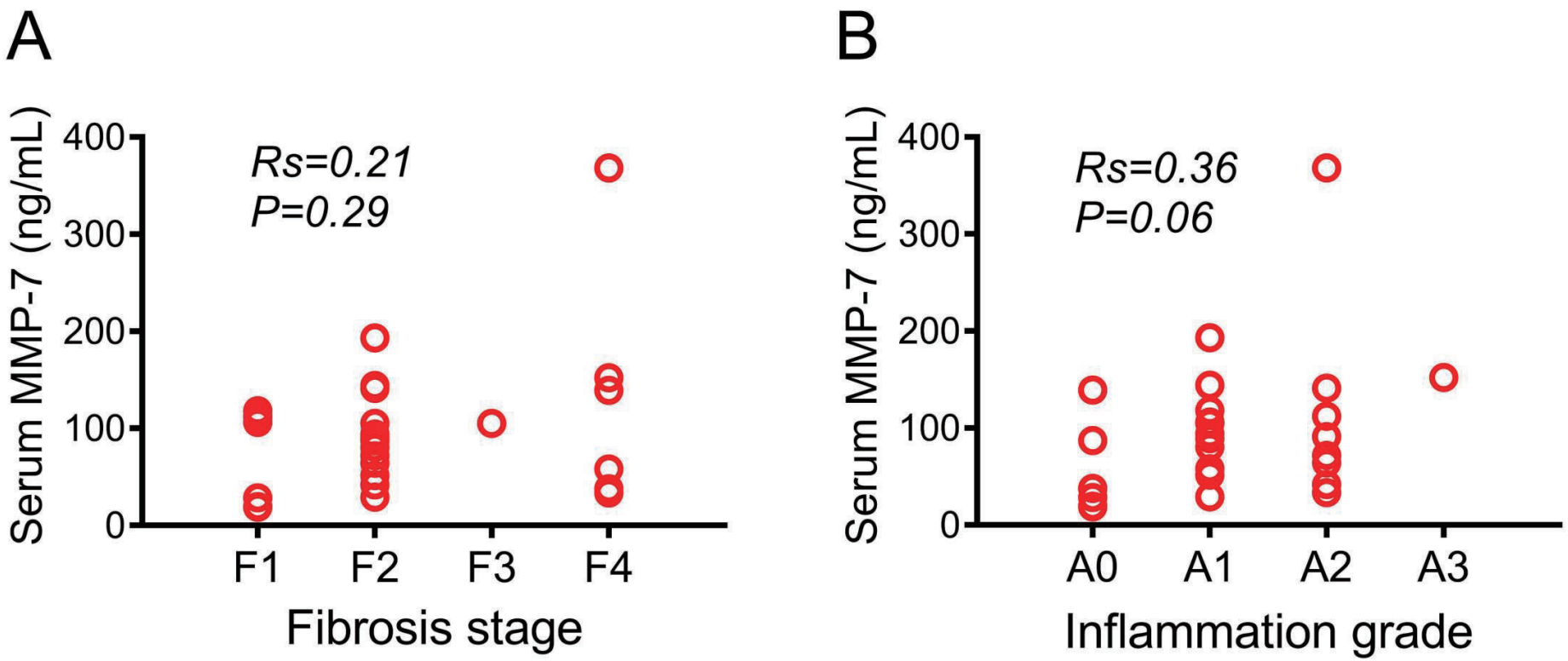


Table 1. Demographics and laboratory characteristics among BA at diagnosis, non-BA, and NC

	BA [n=27]	Non-BA [n=20]	NC [n=29]	*P* value*
Age, median, days after birth (range)	56 (24-170)	51 (14-162)	49 (14-147)	*0.93*
Sex, n				
Male	8	13	18	*0.02*
Female	19	7	11	
Serum concentration, median (range)				
Alanine aminotransferase, U/L	78 (15-363)	69 (14-1442)	13 (7-34)	*0.94*
γ-Glutamyltransferase, U/L	438 (123-1793)	124 (28-1153)	87 (35-1279)	*<0.001*
Total bilirubin, mg/dL	8.0 (4.6-14.2)	6.6 (2.6-22.7)	3.1 (0.7-9.6)	*0.16*
Direct bilirubin, mg/dL	5.3 (2.1-10.4)	3.7 (1.5-16.5)	0.2 (0.1-0.3)	*0.11*
Total bile acids, μmol/L	120 (54-251) [#]	118 (29-343) [##]	N/A	*0.73*

BA, biliary atresia; Non-BA, non-biliary atresia cholestatic controls; NC, normal controls; *, BA vs. Non-BA; [#], n=21; [##], n=15; N/A, not available.

Table 2. Demographics and laboratory characteristics before and after KP compared between BA-KP and BA-LT

	Before KP			1 week after KP			4 weeks after KP		
	BA-KP [n=18]	BA-LT [n=9]	*P* value	BA-KP [n=14]	BA-LT [n=5]	*P* value	BA-KP [n=10]	BA-LT [n=3]	*P* value
Age, median, days after birth (range)	46 (24-111)	86 (45-170)	*0.009*						
Gender, n									
Male	4	4	*0.37*						
Female	14	5							
Serum concentration, median (range)									
Alanine aminotransferase, U/L	67 (15-238)	121 (42-363)	*0.16*	95 (24-291)	99 (38-337)	*0.88*	65 (20-343)	74 (60-127)	*0.94*
γ-Glutamyltransferase, U/L	401 (123-1793)	651 (177-1175)	*0.43*	447 (124-1072)	527 (135-1336)	*0.89*	637 (268-1168)	752 (103-1628)	*0.81*
Total bilirubin, mg/dL	8.7 (4.6-14.2)	7.3 (4.9-13.9)	*0.35*	3.7 (2.3-10.4)	5.4 (3.1-9.3)	*0.38*	1.1 (0.5-11.6)	5.7 (1.9-6.6)	*0.07*
Direct bilirubin, mg/dL	5.6 (2.1-10.4)	5.0 (3.1-9.5)	*0.97*	2.5 (1.3-7.6)	3.5 (2.1-6.5)	*0.31*	0.5 (0.2-8.3)	3.9 (1.0-5.9)	*0.07*
Total bile acids, μmol/L	120 (76-143) [#]	125 (54-251) [##]	*0.4*	N/A	N/A	N/A	N/A	N/A	N/A

BA-KP, biliary atresia with only Kasai portoenterostomy; BA-LT, biliary atresia with liver transplantation after Kasai portoenterostomy; KP, Kasai portoenterostomy; [#], n=14; [##], n=6; N/A, not available.

Table 3. Differences in diagnostic accuracy and cutoff value for serum MMP-7 in diagnosing BA in previous reports and the present study

Author and published year	Country	ELISA kit	AUC	Cutoff value	Sensitivity, %	Specificity, %
Lertudomphonwanit, et al. 2017	USA	N/A (Multiplex)	0.97	N/A	97	91
Yang, et al. 2018	China	Cloud-Clone, China	0.99	52.85	98.7	95
Wu, et al. 2018	Taiwan	R&D, USA	0.96	1.43	97.3	83.2
Jiang, et al. 2019	China	Cloud-Clone, China	0.98	10.4	95.2	93
Present study	Japan	R&D, USA	0.99	18.6	100	90

MMP-7, matrix metalloproteinase-7; BA, biliary atresia; ELISA, enzyme-linked immunosorbent assay;
AUC, area under the receiver operating characteristic curve; N/A, not available.