Hepatology International (2022) 16:954–963
https://doi.org/10.1007/s12072-022-10322-x

ORIGINAL ARTICLE

Dynamic analysis of serum MMP-7 and its relationship with disease progression in biliary atresia: a multicenter prospective study

Shuiqing Chi[1] · Peipei Xu[1] · Pu Yu[2] · Guoqing Cao[2] · Haibin Wang[3] · Yongqin Ye[4] · Shuai Li[1] · Yun Zhou[1] · Xiangyang Li[1] · Ying Zhou[1] · Xi Zhang[1] · Huizhong Niu[5] · Lei Xu[6] · Pengcheng Cai[6] · Shaotao Tang[1]

Received: 31 August 2021 / Accepted: 28 November 2021 / Published online: 22 June 2022

Abstract

Purpose We aimed to assess the dynamic changing trend of serum matrix metalloproteinase-7 (MMP-7) in biliary atresia (BA) patients from diagnosis to LTx to further elucidate its clinical value in diagnosis and prognoses and its relationship with disease progression.

Methods In this multicentre prospective study, 440 cholestasis patients (direct bilirubin level of > 17 μmol/L) were enrolled. Serum MMP-7 levels were measured using an enzyme-linked immunosorbent assay at diagnosis, 1 week, 2 weeks, 1 month, 6 weeks, 2 months, 3 months, 6 months and then every 6 months post-KPE. The medical record at each follow-up visit for post-Kasai portoenterostomy patient was collected and analyzed.

Results Using a cut-off value of > 26.73 ng/mL, serum MMP-7 had an AUC of 0.954 in BA neonates and 0.983 in BA infants. A genetic mutation (G137D) was associated with low MMP-7 levels in serum of BA patients. MMP-7 showed a mediation effect on the association between inflammation and liver fibrosis in BA patients. Four dynamic patterns of serum MMP-7 post-KPE were associated with prognosis. Serum MMP-7 was the only significant predictor at 6 weeks post-KPE and the most accurate predictor at 3 months post-KPE of survival with the native liver in 2 years.

Conclusion As one of the critical factors associated with BA occurrence and progression, serum MMP-7 can be used for early diagnosis of BA and post-KPE MMP-7 level is the earliest prognostic biomarker so far.

Keywords Biliary atresia · Cholestatic liver diseases · Diagnosis · Prognosis · MMP7 · Kasai portoenterostomy · Neonates · Diagnostic biomarker · Dynamic monitoring · Native liver survival

Shuiqing Chi, Peipei Xu and Pu Yu contributed equally to this study.

✉ Shaotao Tang
tshaotao83@hust.edu.cn

1 Department of Paediatric Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

2 Department of Neonatal Surgery, Xi'an Children's Hospital, Xi'an, China

3 Department of Paediatric Surgery, Wuhan Children's Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

4 Department of General Surgery, Shenzhen Children's Hospital General Surgery Ward, Shenzhen, China

5 Department of Paediatric General Surgery, Hebei Children's Hospital of Hebei Medical University, Hebei, China

6 Department of Clinical Laboratory, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Introduction

Biliary atresia (BA) is one of the most severe cholestatic liver diseases in infants and the most frequent indication for pediatric liver transplantation (LTx) globally [1–3]. KPE could establish biliary enteric drainage and offer the potential for long-term survival or postpone LTx in patients with BA. Early diagnosis and timely Kasai portoenterostomy (KPE) are correlated with favorable prognoses [4–7]. Unfortunately, similar clinical manifestations and overlapping examination results make an early and prompt diagnosis of BA extremely challenging [8, 9]. In addition, the cost-effectiveness of BA screening is still debatable, and the required infrastructure for screening is currently unavailable in most countries and regions [10]. Several studies have confirmed the diagnostic value of serum matrix metalloproteinase-7 (MMP-7) for BA and recovered the association between serum MMP-7 and bile duct epithelial injury and

liver fibrosis [11–14]. A previous research reported that BA neonates have lower serum MMP-7 levels; thus, more evidence is needed to prove serum MMP-7's diagnostic value in BA neonates [13].

Failed KPE is common in BA patients, and the vast majority of liver transplants occur in BA children less than 2 years of age [3]. A Markov model simulation found an increase of 17.45 additional expected life years when an LTx was performed in a patient with a pediatric end stage liver disease (PELD) score of 15–25 with one or fewer systemic complications as compared to those with PELD scores > 25 who had more than one systemic complication [15]. Identifying early predictors of progressive liver fibrosis would help surgeons plan individualized treatment interventions and determine the appropriate time for performing LTx. Clearance of jaundice at 3 months post-KPE is widely used in the evaluation of the prognosis of BA patients [16]. Therefore, this time point was chosen to evaluate the capacity of each prognostic factor in the following studies. Few studies reported the prediction value of dynamic patterns of serum biomarker levels in post-KPE BA infants for LTx.

In this multicenter prospective study, we evaluated the role of serum MMP-7 in diagnosing BA in cholestatic neonates. We also investigated the utility of dynamic patterns of serum MMP-7 for predicting 2-year survival with native liver (SNL) in post-KPE BA patients.

Materials and methods

Study design and population

From August 2017 to February 2020, a total of five hospitals participated in this prospective study (Department of Pediatric Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan; Department of Neonatal Surgery, Xi'an Children's Hospital, Xi'an; Department of Pediatric Surgery, Wuhan Children's Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan; Department of General Surgery, Shenzhen Children's Hospital, Shenzhen; Department of General Surgery, Hebei Children's Hospital). A total of 440 cholestasis patients (direct bilirubin level of > 17 μmol/L) within 6 months of age with unclear diagnoses were included in this study [17]. Exclusion criteria included refusal to perform the MMP-7 test and known diagnosis/cause of cholestasis before the test. All patients underwent a workup for cholestasis, including general information, blood tests, urine tests, abdominal sonograms, and metabolic or genetic workups, and all serum MMP-7 level tests were performed in Wuhan Union Hospital. The diagnosis of BA was confirmed by intraoperative cholangiography, hepatic subcapsular spider-like telangiectasis (HSST) signs [18] and pathological pictures. The final diagnosis for each included patient was made by pediatric hepatologists and pediatric surgeons together. The diagnostic criteria for BA were the same as those noted in a previous study [12]. BA was excluded in all infants with neonatal hepatitis and idiopathic cholestasis by laparoscopic-assisted cholangiography and liver biopsy. We developed a neonate cohort ($n = 65$) from 440 cholestasis patients as validation cohort one and cholestasis patients without age limit as validation cohort two ($n = 440$) (Fig. 1a). A total of 191 BA patients without age limit who underwent KPE in the same period were included in the follow-up study. All feces samples of BA patients were collected before discharge (usually 2 weeks post-KPE), and fecal MMP-7 levels were tested. Urine samples were collected daily before discharge, and urinary MMP-7 levels were tested. Serum samples were collected for MMP-7 and liver function tests weekly before discharge. Follow-up of BA patients was performed at 1 month, 6 weeks, 2 months, 3 months, 6 months, and then every 6 months post-KPE. Serum MMP-7 levels, liver function tests, Fibrotouch, and abdominal ultrasonography were conducted in BA patients at each follow-up visit. The follow-up endpoint was the date of LTx or death. SNL was defined as normal bilirubin levels and no cholestatic complications at the second year visit post-KPE. Eighty-three BA patients who had a follow-up of less than 2 years or were lost to follow-up after discharge were excluded from the dynamic trend analysis (Fig. 1b). Informed and written consent to participate in the study was obtained from all patients' parents. All included patients received the unified post-KPE treatment plan in all centers. (ursodesoxycholic acid and Reducedglutathione for 1 year, and Tebipenem Pivoxil for 6 months).

MMP-7 sample acquisition and measurement

There were three ways to obtain MMP-7 serum samples in the current study: (1) No transportation required (blood sample from the Wuhan Union Hospital): blood samples from 224 subjects were collected in a coagulation-promoting tube and placed in a 4 °C refrigerator. (2) Transportation time less than 12 h (other two hospitals in Wuhan): the blood samples from 83 subjects were gathered, stored in an ice box (≤ 4 °C) and transported to Wuhan Union Hospital for tests. For the above two situations, the blood samples were tested directly after centrifugation (4000 rpm/5 min) at 4 °C. (3) Transportation time greater than 12 h: serum samples from 133 subjects were packed in a thermally isolated box filled with dry ice and transported to Wuhan Union Hospital. Samples were tested directly after they were received.

According to the protocol, serum, urinary and fecal MMP-7 levels were measured using a Human Total MMP-7 Quantikine ELISA Kit (R&D, USA). The dilution

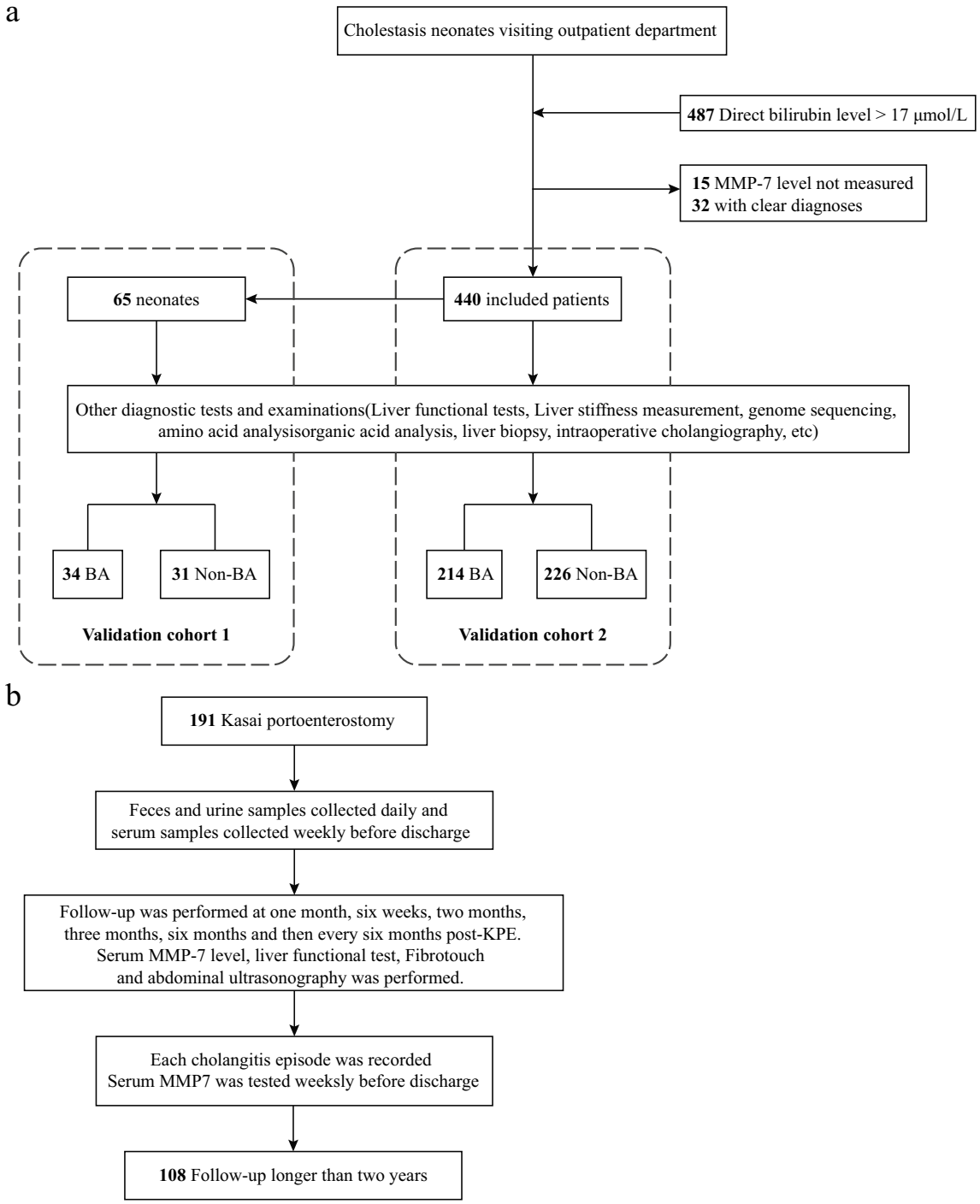

Fig. 1 **a** Flow chart of patient selection, diagnostic process and **b** flow chart of the follow-up study

times were 8 and 32 for serum samples. Feces samples were washed with PBS three times immediately after collection. The final concentration of feces was 1 g to 9 mL PBS, and the sample was subjected to ultrasound for 30 s. The feces solution was centrifuged for 5 min at 5000*g*, and the supernatant was used for ELISA tests.

MMP-7 expression and gene polymorphism

All exons and promoter regions of MMP-7 in 7 patients with low serum MMP-7 levels (LSM, < 26.73 ng/mL) and 33 patients (randomly chosen from 207 BA patients with high serum MMP-7 levels) with high serum MMP-7 levels

(HSM, > 26.73 ng/m) were sequenced using the Sanger method.

Inflammation grades and fibrosis classifications

The liver pathology of 256 patients (BA = 191; non-BA = 65, laparoscopic biliary lavage) undergoing surgery was analyzed and recorded independently by two pathologists, and discrepancies were resolved by discussion. The stages of inflammation and fibrosis classifications were defined according to the Scheuer scale system, ranging from G0 to 4 and S0 to 4 [12]. Inflammation grades are: G0: no inflammation; G1: mild inflammation; G2: moderate inflammation; G3: moderate-to-severe inflammation; and G4: severe inflammation. Fibrosis classifications are: S0: no fibrosis; S1: enlarged fibrotic portal tracts; S2: portal fibrosis with rare septa; S3: fibrosis with architectural distortion, but no obvious cirrhosis; and S4: numerous fibrous septal with pseudo lobule formation.

Statistical analysis

Categorical variables are described as frequencies and percentages; continuous variables are described as the mean (standard deviation) or median (interquartile range) according to the distribution of the data. The diagnostic cut-off value of serum MMP-7 was determined by receiver operating characteristic (ROC) analyses and area under the curve (AUC). Spearman's correlation was used to analyze the correlation between MMP-7 and different factors. The stepwise multivariable linear regression model was built considering all significant variables from the univariate regression. Mediation effect of MMP-7 on the association between inflammation and liver fibrosis was tested by Bootstrap proposed by Preacher and Hayes [19]. Kaplan–Meier survival curves and log-rank tests were used to determine the ability of different factors to predict prognoses. Significant differences between two groups were tested by Student's *t* test or the Mann–Whitney *U* test. The data were analyzed using SPSS 26.0, and $p < 0.05$ was used to determine significance.

Ethics approval

The research program was approved by the Institutional Review Board of each center, and this clinical trial was approved and registered in the Chinese clinical trial registry (Number: ChiCTR1900028456). The medical records of all included patients were collected and analyzed.

Results

Demographic characteristics

Of the 440 patients, 214 were diagnosed with BA, and 226 non-BA jaundice patients were recognized as the control group. All included patients were followed up to confirmation of diagnoses, and the diagnoses of 440 patients are listed in Supplementary Table 1. The baseline demographic, clinical, and laboratory profiles of the included patients are shown in Table 1.

Age-related changes and optimal threshold value of serum MMP-7 levels

To investigate whether MMP-7 was age related, we divided the included patients into three age groups (Group A: 4–28 days, $n = 65$, Group B: 29–60 days, $n = 234$, Group C: ≥ 61 days, $n = 141$). Analysis based on age groups showed that BA patients in group C had significantly higher serum

Table 1 Demographics and laboratory findings of the study population

	BA ($n = 214$)	Non-BA ($n = 226$)	*p*
Age, median (IQR), days	50 (35–66)	50 (35–65.25)	0.975
4–28	34	31	0.587
29–60	113	121	
61–189	67	74	
MMP-7, ng/mL	61.26 (47.87–102.04)	12.57 (8.22–18.19)	< 0.001
Sex, *n* (%)			< 0.001
Male	91 (42.52)	139 (61.50)	
Female	123 (57.48)	87 (38.50)	
TB, μmol/L	180.85 (137.40–224.58)	133.30 (94.45–185.80)	< 0.001
DB, μmol/L	119.30 (86.83–153.53)	76.05 (35.33–117.23)	< 0.001
ALT, U/L	110.00 (62.75–193.50)	59.00 (29.00–120.00)	< 0.001
AST, U/L	171.00 (104.00–280.00)	108.00 (55.00–172.00)	< 0.001
γ-GT, U/L	454.50 (239.95–776.83)	131.00 (79.00–210.65)	< 0.001
PLT, G/L	392.50 (229.75–486.50)	350.00 (269.00–446.00)	0.022

MMP-7 levels than those in group A and group B. No significant differences were identified between group A and group B. In non-BA patients, no age-related differences were noted.

Considering the progression features of BA and to eliminate the potential age interference, we calculated the cut off values of serum MMP-7 separately for cholestasis neonates. In neonate cholestasis patients ($n=65$, validation cohort one; BA = 34, non-BA = 31), using a cut off value of > 26.73 ng/mL, serum MMP-7 had a sensitivity = 91.2%, specificity = 100%, and AUC of 0.954 [95% CI 0.897–1.000]. (Supplementary Fig. 1a) We further examined the diagnostic accuracy of using > 26.73 ng/mL in all patients without age limit ($n=440$, validation cohort two; BA = 214, non-BA = 226). Using a cut-off value of > 26.73 ng/mL in all cholestasis patients, serum MMP-7 had a sensitivity = 96.7%, specificity = 95.6%, and AUC of 0.983 [95% CI 0.970–0.996] (Supplementary Fig. 1b). The positive predictive value (PPV), negative predictive value (NPV), and diagnostic efficiency of serum MMP-7 level > 26.73 ng/mL for predicting BA were 95.39%, 96.86%, and 96.14%, respectively.

Reasons for low serum MMP-7 level in BA patients

Although serum MMP-7 has high diagnostic accuracy, seven (3.27%) BA patients in our cohort had serum MMP-7 lower than 26.73 ng/mL. To assess the reason for the low MMP-7 expression level in these BA patients, we performed polymorphism sequencing on exons and promoter regions of MMP-7. The heterozygote mutation percentage of c.410G > A (rs17884789 C > T) in exon 3 was significantly increased in LSM patients compared with HSM patients (42.86% vs 3.03%, $p=0.002$) (Supplementary Table 2).

Correlation of serum MMP-7 levels with multiple clinical characteristics

Serum MMP-7 levels at diagnosis were found to positively correlate with total bilirubin (TBIL), direct bilirubin (DBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (γ-GT) at diagnosis and fibrosis classification and inflammation grade at KPE in BA patients (Supplementary Table 3). The multivariable linear regression model showed that the γ-GT level at diagnosis and inflammation grades and fibrosis classifications at KPE were significant factors associated with serum MMP-7 levels at diagnosis (Supplementary Table 4). Moreover, MMP-7 showed a significant mediation effect with an effect ratio of 0.35 on the association between inflammation and liver fibrosis in BA infants (supplementary Fig. 2 and Table 2); however, no mediation effect was identified in non-BA infants.

Table 2 Mediation effect of MMP-7 on the association between inflammation and liver fibrosis in BA and non-BA infants

BA	Fibrosis classification		Fibrosis classification		Serum MMP7	
	B	*t*	*B*	*t*	*B*	*t*
Inflammation grades	0.1189	2.0294*	0.1839	3.6620*	27.4504	9.0034*
Age	0.0069	4.6708*	0.0079	5.5638*	0.4125	4.7769*
Serum MMP7	0.0024	0.0366*	–	–	–	–
	Effect	Boot SE	Boot LLCI	Boot ULCI	Effect ratio	
MMP7	0.0649	0.0293	0.0108	0.1253	0.3529	
Direct effect	0.1189	0.0586	0.0034	0.2344	0.6465	
Total effect	0.1839	0.0502	0.0849	0.2828	–	
Non-BA	Fibrosis classification		Fibrosis classification		Serum MMP7	
	t	*t*	*B*	*t*	*B*	*t*
Inflammation grades	0.3186	2.0765*	0.3585	3.0673*	6.4659	5.7075*
Age	0.0011	0.2666	0.0009	0.22	−0.0329	−0.8548
Serum MMP7	0.0062	0.4061	–	–	–	–
	Effect	Boot SE	Boot LLCI	Boot ULCI	Effect ratio	
MMP7	0.0399	0.1033	−0.1558	0.2493	–	
Direct effect	0.3186	0.1736	0.0008	0.6841	–	
Total effect	0.3585	0.138	0.118	0.659	–	

*$p < 0.05$

At 3 months post-KPE, serum MMP-7 levels were positively correlated with TBIL (Correlation coefficient: 0.483), DBIL (0.433), γ-GT (0.638), APRI (0.453), Fibro touch (0.672). The multivariable linear regression model showed that total bilirubin, Fibro Touch results and APRI were significantly associated with serum MMP-7 levels at 3 months post-KPE (Supplementary Table 5).

Dynamic changes in serum MMP-7 in BA patients post-KPE

No significant differences were identified between the demographics of the included ($n = 108$) and excluded patients ($n = 83$) and SNL percentages of each centre. 62 (57.41%) and 51 (47.22%) post-KPE BA patients had event-free survival with their native liver (SNL) during the 1st and 2nd-year post-KPE, respectively. The dynamic trends of serum MMP-7 levels in BA patients post-KPE with different prognoses (SNL vs. non-SNL) are summarized in Fig. 2a. Significant differences between the two groups began to appear from 4 weeks post-KPE. After analyzing the dynamic characteristics of post-KPE serum MMP-7 in each BA patients, we observed four patterns. MMP-7 pattern 1 ($n = 11$, 10.19%) involved a rapid decline and then gradually increased or remained relatively stable at a low level (below the postoperative level), finally presenting a downward trend (Supplementary Fig. 3a). MMP-7 pattern 3 ($n = 29$, 26.85%) showed elevated or fluctuating MMP-7 levels around a relatively high level and then a stable downward trend (Supplementary Fig. 3b). MMP-7 pattern 4 ($n = 51$, 47.22%) involved a drastic fluctuation in the first 1–2 months post-KPE and subsequently elevated MMP-7 levels, even though the jaundice-free status ever achieved post-KPE (Supplementary Fig. 3c). MMP-7 pattern 4 ($n = 17$, 15.74%) involved a continuously rising serum MMP-7 level post-KPE and usually had persistent jaundice (Supplementary Fig. 3d). The percentage of SNL patients at the 2-year visit in the dynamic pattern 1–4 is 100%, 79.31%, 31.37%, and 5.88%, respectively. No cholangitis, thrombocytopenia, splenomegaly, or esophageal varices were found in patients with MMP-7 pattern 1, while the incidence of the above complications was the highest in patients with MMP-7 pattern 4 (Table 3). Survival curves with log-rank test showed that BA patients with different MMP7 patterns have different SNL times (Fig. 2b).

To determine the reason for the rapid decline in serum MMP-7 levels in some BA patients post-KPE, we analyzed MMP-7 levels in urine and feces samples of 40 post-KPE BA patients. Continuously low concentrations of MMP-7 were detected in urine samples of BA patients, and no correlation was found between serum and urinary MMP-7 levels. However, in response to the two changing directions (increase/decrease) in serum MMP-7 levels, two fecal MMP-7 level dynamic patterns were observed. The detailed post-KPE serum and fecal MMP-7 levels of two representative patients are shown in Fig. 2c, d. Patient 1 had a rapidly increased fecal MMP-7 level with a decreasing/fluctuating serum MMP-7 level (fecal pattern 1, $n = 31$, Fig. 2c). Patient 2 had a stable low fecal MMP-7 level; however, her serum MMP-7 level increased continuously after KPE (fecal pattern 2, $n = 9$, Fig. 2d).

The power of postoperative clinical indicators to predict SNL at 2 years post-KPE

The ROC analyses showed varying degrees of power for serum MMP-7 levels, total bilirubin, direct bilirubin, γ-GT, Fibro Touch levels, and APRI post-KPE in predicting SNL at 2 years post-KPE (Fig. 3). Delong's test showed that serum MMP-7 level at 6 weeks (AUC (95% CI) 0.796 (0.707–0.867), $p < 0.01$) and 3 months (AUC (95% CI) 0.861 (0.781–0.920), $p < 0.05$) post-KPE has the highest prognostic value among all current minimally invasive indicators. However, the prediction ability of serum MMP-7 did not increase when combined with other indicators at 6 weeks post-KPE. The Cox regression results showed that serum MMP-7 level, total bilirubin, and APRI at 3 months after KPE were significant indicators of SNL at 2 years post-KPE and that serum MMP-7 level had the highest hazard ratio (5.34, 95% CI [2.50–11.39]) (Supplementary Table 6).

Discussion

The results of this study showed that serum MMP-7 could be applied in neonate BA diagnosis. High sensitivity and specificity were maintained when applying the optimal cut-off value in neonate cholestasis patients to all patients for BA diagnosis. To find more BA patients at an early stage, we recommend using 26.73 ng/mL for BA diagnosis. Several studies proved the diagnostic value of serum MMP-7 levels in BA patients, but the diagnostic values differ greatly [11, 13, 14]. The inconsistency of the cut-off value of serum MMP-7 among different centers could be attributed to the different ELISA kits, differences between laboratories, different disease distributions in the control group, and different average ages of the included patients. Although highly sensitive, a small part of BA patients could not be diagnosed by MMP-7. Our sequencing results showed that MMP-7 (rs17884789 C > T) in BA patients associated with a low serum MMP-7 concentration. A previous study focused on liver cirrhosis reported that this SNP could lead to the extracellular membrane's distribution of MMP-7 and rarely secreted into the blood [20]. However, the MMP-7 (rs17884789 C > T) mutation was only identified in three of seven patients in the LSM group. Whether the low serum MMP-7 levels in another four

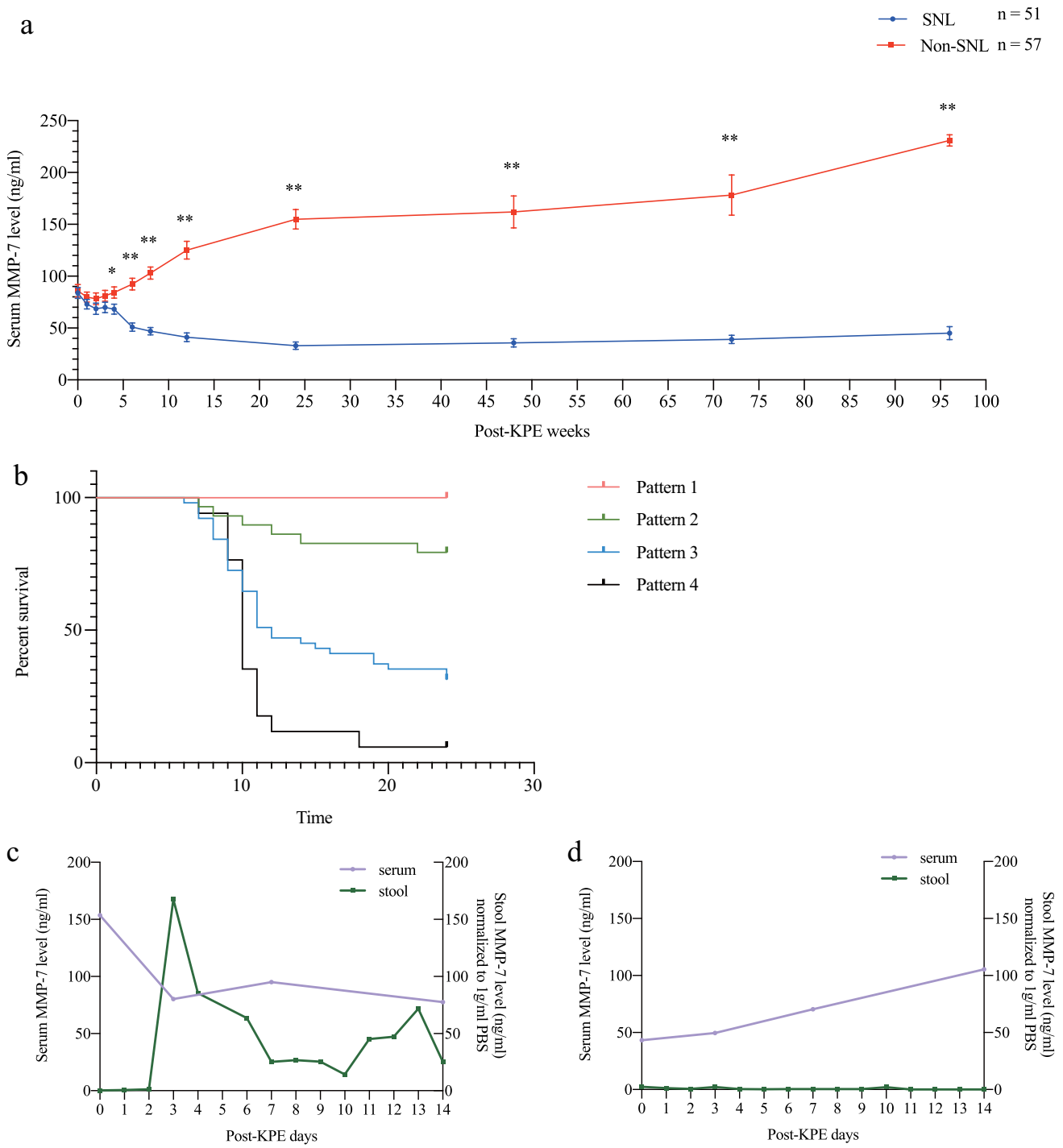

Fig. 2 **a** Dynamic changes in serum MMP-7 levels after hepatoportoenterostomy in different prognosis groups. **b** Survival curves of BA patients with different post-KPE dynamic patterns. Dynamic patterns of fecal MMP-7 levels, $^{*}p < 0.05$, $^{**}p < 0.001$ (**c**) pattern 1 (**d**) pattern 2

Table 3 Incidence of complications in BA patients with different post-KPE serum MMP-7 patterns

	Pattern 1 ($n = 11$)	Pattern 2 ($n = 29$)	Pattern 3 ($n = 51$)	Pattern 4 ($n = 17$)	p value
Cholangitis	0 (0.00%)	7 (24.14%)	18 (35.29%)	13 (76.47%)	< 0.001
Thrombocytopenia	0 (0.00%)	7 (24.14%)	20 (39.22%)	12 (70.59%)	0.001
Splenomegaly	0 (0.00%)	10 (34.48%)	26 (50.98%)	13 (76.47%)	< 0.001
Esophageal varices	0 (0.00%)	8 (27.59%)	17 (33.33%)	10 (58.82%)	0.012

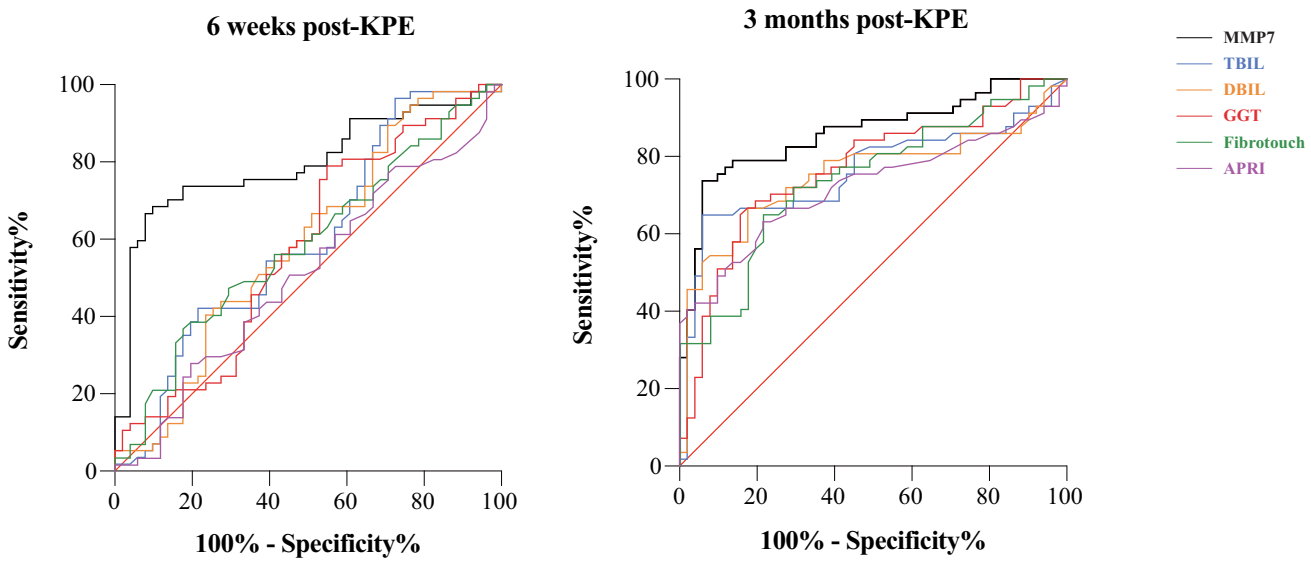

Fig. 3 ROC curves of different post-KPE indicators at 6 and 3 months post-KPE for prognosis prediction

cases were related to the polymorphism of other introns or exons needs further study. Almost no T alle exists at SNP rs17884789 except for the Asian population (1000 genomes project phase 3 allele frequencies from the ensemble), which indicated serum MMP-7 might have higher diagnostic accuracy in other races.

Chatmanee et al. found that preoperative serum MMP7 levels were correlated with inflammation classifications [12]. Jiang et al. reported that the preoperative serum MMP-7 level was correlated with the fibrosis stage in liver biopsies, whereas no significance was found within different inflammation grades [11]. In the current study, we found that serum MMP-7 level at diagnosis positively correlated with both inflammation and fibrosis grades. We also found a significant mediation effect of serum MMP-7 between inflammation and fibrosis in BA patients, which suggested that MMP-7 was produced by severe bile duct inflammation and could promote liver fibrosis. Moreover, serum MMP-7 at 3 months post-KPE was positively associated with indicators that reflect the degree of liver fibrosis (total bilirubin levels, Fibro Touch results, and APRI). These results indicated that MMP-7 could be a factor that participates in the whole course of BA occurrence and development rather than the result of fibrosis. Further work is required to find out whether MMP-7 promoted liver fibrosis through the E-cadherin/β-catenin pathway, like kidney fibrosis [21, 22].

There are two ways to clear MMPs in the human body, degradation clearance and excretion with urine or feces. A-2 Macroglobulin was the major plasma inhibitor of MMPs, and TIMP is a key inhibitor in tissues [23]. In idiopathic pulmonary fibrosis and kidney fibrosis, MMP-7 levels in serum or urine correlate very well with clinical symptoms as well as prognosis [7, 22, 24]. In clostridioides difficile infection, fecal MMP-7 levels served as biomarkers determining disease severity [25]. Two previous studies reported that MMP-7 levels at 6 months post-KPE were positively correlated with the severity of liver fibrosis and native liver survival rates [13, 26]. To find more substantial evidence that can explain the mediation effect of MMP-7, we performed dynamic monitoring of serum, urinary, and fecal MMP-7 levels in post-KPE BA patients. Different dynamic patterns of serum MMP-7 corresponded well to clinical symptoms (cholangitis and liver cirrhosis complications) and prognoses. A continuously high level of serum MMP-7 post-KPE was more frequently seen in patients with cholangitis, liver cirrhosis complications, and needed LTx in 2 years. Moreover, a high fecal MMP-7 level post-KPE is usually accompanied by a decrease in serum MMP-7. Persistently undetectable low levels of fecal MMP-7 would lead to continuously high serum MMP-7 levels, while there was no correlation between serum and urinary MMP-7 levels. Thus, the observed decrease in serum MMP-7 levels post-KPE was attributed to extrahepatic bile duct surgical reconstruction, which relieved biliary obstruction. A portion of MMP-7 could be excreted via the reconstructed bile duct. The massive excretion of MMP-7 through feces could maintain a low level of MMP-7 in the liver and bile duct, thus the fibrosis remained stable or even resolved over time in post-KPE BA patients. While the accumulation of MMP-7 in bile ducts, liver, or serum was related to progressive liver fibrosis and would lead to end-stage liver disease rapidly.

Prior studies have shown that TBIL, γ-GT, APRI, liver stiffness, fibrosis gene signature, interleukin-8, interleukin 12B, inflammation gene signature, and collagen hybridizing

peptide levels could predict the prognoses of BA patients post-KPE [27–34]. In the current study, serum MMP-7 levels at 6 weeks and 6 months post-KPE made the most accurate predictions among all the indicators according to Delong's test of the ROC curves, and combination with other indicators would not increase the prediction accuracy. This finding highlighted MMP-7's reflective ability in the early stage of bile duct injury before the bile duct obstruction develops, while other indicators (TBIL, DBIL, GGT, APRI, Fibro touch) could only reflect the result of bile duct obstruction and liver fibrosis. Similar to MMP-7's early diagnostic ability, this evidence suggested that MMP-7 is the forerunner of the known indicators related to BA progression. As a much less invasive detection method, serum and fecal MMP-7 level measurement could optimize pre-transplant care and maximize transplant outcomes of BA patients. Further work is required to explore the metabolic pathway and pathogenesis of MMP-7 in the liver of BA patients.

Several limitations of the current study are worth noting. First, most included patients were from two provinces of China (Hubei and Shaanxi), potentially limiting the applicability of the study's results to other populations or ethnic groups. Second, the prospective study randomly included BA and non-BA patients who visited the outpatient department. Thus, there were some inconsistencies in the demographics of the two groups. However, no significant difference was identified in the average age between the two groups, given that serum MMP-7 levels are age-related in BA patients. Third, the heterogeneity of the sample storage, transportation, and multicenter nature of the study slightly decreases the credibility of the results, but increases the study findings' external validity or generalizability. Finally, some patients were lost to follow-up, which may introduce some bias to the study.

To the best of our knowledge, the present study has been the first prospective, multicenter, large sample size study focused on the clinical application value of MMP-7 reported to date, providing a valuable basis for MMP-7's potential for cholestatic neonates differential diagnosis and an earliest prognostic biomarker in BA. Dynamic monitoring of serum and fecal MMP-7 post-KPE is necessary for planning individualized treatment interventions and determine the appropriate time for performing LTx.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12072-022-10322-x.

Author contributions All authors contributed to the study conception and design. SQC and PPX collected, analyzed the data, and drafted the manuscript; PY collected the data and provided analytical oversight; PPX, SQC, PY and STT designed and supervised the study; HBW, YQY, GQC, SL, YZ, XZ, XYL, HZN, LX and PCC revised the manuscript for important intellectual content; GQC and SL offered technical or material support; STT provided administrative support; all authors read and approved the final manuscript.

Funding National Natural Science Foundations of China (Grant nos. 81670511 and 81270481).

Availability of data and material The data can be obtained by reasonable request from the corresponding author. STT has access to all data.

Code availability Not applicable.

Declarations

Conflict of interest Shuiqing Chi, Peipei Xu, Pu Yu, Guoqing Cao, Haibin Wang, Yongqin Ye, Shuai Li, Yun Zhou, Xiangyang Li, Ying Zhou, Xi Zhang, Huizhong Niu, Lei Xu, Pengcheng Cai, Shaotao Tang declare no competing interests.

Ethics approval The research program was approved by the Institutional Review Board of each center, and this clinical trial was approved and registered in the Chinese clinical trial registry.

Consent to participate Informed and written consent to participate in the study was obtained from all patients' parents.

Consent for publication All authors agreed to publish this manuscript.

References

1. Bezerra JA, Wells RG, Mack CL, Karpen SJ, Hoofnagle JH, Doo E, et al. Biliary atresia: clinical and research challenges for the twenty-first century. Hepatology 2018;68:1163–1173
2. Feldman AG, Mack CL. Biliary atresia: clinical lessons learned. J Pediatr Gastroenterol Nutr 2015;61:167–175
3. Sundaram SS, Mack CL, Feldman AG, Sokol RJ. Biliary atresia: Indications and timing of liver transplantation and optimization of pretransplant care. Liver Transpl 2017;23:96–109
4. Lopez RN, Ooi CY, Krishnan U. Early and peri-operative prognostic indicators in infants undergoing hepatic portoenterostomy for biliary atresia: a review. Curr Gastroenterol Rep 2017;19:16
5. Obayashi J, Kawaguchi K, Manabe S, Nagae H, Wakisaka M, Koike J, et al. Prognostic factors indicating survival with native liver after Kasai procedure for biliary atresia. Pediatr Surg Int 2017;33:1047–1052
6. Song Z, Dong R, Shen Z, Chen G, Yang Y, Zheng S. Surgical outcome and etiologic heterogeneity of infants with biliary atresia who received Kasai operation less than 60 days after birth: a retrospective study. Medicine (Baltimore) 2017;96:e7267
7. Tyraskis A, Davenport M. Steroids after the Kasai procedure for biliary atresia: the effect of age at Kasai portoenterostomy. Pediatr Surg Int 2016;32:193–200
8. Hartley JL, Davenport M, Kelly DA. Biliary atresia. Lancet 2009;374:1704–1713
9. Sokol RJ, Shepherd RW, Superina R, Bezerra JA, Robuck P, Hoofnagle JH. Screening and outcomes in biliary atresia: summary of a National Institutes of Health workshop. Hepatology 2007;46:566–581
10. Harpavat S, Garcia-Prats JA, Anaya C, Brandt ML, Lupo PJ, Finegold MJ, et al. Diagnostic yield of newborn screening for biliary atresia using direct or conjugated bilirubin measurements. JAMA 2020;323:1141–1150
11. Jiang J, Wang J, Shen Z, Lu X, Chen G, Huang Y, et al. Serum MMP-7 in the diagnosis of biliary atresia. Pediatrics 2019;144:e20190902

12. Lertudomphonwanit C, Mourya R, Fei L, Zhang Y, Gutta S, Yang L, et al. Large-scale proteomics identifies MMP-7 as a sentinel of epithelial injury and of biliary atresia. Sci Transl Med 2017;9:eaan8462
13. Wu JF, Jeng YM, Chen HL, Ni YH, Hsu HY, Chang MH. Quantification of serum matrix metallopeptide 7 levels may assist in the diagnosis and predict the outcome for patients with biliary atresia. J Pediatr 2019;208:30–37.e31
14. Yang L, Zhou Y, Xu PP, Mourya R, Lei HY, Cao GQ, et al. Diagnostic accuracy of serum matrix metalloproteinase-7 for biliary atresia. Hepatology 2018;68:2069–2077
15. Arnon R, Leshno M, Annunziato R, Florman S, Iyer K. What is the optimal timing of liver transplantation for children with biliary atresia? A Markov model simulation analysis. J Pediatr Gastroenterol Nutr 2014;59:398–402
16. Shneider BL, Brown MB, Haber B, Whitington PF, Schwarz K, Squires R, et al. A multicenter study of the outcome of biliary atresia in the United States, 1997 to 2000. J Pediatr 2006;148:467–474
17. Russo P, Magee JC, Boitnott J, Bove KE, Raghunathan T, Finegold M, et al. Design and validation of the biliary atresia research consortium histologic assessment system for cholestasis in infancy. Clin Gastroenterol Hepatol 2011;9:357–362.e352
18. Zhou Y, Jiang M, Tang ST, Yang L, Zhang X, Yang DH, et al. Laparoscopic finding of a hepatic subcapsular spider-like telangiectasis sign in biliary atresia. World J Gastroenterol 2017;23:7119–7128
19. Preacher KJ, Hayes AF. SPSS and SAS procedures for estimating indirect effects in simple mediation models. Behav Res Methods Instrum Comput 2004;36:717–731
20. Hung TM, Chang SC, Yu WH, Wang YW, Huang C, Lu SC, et al. A novel nonsynonymous variant of matrix metalloproteinase-7 confers risk of liver cirrhosis. Hepatology 2009;50:1184–1193
21. Ke B, Fan C, Yang L, Fang X. Corrigendum: matrix metalloproteinase-7 and kidney fibrosis. Front Physiol 2017;8:192
22. Zhou D, Tian Y, Sun L, Zhou L, Xiao L, Tan RJ, et al. Matrix metalloproteinase-7 is a urinary biomarker and pathogenic mediator of kidney fibrosis. J Am Soc Nephrol 2017;28:598–611
23. Mocchegiani E, Giacconi R, Costarelli L. Metalloproteases/antimetalloproteases imbalance in chronic obstructive pulmonary disease: genetic factors and treatment implications. Curr Opin Pulm Med 2011;17(Suppl 1):S11-19
24. Song JW, Do KH, Jang SJ, Colby TV, Han S, Kim DS. Blood biomarkers MMP-7 and SP-A: predictors of outcome in idiopathic pulmonary fibrosis. Chest 2013;143:1422–1429
25. Golizeh M, Winter K, Roussel L, Landekic M, Langelier M, Loo VG, et al. Fecal host biomarkers predicting severity of *Clostridioides difficile* infection. JCI Insight 2021;6:e142976
26. Kerola A, Lampela H, Lohi J, Heikkilä P, Mutanen A, Hagström J, et al. Increased MMP-7 expression in biliary epithelium and serum underpins native liver fibrosis after successful portoenterostomy in biliary atresia. J Pathol Clin Res 2016;2:187–198
27. Hukkinen M, Kerola A, Lohi J, Jahnukainen T, Heikkilä P, Pakarinen MP. Very low bilirubin after portoenterostomy improves survival of the native liver in patients with biliary atresia by deferring liver fibrogenesis. Surgery 2019;165:843–850
28. Jaramillo C, Guthery SL, Lowichik A, Stoddard G, Kim T, Li Y, et al. Quantitative liver fibrosis using collagen hybridizing peptide to predict native liver survival in biliary atresia: a pilot study. J Pediatr Gastroenterol Nutr 2020;70:87–92
29. Koga H, Wada M, Nakamura H, Miyano G, Okawada M, Lane GJ, et al. Factors influencing jaundice-free survival with the native liver in post-portoenterostomy biliary atresia patients: results from a single institution. J Pediatr Surg 2013;48:2368–2372
30. Luo Z, Shivakumar P, Mourya R, Gutta S, Bezerra JA. Gene expression signatures associated with survival times of pediatric patients with biliary atresia identify potential therapeutic agents. Gastroenterology 2019;157:1138-1152.e1114
31. Pakarinen MP, Johansen LS, Svensson JF, Bjørnland K, Gatzinsky V, Stenström P, et al. Outcomes of biliary atresia in the Nordic countries—a multicenter study of 158 patients during 2005–2016. J Pediatr Surg 2018;53:1509–1515
32. Shneider BL, Magee JC, Karpen SJ, Rand EB, Narkewicz MR, Bass LM, et al. Total serum bilirubin within 3 months of hepatoportoenterostomy predicts short-term outcomes in biliary atresia. J Pediatr 2016;170:211–217.e211–e212
33. Superina R, Magee JC, Brandt ML, Healey PJ, Tiao G, Ryckman F, et al. The anatomic pattern of biliary atresia identified at time of Kasai hepatoportoenterostomy and early postoperative clearance of jaundice are significant predictors of transplant-free survival. Ann Surg 2011;254:577–585
34. Webb NL, Jiwane A, Ooi CY, Nightinghale S, Adams SE, Krishnan U. Clinical significance of liver histology on outcomes in biliary atresia. J Paediatr Child Health 2017;53:252–256