

# Noninvasive Evaluation of Liver Fibrosis and Portal Hypertension After Successful Portoenterostomy for Biliary Atresia

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We investigated noninvasive follow-up markers for histologic liver fibrosis and portal hypertension (PH) in patients with biliary atresia after successful portoenterostomy (PE). Among children with bilirubin <20  $\mu\text{mol/L}$  after PE ( $n = 39$ ), Metavir fibrosis stage was evaluated at PE and in follow-up protocol liver biopsies ( $n = 83$ ). PH was defined as endoscopically confirmed esophageal varices or thrombocytopenia associated with splenomegaly. The accuracy of liver biochemistry and stiffness in detecting liver fibrosis and PH was analyzed by the area under the receiving operating characteristic curve (AUROC) and multiple regression models. During a median native liver survival of 8.3 years (interquartile range 2.5–10.8 years), cirrhosis (Metavir F4) had developed in 51% of patients and PH in 54% of patients. Cirrhosis was equally common in all age tertiles of 1.2–2.1 years ( $n = 10/27$ ), 3.9–5.8 years ( $n = 12/28$ ), and 9.0–14 years ( $n = 12/28$ ). In the two oldest age tertiles, histologic liver fibrosis had progressed further in patients with PH than without PH ( $P < 0.001$ ). PH was accurately predicted by the aspartate aminotransferase-to-platelet ratio index (APRI) (cutoff, 0.70; AUROC, 0.92), bile acids (cutoff, 49  $\mu\text{mol/L}$ ; AUROC, 0.91), and liver stiffness (cutoff, 16.9 kPa; AUROC, 0.89;  $P < 0.001$  each) across all age tertiles. Liver stiffness was the most accurate predictor of cirrhosis overall (AUROC, 0.82;  $P < 0.001$ ), whereas bilirubin was >11  $\mu\text{mol/L}$  in the youngest tertile (AUROC, 0.91;  $P < 0.001$ ), bile acids was >80  $\mu\text{mol/L}$  in the middle tertile (AUROC, 0.81;  $P = 0.009$ ), and liver stiffness was >24 kPa in the oldest age tertile (AUROC, 0.96;  $P = 0.002$ ). **Conclusion:** After successful PE, development of PH associates with progression of liver fibrosis and can be accurately detected by APRI and stiffness. Liver stiffness most accurately identified cirrhosis in older children, whereas biochemical markers of cholestasis closely reflected histologic cirrhosis in younger children. (*Hepatology Communications* 2019;3:382–391).

**B**iliary atresia (BA) is a progressive fibroinflammatory cholangiopathy presenting exclusively in newborns and accounting for the majority of pediatric liver transplantations (LTs) worldwide.<sup>(1)</sup> Currently, clearance of jaundice (COJ) rates after portoenterostomy (PE) exceed 50%,<sup>(2–4)</sup> and up to 25% of patients survive with their native liver (NL) until adulthood.<sup>(3,5)</sup> Although patients who still have cholestasis after PE rapidly progress

to end-stage liver disease and require LT for survival,<sup>(4,6)</sup> the disease course is far more variable after COJ. Following a successful PE, most patients survive with their NLs for many years.<sup>(2–5)</sup> However, during their disease course, the majority of children gradually progress to cirrhosis and develop symptoms of portal hypertension (PH), whereas others remain free from liver disease complications during childhood.<sup>(7–9)</sup>

*Abbreviations:* APRI, aspartate aminotransferase-to-platelet ratio index; AUROC, area under the receiver operating characteristic curve; BA, biliary atresia; BALF, BA liver fibrosis; CI, confidence interval; COJ, clearance of jaundice; GGT, gamma-glutamyltransferase; IQR, interquartile range; LT, liver transplantation; NL, native liver; PE, portoenterostomy; PH, portal hypertension; ROC, receiver operating characteristic; sens., sensitivity; spec., specificity.

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Accurate noninvasive tests for progressive liver fibrosis and the main complications of chronic liver disease would help to guide the post-PE follow-up of patients with normalized bilirubin levels and to serve as meaningful outcome measures for interventional clinical trials. The limited number of previous studies analyzing such tests has also included children after failed PE without a systematic longitudinal assessment of postoperative liver histology,<sup>(10-12)</sup> rendering the results unreliable. Liver stiffness is considered a useful predictor of fibrosis stage in children with various liver diseases<sup>(13-15)</sup> and has been reported to reflect the presence of esophageal and gastric varices after successful PE.<sup>(16)</sup> A previous study combining patients with both successful and failed PE found an equation based on liver biochemistry accurate in predicting the degree of histologic fibrosis.<sup>(10)</sup> To systematically assess the accuracy of different noninvasive tests in reflecting fibrosis stage and the main complications of liver disease longitudinally after COJ, we related follow-up liver biochemistry and liver stiffness with histologic liver fibrosis and PH in a national cohort of patients with BA who had cleared their jaundice after PE and underwent protocol liver biopsies and endoscopic variceal surveillance.

## Patients and Methods

### ETHICS

The study protocol was approved by the hospital ethical committee (protocol number 345/03/1372008). Institutional approval was renewed on July 21, 2017 (§68 HUS/149/2017).

## PATIENTS AND STUDY DESIGN

Helsinki University Children's Hospital is a nationwide referral center for pediatric hepatobiliary conditions and has had a pediatric LT program since 1987. In the present study, we included all patients with BA managed in Helsinki between 1987 and 2016 and who had cleared their jaundice after PE (defined as normalization of total bilirubin below 20  $\mu\text{mol/L}$  postoperatively) and had undergone at least one follow-up liver biopsy. The diagnosis of BA was confirmed by intraoperative cholangiography when possible and histopathology of the biliary remnant in all cases. At last follow-up, current medications and the total number of cholangitis episodes during follow-up were registered. Cholangitis was defined as febrile illness ( $>38^{\circ}\text{C}$ ) without any other identifiable cause treated with intravenous antibiotics.

## LIVER HISTOLOGY

All available follow-up liver biopsies ( $n = 83$ ) and biopsies taken at PE ( $n = 34$ ) of patients with BA who had cleared their jaundice were analyzed. Before centralization of BA management in 2005, follow-up biopsies were taken inconsistently. After 2005, protocol follow-up biopsies were scheduled 1, 5, and 10 years after PE and at transition as well as when clinically indicated. The specimens were mainly core needle biopsies taken percutaneously under ultrasound guidance in general anesthesia for endoscopic variceal surveillance.<sup>(17)</sup> Blood count, liver biochemistry, and coagulation parameters were checked prior to biopsy, and patients were followed overnight in the hospital after the biopsy. Biopsies were graded for fibrosis using Herovici as well as hematoxylin and eosin staining and applying the Metavir staging where stage F1

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corresponds to fibrous portal expansion without formation of septa, stage F2 indicates portal fibrosis with occasional portal-to-portal septa, stage F3 indicates portal fibrosis with numerous septa, and stage F4 represents cirrhosis.<sup>(18)</sup> Biopsies containing at least 10 portal areas were considered representative and included in the analyses. The specimens were re-reviewed by two experienced pediatric pathologists blinded to clinical data until consensus was reached. The difference between Metavir stages at follow-up and at PE was defined as the change of Metavir stage after PE.

## FOLLOW-UP LIVER BIOCHEMISTRY

Liver biochemistry, including blood platelet count, serum bile acids, and plasma concentrations of total and conjugated bilirubin, albumin, prealbumin, and gamma-glutamyltransferase (GGT), were registered at the time of follow-up liver biopsies. The aspartate aminotransferase (AST)-to-platelet ratio index (APRI) was calculated according to the following formula:  $([\text{AST}/\text{upper normal limit of normal}] \times 100) / \text{platelet count } (10^9/\text{L})$ .<sup>(19)</sup> The biliary atresia liver fibrosis (BALF) score was calculated as  $7.196 + (1.438 \times \log_e \text{ total bilirubin}) + (0.434 \times \log_e \text{ GGT}) - (3.491 \times \log_e \text{ albumin}) - (0.670 \times \log_e \text{ age})$ .<sup>(10)</sup>

## LIVER STIFFNESS MEASUREMENT

Liver stiffness was measured by experienced pediatric radiologists at the time of liver biopsy with a transient elastography device (FibroScan; Echosens, Paris, France).<sup>(20)</sup> The size of the probe was chosen according to the manufacturer's recommendations based on the patients' thoracic perimeter. Results were based on a median of 10 validated measurements and reported in kilopascals. Only liver stiffness measurements with a success rate >60% and a ratio of interquartile range (IQR) and median <30% were included.<sup>(20)</sup> All measurements were preprandial and performed with patients awake and without sedation.

## PH

Development of esophageal varices was monitored by performing annual upper gastrointestinal endoscopies, initiating at 12 months of age or at a younger age if bleeding were suspected.<sup>(4)</sup> Abdominal ultrasound

was performed every 3 to 12 months at outpatient visits. PH was defined as occurrence of varices at any time during follow-up or thrombocytopenia ( $<150 \times 10^9/\text{L}$ ) in association with splenomegaly in ultrasound (spleen length  $\geq 2 z$  scores above the age- and sex-specific reference values).<sup>(8,21)</sup> The detection age of PH and its presence at the time of each liver biopsy was registered.

## STATISTICAL METHODS

Continuous variables were expressed as mean or median values with IQRs. Spearman rank correlation was used to examine associations between variables, and the Mann-Whitney U test was used to compare continuous variables. Follow-up liver biopsies and liver function test results were divided into tertiles according to age at examination. Receiver operating characteristic (ROC) curves and areas under the ROC (AUROCs) curves were used to evaluate the ability of liver biochemistry and stiffness to detect histologic fibrosis as well as the presence of PH. In order to evaluate combinations of different liver function tests and stiffness for detection of cirrhosis (Metavir stage F4) and PH, natural logarithms of liver function tests and stiffness were included as covariates in multiple logistic regression models. Only covariates showing correlations <0.70 with each other were included in the same model. The predicted probabilities from logistic regression models were used to plot the respective ROC curves, and the multivariate models showing highest accuracy were used to create equations predicting the outcomes.<sup>(10)</sup> The optimal cut-off values were calculated using the maximum sum of specificity and sensitivity. The level of significance was set at  $P < 0.05$ , and all analyses were carried out with SPSS version 24.

## Results

### PATIENT CHARACTERISTICS

A total of 40 children with BA who cleared their jaundice after PE were identified; these children comprised 63% of all patients with BA treated from 1987 to 2016. One patient who underwent successful PE before 2005 but no follow-up liver biopsy was excluded. Type 3 BA was present in 90% of patients, and any associated malformations were detected in 39% of patients. After PE was performed (at a median age of 58 days), patients had cleared their jaundice by 2.5 months (IQR, 1.0-4.1

TABLE 1. PATIENT CHARACTERISTICS

	All Patients (n = 39)
Age at last follow-up or at LT (years)	8.3 (2.5-10.8)
Age at PE (days)	58 (29-78)
Type of biliary atresia, n (%)	
1 or 2	4 (10)
3	35 (90)
Associated malformations, n (%)	15 (39)
Polysplenia, n (%)	7 (5.9)
Time to COJ (months)	2.5 (1.0-4.1)
Metavir fibrosis stage at PE,* n (%)	
1	2 (5.7)
2	18 (51)
3	11 (31)
4	3 (8.6)
Follow-up biopsies per patient, n	3 (2-4)
Metavir fibrosis stage in follow-up biopsies, n (%)	
1	13 (16)
2	21 (25)
3	13 (16)
4	36 (43)
Ursodeoxycholic medication at last follow-up, n (%)	38 (97)
Prophylactic antibiotics at last follow-up, n (%)	35 (90)
Cholangitis episodes after PE, n (%)	2 (0-5)
Portal hypertension, n (%)	21 (54)
Age at portal hypertension detection (years)	1.3 (1.1-3.7)
Esophageal varices, n (%)	20 (51)
Age at detection of esophageal varices (years)	1.8 (1.2-5.2)
LT, n (%)	7 (18)
Age at LT (years)	7.4 (2.5-10.1)

Continuous data are reported as median with IQR.

\*Liver histology at PE available for 34 patients.

months) (Table 1). PH was first detected at a median age of 1.2 years (1.1-3.6 years) in 54% of patients, and 18% received LT during follow-up (Table 1). Apart from 1 patient who underwent LT soon after detection of PH, esophageal varices were noticed during follow-up in all others with PH. At the time of the study, no patient was listed for LT.

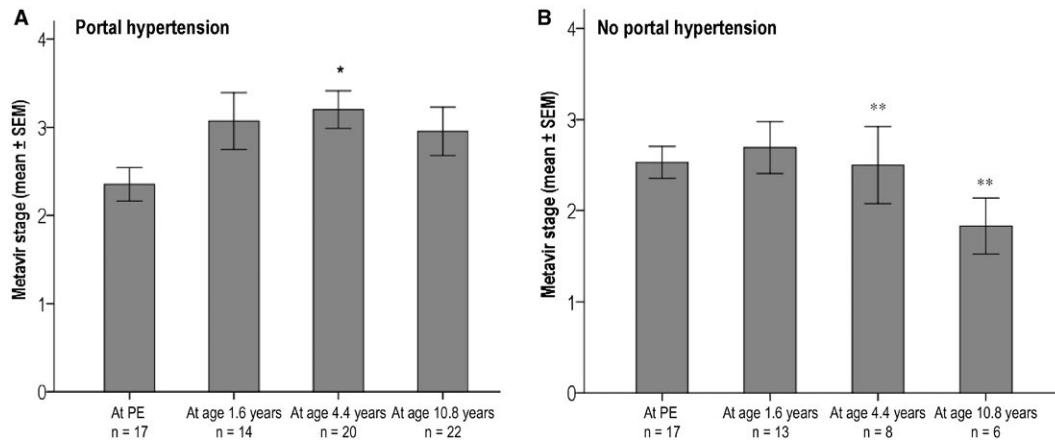
## FOLLOW-UP LIVER BIOPSIES AND PROGRESSION OF FIBROSIS

We analyzed 83 follow-up liver biopsies (median of three biopsies/patient) (Table 1). Grouped

according to age tertiles, the follow-up specimens were taken at a median age of 1.6 years (IQR, 1.2-2.1 years; n = 27), 4.4 years (IQR, 3.9-5.8 years; n = 28), or 10.8 years (IQR, 9.0-14.0 years; n = 28). At the time of biopsy, PH was present in 61% (n = 51) of patients, while grade 2 or greater esophageal varices were present in 57% (n = 47) of patients. Age at biopsy showed no correlation with Metavir fibrosis stage ( $r = -0.056$ ;  $P = 0.613$ ), and the proportions of liver biopsies showing high-grade fibrosis (Metavir stages F3 and F4) as well as cirrhosis (Metavir stage F4) were similar across the three age tertiles (63%, 61%, and 54% for Metavir F3 and F4; 37%, 43%, and 43% for Metavir F4, respectively); the difference was not significant (NS). Patients who developed PH during follow-up showed higher Metavir fibrosis stage in their follow-up biopsies compared to PE (mean stage, 3.0 [IQR, 2.0-4.0] versus 2.4 [IQR, 2.0-3.0];  $P = 0.014$ ), whereas no longitudinal change in Metavir fibrosis stage was observed in patients remaining free from PH (mean stage, 2.4 [IQR, 2.0-3.0] versus 2.5 [IQR, 2.0-3.0]), and the difference was NS (Fig. 1). In the two oldest age tertiles (median age of 4.4 and 10.8 years), Metavir fibrosis stage increased after PE significantly more among patients with PH than patients without PH (mean stage, 1.0 [IQR, 0.0-2.0] and mean stage, 1.8 [IQR, 1.5-2.0] versus mean stage, 0.43 [IQR, 0.0-2.0] and mean stage, -0.67 [IQR, -1.0-0.0];  $P < 0.001$  for both). The total number of cholangitis episodes during follow-up showed no difference between patients with and without PH or between patients with and without cirrhosis in their last follow-up liver biopsy.

## LIVER STIFFNESS AND LIVER BIOCHEMISTRY IN RELATION TO FIBROSIS STAGE AND PH

Liver biochemistry was measured within a median of 1.0 day (IQR, 1.0-1.0 day) of liver biopsies. A total of 58 liver stiffness measurements among 34 patients (87% of patients) were considered reliable and included for further analyses. They were performed within a median of 1.0 day (IQR, 1.0-48 days) of follow-up liver biopsies. Liver stiffness values and all biochemical values correlated with Metavir fibrosis stage (liver stiffness,  $r = 0.482$ ,  $P < 0.001$ ; bile acids,  $r = 0.479$ ,  $P < 0.001$ ; total bilirubin,  $r = 0.423$ ,



**FIG. 1.** Progression of liver fibrosis. Patients who (A) developed (n = 21) or (B) did not develop (n = 18) portal hypertension during follow-up according to age tertiles are shown. \**P* < 0.05 compared to PE, \*\**P* < 0.05 compared to patients with portal hypertension for the change of Metavir fibrosis stage after PE. Data represent mean ± SEM.

**TABLE 2. LIVER BIOCHEMISTRY AND STIFFNESS IN RELATION TO HISTOLOGIC CIRRHOSIS (METAVIR STAGE 4) AND PORTAL HYPERTENSION AT TIME OF FOLLOW-UP LIVER BIOPSY**

Liver Function Test	Measurements (n)	Cirrhosis	No Cirrhosis	<i>P</i> Value	Portal Hypertension	No Portal Hypertension	<i>P</i> Value
Bilirubin (μmol/L)	83	17 (11-35)	7.0 (4.0-13)	<0.001	14 (10-26)	5.0 (4.0-8.5)	<0.001
GGT (U/L)	83	136 (58-308)	39 (25-92)	0.001	125 (41-307)	32 (19-57)	<0.001
Bile acids (μmol/L)	75	126 (39-193)	28 (14-56)	<0.001	126 (52-198)	20 (14-30)	<0.001
Prealbumin (mg/L)	77	119 (98-163)	155 (133-171)	0.008	126 (96-155)	166 (146-180)	<0.001
APRI	83	1.79 (1.15-2.68)	0.58 (0.35-1.52)	<0.001	1.84 (1.30-2.41)	0.50 (0.26-2.41)	<0.001
Platelets (× 10 <sup>9</sup> /L)	83	117 (82-167)	192 (108-280)	0.001	101 (64-160)	252 (118-290)	<0.001
Liver stiffness (kPa)	58	38.2 (23.9-60.9)	11.9 (6.3-23.7)	<0.001	31 (17.9-60.1)	8.1 (5.6-13.4)	<0.001

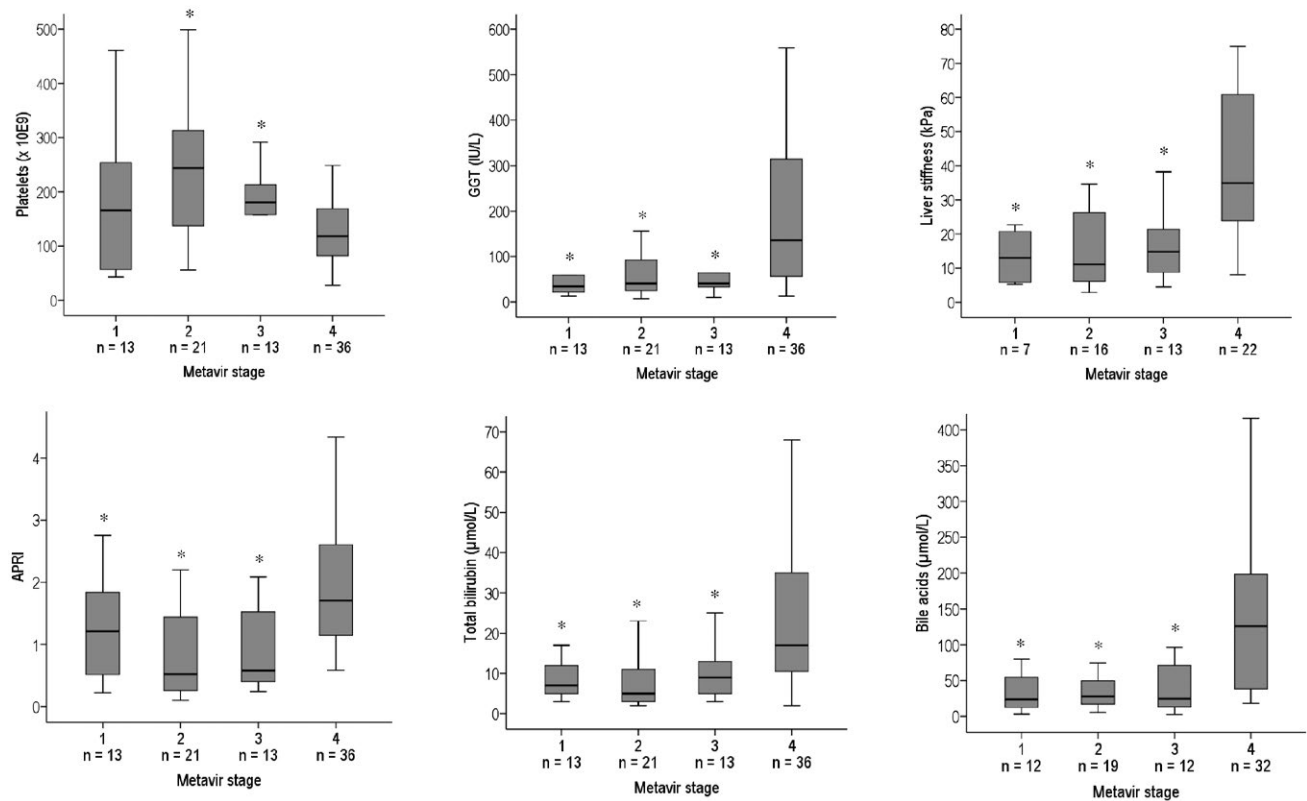
*P* values from Mann-Whitney U test are reported.

*P* < 0.001; APRI, *r* = 0.396, *P* < 0.001; GGT, *r* = 0.358, *P* = 0.001; platelets, *r* = -0.301, *P* = 0.006; prealbumin, *r* = -0.287, *P* = 0.011). All biochemical values and liver stiffness values were significantly altered in the presence of cirrhosis or PH (Table 2).

### PREDICTION OF LIVER FIBROSIS

None of the studied liver biochemistries, APRI, or liver stiffness were able to differentiate between Metavir fibrosis stages F1 through F3 (Fig. 2). In the whole cohort, liver stiffness was the most accurate predictor of Metavir stage F4, with an AUROC

value of 0.82 (Table 3; Fig. 3). Bilirubin, bile acids, and APRI performed best in the youngest age tertile, whereas bile acids and liver stiffness performed best in the middle tertile (Table 3). Although liver stiffness was an inaccurate predictor of fibrosis in the youngest age tertile, it predicted cirrhosis with a moderate accuracy in the middle tertile and with a high accuracy in the oldest age tertile (Table 3; Fig. 3). In simple logistic regression, the highest coefficients for prediction of cirrhosis were obtained for the natural logarithms of bile acids, APRI, and liver stiffness (Supporting Table S1). The multiple regression models showing the highest accuracy for



**FIG. 2.** Liver biochemistry and stiffness in relation to Metavir fibrosis stage. \* $P < 0.05$  compared to Metavir stage F4. The number of liver biopsies for each analysis is given on the  $x$  axis. The length of the box represents the interquartile range and the line through the middle of each box represents the median. Error bars show  $\pm$  SEM.

prediction of cirrhosis included the natural logarithms of bile acids, liver stiffness, prealbumin, and platelets as covariates, resulting in AUROC values of 0.98-0.88 with respective positive and negative predictive values of 0.90 and 0.84 for the whole cohort (Table 3). The accuracy of the published BALF score<sup>(10)</sup> remained below 0.75 for each age subgroup (Table 3).

## PREDICTION OF PH

APRI, bile acids, and liver stiffness were accurate predictors of PH, and they performed well across all age tertiles (Table 4; Fig. 3). GGT was accurate in younger age tertiles, whereas liver stiffness had the highest accuracy in older age tertiles (Table 4). In logistic regression, the highest coefficient for prediction of PH was obtained for the natural logarithm of APRI (Supporting Table S2). The multiple regression model showing the highest accuracy for prediction of

PH included the natural logarithms of APRI, liver stiffness, and age, and the equation derived from the model was a highly accurate predictor of PH for each age tertile (Table 4), with respective positive and negative predictive values of 0.97 and 0.95 for the whole cohort. For the prediction of grade 2 or greater esophageal varices, this score resulted in an AUROC value of 0.92 (95% confidence interval [CI], 0.84-1.00), with an optimal cut-off value of 0.63, sensitivity (sens.) of 0.94, specificity (spec.) of 0.89, and respective positive and negative predictive values of 0.88 and 0.92.

## Discussion

In this study, we longitudinally evaluated noninvasive markers of histologic liver fibrosis and PH among patients with BA who had cleared their jaundice after PE. Although APRI, bile acids, and liver stiffness were highly accurate in detecting the presence of PH

TABLE 3. PREDICTORS OF CIRRHOSIS (METAVIR STAGE 4) AFTER PORTOENTEROSTOMY

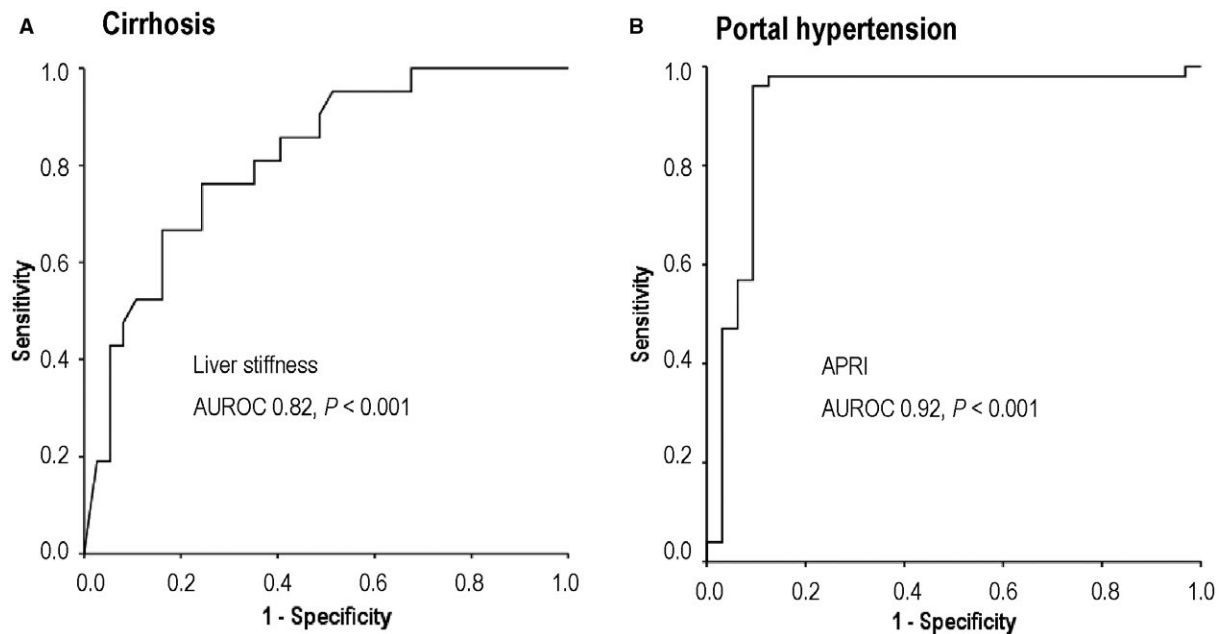
Liver Function Test	All Biopsies		Age 1.6 (1.2-2.1) Years		Age 4.4 (3.9-5.8) Years		Age 10.8 (9.0-14.0) Years	
	Biopsies (n)	AUROC (95% CI)	Biopsies (n)	AUROC (95% CI)	Biopsies (n)	AUROC (95% CI)	Biopsies (n)	AUROC (95% CI)
Bilirubin (μmol/L)	83	0.76 (0.65-0.87) <sup>‡</sup>	27	0.91 (0.79-1.00) <sup>‡</sup>	28	0.67 (0.46-0.88)	28	0.70 (0.50-0.90)
GGT (IU/L)	83	0.72 (0.61-0.84) <sup>‡</sup>	27	0.71 (0.51-0.90)	28	0.72 (0.52-0.91)	28	0.75 (0.55-0.96) <sup>*</sup>
Bile acids (μmol/L)	75	0.79 (0.69-0.89) <sup>‡</sup>	25	0.86 (0.70-1.00) <sup>‡</sup>	16	0.81 (0.64-0.97) <sup>‡</sup>	24	0.70 (0.49-0.91)
Precalburin (mg/L)	77	0.68 (0.55-0.81) <sup>‡</sup>	25	0.81 (0.60-1.00) <sup>*</sup>	26	0.61 (0.29-0.8)	26	0.62 (0.38-0.86)
APRI	83	0.77 (0.67-0.87) <sup>‡</sup>	27	0.59 (1.00, 0.51)	28	0.77 (0.59-0.94) <sup>*</sup>	28	0.73 (0.54-0.93) <sup>*</sup>
Platelets (× 10 <sup>9</sup> /L)	83	0.72 (0.61-0.83) <sup>‡</sup>	27	0.87 (0.72-1.00) <sup>‡</sup>	28	0.71 (0.52-0.91)	28	0.66 (0.45-0.86)
Liver stiffness (kPa)	58	0.82 (0.70-0.93) <sup>‡</sup>	20	0.73 (0.48-0.97)	21	0.79 (0.59-0.99) <sup>*</sup>	17	0.96 (0.86-1.00) <sup>‡</sup>
BALF score	74	0.73 (0.61-0.86) <sup>‡</sup>	23	0.75 (0.55-0.96) <sup>*</sup>	24	0.69 (0.47-0.92)	27	0.74 (0.53-0.96) <sup>*</sup>
Combination of bile acids, prealbumin, liver stiffness, and platelets <sup>§</sup>	52	0.88 (0.79-0.97) <sup>‡</sup>	19	0.87 (0.70-1.00) <sup>*</sup>	19	0.83 (0.64-1.00) <sup>*</sup>	14	0.98 (0.92-1.00) <sup>‡</sup>

AUROC values with 95% CIs are reported for all available liver biopsies as well as for subgroups based on age tertiles at biopsy. Age at biopsy reported as median with IQR. \*P < 0.05, †P < 0.01, ‡P ≤ 0.001. §Based on the equation -4.063 + (0.476 × log<sub>e</sub> Bile acids) + (1.241 × log<sub>e</sub> Liver stiffness) + (1.605 × log<sub>e</sub> Prealbumin) - (2.035 × log<sub>e</sub> Platelets).

regardless of patient age, the accuracy of the fibrosis markers varied more across the age tertiles, and none were able to discriminate between Metavir stages F1 through F3. Liver stiffness was the most accurate overall predictor of Metavir stage F4. Among children 1 to 2 years of age, bilirubin, bile acids, and APRI detected cirrhosis with good accuracy, whereas among school-aged children, liver stiffness was highly accurate for cirrhosis.

The progression of liver fibrosis and associated complications largely determine the prognosis of BA, which is profoundly distinct between patients who still have cholestasis compared to those whose bilirubin normalizes after PE.<sup>(6,10,22)</sup> After failed PE, patients nearly uniformly require LT by the age of 2 years,<sup>(1)</sup> and consequently, the use of noninvasive methods to predict their inevitable and rapid progression toward end-stage liver disease may be less useful. On the other hand, after a successful PE and prolonged NL survival, some patients progress rapidly to cirrhosis, whereas in others, liver fibrosis may even resolve over time.<sup>(10)</sup> In our patient sample, Metavir fibrosis stage was unrelated to age and remained stable among patients free from PH, whereas it progressed during follow-up in others, demonstrating how development of PH parallels the progression of histologic liver fibrosis after successful PE.

Reliable noninvasive markers of liver fibrosis would promote performance of interventional clinical trials designed to prolong NL survival in BA while sparing children from the burden and potential risks related to liver biopsies.<sup>(23,24)</sup> Although liver stiffness appears to be one of the most promising noninvasive methods reflecting fibrosis stage, it is influenced both by the etiology of liver disease and patient age.<sup>(25)</sup> Accordingly, the reported cutoffs and AUROC values for pediatric cirrhosis overlap markedly.<sup>(14,15,26)</sup> Previously determined cutoffs for cirrhosis at the time of PE are slightly lower compared to our results.<sup>(27,28)</sup> Because histologic inflammation and cholestasis are prominent at PE<sup>(4)</sup> and both may influence liver stiffness,<sup>(29-31)</sup> results obtained at the time of initial surgery are not directly comparable to the postoperative period after COJ. Further, the published BALF score based on albumin, GGT, and bilirubin reflecting follow-up fibrosis stage in an unselected sample of patients with BA showed no diagnostic accuracy in our cohort comprising solely patients with successful PE.<sup>(10)</sup> These findings demonstrate that patients



**FIG. 3.** AUROCs for the best predictors of cirrhosis. (A) Cirrhosis (Metavir stage F4; n = 58 measurements). (B) Portal hypertension (n = 83 measurements).

**TABLE 4. FOLLOW-UP LIVER BIOCHEMISTRY AND LIVER STIFFNESS IN THE PREDICTION OF PORTAL HYPERTENSION**

Liver Function Test	All Measurements			Age 1.6 (1.2-2.1) Years		Age 4.4 (3.9-5.8) Years		Age 10.8 (9.0-14.0) Years	
	n	AUROC (95% CI)	Best Cutoff (sens., spec.)	n	AUROC (95% CI)	n	AUROC (95% CI)	n	AUROC (95% CI)
Total bilirubin (μmol/L)	83	0.83 (0.73-0.92) <sup>‡</sup>	6.5 (0.90, 0.66)	27	0.86 (0.72-1.00) <sup>†</sup>	28	0.78 (0.61-0.96)*	28	0.82 (0.63-1.00)*
GGT (U/L)	83	0.79 (0.69-0.89) <sup>‡</sup>	85.0 (0.63, 0.88)	27	0.92 (0.82-1.00) <sup>‡</sup>	28	0.93 (0.84-1.00) <sup>‡</sup>	28	0.78 (0.58-0.98)*
Bile acids (μmol/L)	75	0.91 (0.84-0.97) <sup>‡</sup>	48.9 (0.77, 0.94)	25	0.95 (0.88-1.00) <sup>‡</sup>	26	0.94 (0.84-1.00) <sup>‡</sup>	24	0.90 (0.77-1.00) <sup>†</sup>
APRI	83	0.92 (0.85-1.00) <sup>‡</sup>	0.70 (0.98, 0.88)	27	0.94 (0.85-1.00) <sup>‡</sup>	28	0.94 (0.84-1.00) <sup>‡</sup>	28	0.85 (0.58-1.00) <sup>†</sup>
Prealbumin	77	0.75 (0.64-0.86) <sup>‡</sup>	152 (0.74, 0.70)	25	0.74 (0.53-0.95)	26	0.80 (0.62-0.97)*	26	0.86 (0.71-1.00) <sup>†</sup>
Platelets (× 10 <sup>9</sup> /L)	83	0.91 (0.85-0.97) <sup>‡</sup>	164 (0.89, 0.78)	27	0.82 (0.67-0.98) <sup>†</sup>	28	0.85 (0.71-1.00) <sup>†</sup>	28	0.99 (0.95-1.00) <sup>‡</sup>
Liver stiffness (kPa)	58	0.89 (0.81-0.97) <sup>‡</sup>	16.9 (0.77, 0.87)	20	0.84 (0.67-1.00)*	21	0.94 (0.84-1.00) <sup>‡</sup>	17	0.96 (0.87-1.00) <sup>†</sup>
Age (years)	83	0.71 (0.59-0.82) <sup>†</sup>	2.16 (0.88, 0.50)	27	0.68 (0.46-0.90)	28	0.57 (0.35-0.80)	28	0.52 (0.25-0.79)
Combination of APRI, liver stiffness, and age <sup>§</sup>	58	0.98 (0.94-1.00) <sup>‡</sup>	0.44 (0.97, 0.96)	20	0.99 (0.96-1.00) <sup>‡</sup>	21	0.95 (0.85-1.00) <sup>‡</sup>	17	1.00 (1.00-1.00) <sup>†</sup>

AUROC values with 95% CIs are reported for all available measurements as well as for subgroups based on age tertiles at time of liver biochemistry measurement. Age at biopsy reported as median with IQR.

\* $P < 0.05$ , <sup>†</sup> $P < 0.01$ , <sup>‡</sup> $P \leq 0.001$ . <sup>§</sup>Based on the equation  $-5.282 + (3.075 \times \log_e \text{APRI}) + (1.715 \times \log_e \text{Liver stiffness}) + (0.251 \times \log_e \text{Age})$ .



achieving COJ are not directly comparable to infants with BA who are waiting for PE nor to older patients who have failed to normalize their bilirubin after PE.

Although liver stiffness was a moderately accurate predictor of cirrhosis in the entire patient cohort, none of the tests studied were able to differentiate between Metavir stages F1 through F3. This finding is in line with previous studies comparing liver stiffness and APRI to Metavir fibrosis stage obtained at PE.<sup>(27,28,32,33)</sup> As in this study, combination scores based on different variables may result in higher accuracy and better discrimination than individual liver function tests but are less feasible for clinical use. In our study, the accuracy of cirrhosis predictors showed variability across the age tertiles. In the youngest age tertile at a median age of 1.6 years, cirrhosis was accurately predicted by bilirubin values  $>11$   $\mu\text{mol/L}$  and bile acid values  $>80$   $\mu\text{mol/L}$ , suggesting cirrhosis develops early without efficient re-establishment of bile flow by PE. The predictive value of liver biochemistry decreased with increasing age; indeed, long-term survivors with NL and compensated cirrhosis may present with almost normal liver biochemistry.<sup>(5)</sup> In older children, however, liver stiffness predicted cirrhosis with high accuracy. Despite including only successfully completed high-quality measurements, we did not find liver stiffness as a statistically significant predictor of cirrhosis in the youngest age group. This may relate to the dynamic nature of liver damage and remodeling among infants soon after PE or inherently poor reproducibility of liver stiffness measurement in small babies. As many as two-thirds of liver stiffness measurements performed among children aged  $<2$  years have been reported to be unreliable.<sup>(25)</sup>

In clinical practice, recognizing significant PH is of particular importance as it influences patient well-being and may warrant endoscopic interventions for esophageal varices.<sup>(8)</sup> Primary prophylaxis of high-risk varices among children has been recommended due to the mortality and morbidity related to acute gastrointestinal bleeding episodes.<sup>(34)</sup> In our series, apart from 1 patient with PH who underwent LT at a young age, esophageal varices were observed in others soon after detection of PH. According to our findings, liver stiffness  $>16.9$  kPa, APRI  $>0.70$ , and bile acids  $>50$   $\mu\text{mol/L}$  discriminated patients with PH with high accuracy. Similar liver stiffness cutoffs have been shown to predict clinically significant varices among patients with BA with normalized

bilirubin.<sup>(16)</sup> In addition, similar liver stiffness and APRI values correlate with PH defined by hepatic venous pressure gradient measurement, supporting the application of these cut-off values into clinical practice.<sup>(35-37)</sup> Overall, APRI was the best predictor of PH. A cutoff of 0.60 would have correctly classified 98% of cases that resulted in six false-positive results, and such a cutoff could be used in clinical practice to select which patients should undergo endoscopic screening for varices.

This study had some limitations, including its retrospective design and some missing liver function test results, although investigations were performed prospectively according to our institutional follow-up protocol. Synchronous reliable liver stiffness measurements were available for only 70% of biopsies. However, because the available measurements fulfilled the recommended methodologic criteria<sup>(20)</sup> and the patient age distribution was relatively uniform, we consider the results reliable and valuable. Other strengths of this study include the protocol-based longitudinal collection of liver biopsies and performance of surveillance endoscopies for reliable detection of PH.

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