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APRi predicts native liver survival by reflecting portal fibrogenesis and hepatic neovascularization at the time of portoenterostomy in biliary atresia

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ABSTRACT

Background: Aspartate aminotransferase-to-platelet ratio index (APRi) may be useful noninvasive prognostic tool in biliary atresia (BA). We studied whether APRi predicts native liver survival and parallels biochemical and immunohistological signs of liver injury and fibrogenesis at the time of Kasai portoenterostomy (PE).
Methods: Serum and liver specimens were obtained at PE from 29 BA patients for liver biochemistry including APRi, histology and immunohistochemical analysis of collagen 1, α-SMA and CD34. APRi values were related to native liver survival and other clinical data as well as serum liver biochemistry, liver histology and immunohistochemistry at PE.
Results: Median age at PE was 63 (range 7–141) days and median APRi was 0.92 (0.13–6.39). APRi had strong positive correlations with patient age ($r = 0.684$, $p < 0.001$) and biochemical signs of hepatocyte injury and cholestasis. APRi showed no significant correlations with Metavir ($r = 0.336$, $p = 0.223$) or Ishak ($r = 0.289$, $p = 0.262$) global fibrosis scores nor with liver collagen 1 expression ($r = 0.260$, $p = 0.222$). In contrast, portal fibrosis score ($r = 0.515$, $p = 0.013$), predominantly portal α-SMA expression ($r = 0.519$, $p = 0.015$) and amount CD34-positive microvessels in the centrizonal region ($r = 0.604$, $p = 0.004$) correlated positively with APRi. Patients ($n = 10$) who underwent liver transplantation had significantly higher APRi at presentation (1.34 vs. 0.77, $p = 0.017$) compared to those who survived with native liver ($n = 19$).
Conclusions: APRi correlates with portal fibrosis, expression of α-SMA and the amount of CD34-positive microvessels, suggesting that APRi predicts native liver survival by reflecting portal myofibroblastic cell activation, fibrogenesis and associated neovascularization.

Biliary atresia is a progressive infantile cholangiopathy with incompletely understood and most likely multifactorial etiology. The primary goal in BA is surgical clearance of jaundice by Kasai portoenterostomy (PE). Even after successful surgical restoration of bile flow the majority of patients end up with liver transplantation by adulthood owing to progressive liver fibrosis and associated complications of portal hypertension [1,2]. Several factors are known to affect outcomes of PE, including patient age and the degree of liver fibrosis at the time of PE [3,4]. Aspartate aminotransferase-to-platelet ratio index (APRi) has been introduced as a useful noninvasive tool to evaluate liver fibrosis and prognosis in BA [5,6]. Less is known about pathophysiological basis for prognostic power of APRi measured at the time of PE. To this end, we studied how APRi relates to biochemical and immunohistological signs of liver injury and fibrogenesis at the time of PE, and whether APRi predicts native liver survival.

1. Materials and methods

1.1. Patients and study design

This study was performed at the Children's Hospital in Helsinki, Finland. Treatment of BA and pediatric liver transplantations are nationally centralized to our unit [7]. A total of 29 BA patients born between 2000 and 2013 were enrolled. Liver biopsies obtained during PE were collected for immunohistological analysis and medical records were reviewed. Collected clinical data included age at PE, clearance of jaundice, associated malformations, presence of esophageal varices in follow-up upper gastrointestinal endoscopies and indication and timing of liver transplantation. Clearance of jaundice was defined as a decrease in serum bilirubin concentration below 20 μmol/L. All patients underwent endoscopic surveillance for esophageal varices as described previously [8]. Serum aspartate aminotransferase (AST), alanine

Abbreviations: BA, biliary atresia; PE, portoenterostomy; APRi, aspartate aminotransferase-to-platelet ratio index; α-SMA, alpha smooth muscle actin; AST, serum aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GT, gamma-glutamyl transpeptidase.

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aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GT), total and conjugated bilirubin concentration and blood platelets were measured at the time of PE using routine hospital laboratory methods. The APRI was calculated as the serum AST level (U/L)/upper normal × 100/platelet count (10^3/μL) [9]. The ethics committee of the hospital district of Helsinki and Uusimaa approved this study a priori and the study conforms to the principles of the 1975 Declaration of Helsinki.

1.2. Immunohistochemistry and imaging

Altogether 29 biopsies were taken during PE, including 6 core needle biopsies and 23 surgical wedge biopsies. The biopsies were fixed in formalin, embedded in paraffin, sliced, and stained with conventional stains. The representativeness of the biopsy material was considered good: >8 (wedge) or >10 (needle) portal areas were present in 26 (90%) biopsies. Two experienced pediatric liver pathologists, blinded to the clinical patient data, reviewed the specimens until consensus was reached. Histological features were assessed according to a semiquantitative scoring system validated for BA differential diagnosis including liver fibrosis scoring separately in portal (0–4) and lobular (0–2) regions as well as Metavir and Ishak scores [10–13].

Immunostaining for collagen 1 was performed with COL1A2/COL1A1 monoclonal antibody, clone I-8H5 (Abnova Corporation, Taiwan), for α-SMA using Monoclonal Mouse Anti-Human Smooth Muscle Actin, clone 1A4 (Dako, Denmark), for CD34 using Monoclonal Mouse Anti-Human CD34 Class II, clone QBEnd-10 (Dako, Denmark), and for CK7 using SP52 monoclonal antibody (Ventana, Tucson, Arizona, US) and NovoLink Polymer Detection System (Leica Biosystems Newcastle Ltd, Newcastle Upon Tyne, UK).

A Leica DM RXA microscope was used to obtain images of stained sections. A total of five random portal areas were chosen from each section of wedge biopsies (×100 magnification) and all core needle biopsies were photographed as a whole. The proportion of the antibody-positive area (area fraction) was measured by using ImageJ Image Analysis Software [14]. To evaluate neovascularization CD34-positive endothelial cell clusters forming centrizonal microvessels were counted with semiquantitative scoring system. Each biopsy was reviewed and scored for the presence of microvessels on scale of 1–4 [15]. Imaging and all immunohistochemical expression analyses were performed without knowledge of the clinical patient data.

1.3. Statistical analysis

The data are reported as medians and range. Comparisons between groups were performed with Mann–Whitney U-test or Kruskal–Wallis test. Correlations were calculated with Spearman's rank correlation. The predictive value for native liver survival of APRi, collagen 1 expression, Metavir fibrosis stage and portal fibrosis score was assessed with area under the ROC curve (AUROC) analysis with 95% confidence interval (CI). Significance of the AUROC was calculated in relation to the area of 0.5. A *P*-value <0.05 was considered significant. The statistical analyses were made with Statview software (Statview 5.0.1; SAS Institute Inc., CA, US) and SPSS software (IBM SPSS Statistics 19.0.0.1; IBM, Somers, NY, USA).

2. Results

2.1. Patient characteristics

Median age at PE was 63 (7–141) days. Serum bilirubin concentration prior to PE was 175 (50–384) μmol/L and 18 (62%) patients cleared their jaundice. Median overall follow-up time was 5.2 (1.1–14.3) years. The patients (n = 19) surviving with their native liver were followed for 5.1 (1.2–8.8) years and those transplanted (n = 10) for 1.1 (0.4–5.2) years before liver transplantation. Esophageal varices were observed in 15 (52%) patients at follow-up endoscopies. Patient characteristics are summarized in Table 1.

2.2. Correlation of clinical, biochemical and immunohistochemical variables with APRi

Median APRI was 0.92 (0.13–6.39) and it had a strong positive correlation with patient age, but was unrelated to birth weight (Table 2). APRi was related to biochemical signs of hepatocyte injury and cholestasis by showing positive correlations with ALT, ALP and conjugated bilirubin. Interestingly, patients with associated anomalies had significantly lower APRi when compared to patients with isolated disease. The patients with associated anomalies tended to be younger at the time of PE when compared to the rest (49 vs 64 days, p = 0.162), which may partly explain the difference in APRi.

Median Metavir fibrosis score was 2 ranging from 1 to 4. As shown in Table 2, APRi showed no significant correlations with Metavir or Ishak global fibrosis stages nor with collagen 1 expression. In contrast, predominantly portal α-SMA expression and amount of CD34-positive microvessels in the centrizonal region correlated positively with APRi (Table 2). When liver fibrosis was assessed separately in different regions of the hepatic parenchyma, portal area fibrosis with median score of 3 (0–4) correlated positively with APRi whereas lobular fibrosis did not (Table 2).

2.3. Predictive value of APRi for native liver survival

Patients who underwent liver transplantation had significantly higher APRi compared to those who survived with native liver (Table 1). APRi had a better predictive value for native liver survival after PE than any of the immunohistochemical measurements of fibrosis in liver biopsies (Fig. 1). The AUROC curve was 0.774 (95%CI 0.591–0.956, p = 0.017) for APRi, 0.706 (95%CI 0.470- 0.943, p = 0.101) for collagen 1 expression, 0.623 (95%CI 0.368–0.878, p = 0.302) for Metavir fibrosis stage and 0.570 (95%CI 0.304–0.837, p = 0.555) for portal fibrosis score (Fig. 1). Patients, who cleared their jaundice after PE had lower APRi than those who did not, but this difference was not statistically significant (Table 1).

3. Discussion

The majority of BA patients eventually require liver transplantation owing to progressive hepatic fibrosis and associated complications of portal hypertension. The reasons for ongoing liver fibrosis after successful PE remain unclear. Although the patient age and the degree of liver fibrosis are associated with outcomes of PE, more accurate and noninvasive prognostic tools are needed. Liver biopsy is currently the gold standard in evaluating liver fibrosis and cirrhosis, although it carries many

Table 1
Patient characteristics in relation to APRi.

		n	APRi (median and range)	*P*-value
Gender	M	13	0.92 (0.26–2.54)	0.693
	F	16	0.91 (0.13–6.39)	
Subtype	II	3	1.54 (0.77–2.54)	0.363
	III	24	0.91 (0.22–6.39)	
	IIIa	2	0.59 (0.13–1.06)	
Esophageal varices	Yes	15	1.06 (0.13–3.07)	0.432
	No	14	0.83 (0.22–6.39)	
Anomaly associated	Yes	10	0.63 (0.13–1.55)	0.028
	No	19	1.06 (0.35–6.39)	
Clearance of jaundice	Yes	18	0.87 (0.26–2.54)	0.261
	No	11	1.14 (0.13–6.39)	
Native liver survival	NL	19	0.77 (0.13–2.54)	0.017
	LT	10	1.34 (0.31–6.39)	

APRi, aspartate aminotransferase-to-platelet ratio index.