

• RAPID COMMUNICATION •

Diagnostic accuracy of serum biochemical fibrosis markers in children with chronic hepatitis B evaluated by receiver operating characteristics analysis

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Abstract

AIM: To investigate the diagnostic accuracy of potent serum biochemical fibrosis markers in children with chronic hepatitis B evaluated by receiver operating characteristics (ROC) analysis.

METHODS: We determined the serum level of apolipoprotein A-I (APO A-I), haptoglobin (HPT) and a-2 macroglobulin (A2M) with an automatic nephelometer in 63 children (age range 4-17 years, mean 10 years) with biopsy-verified chronic HBeAg-positive hepatitis B. Fibrosis stage and inflammation grade were assessed in a blinded fashion according to Batts and Ludwig. We defined mild liver fibrosis as a score ≤ 2 and advanced fibrosis as a score equal to 3. ROC analysis was used to calculate the power of the assays to detect advanced liver fibrosis (AccuROC, Canada).

RESULTS: Serum concentrations of APO A-I, HPT and A2M were not significantly different in patients with chronic hepatitis B compared to controls. However, APO A-I level of 1.19 ng/L had a sensitivity of 85.7% and a specificity of 60.7% (AUC = 0.7117, $P = 0.035$) to predict advanced fibrosis. All other serum biochemical markers and their combination did not allow a useful prediction. None of these markers was a good predictor of histologic inflammation.

CONCLUSION: Apolipoprotein A-I may be a suitable serum marker to predict advanced liver fibrosis in children with chronic hepatitis B.

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Key words: Chronic hepatitis B; Liver fibrosis; Children;

Apolipoprotein A-I; Haptoglobin; a-2 macroglobulin

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INTRODUCTION

Liver biopsy is regarded as the gold standard for the determination of the stage of liver fibrosis. However, in clinical practice, the use of invasive liver biopsy has several limitations such as complications, sampling variability, low reproducibility and it only provides static information about the fibrotic process^[1-3]. There is a clinical need for noninvasive measurement of liver fibrosis both to diagnose significant hepatic fibrosis and to monitor the effects of antiviral or antifibrotic therapy.

Several matrix-derived surrogate markers of extracellular matrix (ECM) turnover have been studied in adults so far and some were found to be correlated with matrix deposition, while their role as predictors of ECM turnover remains unclear^[4-6]. This applies particularly for children whose serum levels of ECM-derived parameters are usually influenced by body growth^[7,8]. Therefore, there is an urgent need for noninvasive parameters that better define those children who should undergo liver biopsy in order to assess fibrosis stage, to decide whether they should receive antiviral therapy or not, to predict fibrosis progression or to monitor potential antifibrotic treatment. Serum levels of type IV collagen^[9,10], hyaluronic acid^[10-13], laminin^[14], collagen VI^[15], transforming growth factor beta 1 (TGF beta 1)^[16] and metalloproteinases (MMPs) or tissue inhibitors of metalloproteinases (TIMPs)^[10,17] have recently been studied in children, but almost exclusively in patients with secondary biliary fibrosis due to biliary atresia and cystic fibrosis.

To our knowledge, serum fibrosis markers predicting liver fibrosis have not been assessed before in children with chronic hepatitis B (HBV) except our previous studies^[18-20]. From a broad panel of matrix-derived serum markers (collagen IV, collagen VI, PIIINP, laminin-2, hyaluronan, MMP-2, TIMP-1, MMP-9/TIMP1 complex, tenascin-C), the combination of serum hyaluronan and laminin-2 can accurately predict significant liver fibrosis^[18].

In our previous studies, we have reported that serum TGF beta 1 and cystatin C level did not predict advanced liver fibrosis in children with chronic hepatitis B^[19,20].

In the present study, the serum levels of apolipoprotein A-I (APO A-I), haptoglobin (HPT) and a-2 macroglobulin (A2M) were measured in children with chronic hepatitis B and compared to liver histological features to determine whether the measurement of these biochemical tests have any clinical usefulness as markers of liver fibrosis. Receiver operating characteristics (ROC) analysis was used to determine the sensitivity and specificity of the assays in detecting advanced liver fibrosis.

MATERIALS AND METHODS

Patients

The study was carried out in 63 children, including 41 boys and 22 girls (range 4-17 years, mean age 10 years) with biopsy-verified chronic HBeAg-positive hepatitis B prior to antiviral therapy. Other causes of chronic liver diseases, such as HCV coinfection, autoimmune hepatitis and metabolic liver disorders, were excluded. Children with diagnosed liver cirrhosis and evidence of other acute or chronic infections were also excluded from this study. Informed consent was obtained from all the parents of patients and the protocol used was approved by the Ethics Committee of the Medical University of Bialystok, Poland. As a control group, 16 children (mean age 10 years) were included without anamnestic, clinical or laboratory signs of liver or other systemic diseases.

Measurement of serum level of biochemical markers

APO A-I, HPT and A2M were measured in serum samples (obtained after an overnight fast) with an automatic nephelometer (BNII, Dade Behring; Marburg, Germany).

Histological analysis

All children underwent liver biopsy on the day after serum sampling. Liver specimens were fixed in buffered formalin and embedded in paraffin. Histological sections were stained using hematoxylin-eosin, Masson's-Goldner, Masson's trichrome and reticulin. Fibrosis stage and inflammation grade were assessed in a blinded fashion by a single pathologist who was without the knowledge of the patients' laboratory or clinical data. In order to determine specificity and sensitivity of the assay, we arbitrarily defined mild liver fibrosis or inflammation as a score ≤ 2 and advanced liver disease as fibrosis or inflammation score equal to 3 according to Batts and Ludwig^[21].

Statistical analysis

Serum concentrations of biochemical tests were expressed as mean \pm SD. Statistical analysis was performed using the Mann-Whitney two-sample test for nonparametric data. The relationship between the enzymes and liver histology scores was analyzed by the Spearman's rank-correlation test for nonparametric data. Tests were considered statistically significant at $P < 0.05$. Receiver operating characteristics (ROC) analysis (AccuROC, Montreal, Canada) was used

to calculate the power of the assays to detect advanced liver fibrosis. The best cut-off points for the diagnosis of advanced fibrosis are those which maximize the sum of sensitivity and specificity. Sensitivity of the assays was plotted against the false positivity (1-specificity). Comparison of the area under the curve (AUC) was performed using a P -test, which compares the AUC to the diagonal line of no information (AUC 0.5)^[22].

RESULTS

Characteristics of the patients

Selected biochemical and histological data are presented in Table 1.

Table 1 Initial characteristics of children with chronic hepatitis B

Parameters	Mean	SD	Minimum	Maximum
Age (yr)	10	3.41	4	17
ALT (IU/L)	84	57	12	312
AST (IU/L)	68	37	27	264
GGT (IU/L)	15	9	3	69
Bilirubin (μ mol/L)	10.26	4.45	2.74	32.83
APO A-I (g/L)	1.14	0.3	0.5	1.77
HPT (g/L)	0.58	0.36	0.31	1.69
A2M (g/L)	2.27	0.57	1.0	3.59
Staging	1.9	0.56	1	3
Grading	1.49	0.56	1	3

Serum concentration of APO, HPT and A2M

Serum concentrations of APO A-I, HPT and A2M were not significantly different in patients with chronic hepatitis B as compared with the controls (1.14 ± 0.3 vs 1.13 ± 0.2 ; 0.58 ± 0.36 vs 0.71 ± 0.62 , 2.27 ± 0.57 vs 2.53 ± 0.3 , respectively). There were no significant correlations of examined markers with liver fibrosis and inflammation according to Batts and Ludwig^[21].

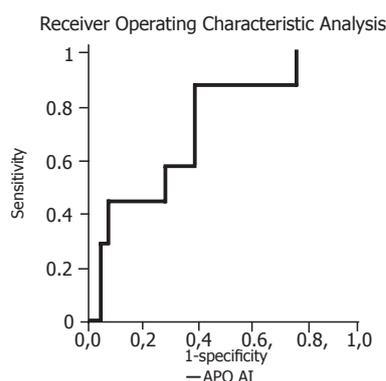
Diagnostic value of biochemical markers for identification of patients with advanced liver fibrosis and inflammation

Seven children (11.1%) had advanced liver fibrosis. The ability of examined biochemical markers to differentiate the children with advanced liver fibrosis from those with mild fibrosis was not significant except APO A-I (Table 2 and Figure 1). Using the APO A-I and the Batts and Ludwig score, 34 and 6 out of 63 children could be correctly allocated either to the group with mild or advanced fibrosis, respectively, potentially avoiding biopsy in 40 (63.5%) of the examined children. HPT and A2M did not reach the predictive power of APO A-I (Table 2). None of the combination of examined markers resulted in a significant increase in sensitivity and specificity for the identification of patients with advanced liver fibrosis. None of these markers was a good predictor of histologic inflammation (Table 3).

Table 2 Diagnostic value of serum markers for predicting advanced fibrosis

Markers	Cut-off (g/L)	Se (%)	Sp (%)	PPV (%)	NPV (%)	AUC	P values
APO A-I	1.19	85.7	60.7	21.4	97.1	0.7117	0.035
HPT	1.01	14.3	87.5	12.5	89.1	0.3878	NS
A2M	1.79	100.0	23.2	14.0	100.0	0.5268	NS

Se: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; AUC: area under curve; NS: no statistical significance.

**Figure 1** ROC curve of ability of serum APO A-I to detect advanced liver fibrosis according to Batts and Ludwig in children with chronic hepatitis B.**Table 3** Diagnostic values of serum markers for predicting advanced inflammation

Markers	Cut-off (g/L)	Se (%)	Sp (%)	PPV (%)	NPV (%)	AUC	P values
APO A-I	0.87	89.7	20.6	49.1	70.0	0.3803	NS
HPT	0.59	34.5	70.6	50.0	55.8	0.4701	NS
A2M	1.6	96.6	14.7	49.1	83.3	0.3813	NS

Se: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; AUC: area under curve; NS: no statistical significance.

DISCUSSION

Infection with the hepatitis B virus remains one of the most important epidemiological problems all over the world. It was estimated that chronic HBV infection, which is the single most common cause of cirrhosis and hepatocellular carcinoma (HCC), affects more than 400 million people worldwide^[23,24].

During the early phase of chronic hepatitis B, the underlying disease is usually subclinical and can be quite mild, particularly in children^[25]. This is referred to as the immune-tolerant phase of infection^[26]. While the natural history of chronic hepatitis B in children is usually marked by an indolent course, advanced disease with significant fibrosis can be found in up to one-third of the children, and decompensated cirrhosis and even HCC have been reported^[27,28]. For this reason, noninvasive diagnosis and monitoring of liver fibrosis in children are urgently needed.

There are several features required for an ideal serum fibrosis marker. It should be liver-specific, independent of metabolic alterations, minimally influenced by impaired urinary and biliary excretion, easy to perform and it should measure either the dynamic processes of fibrogenesis or fibrolysis and reflect the degree of fibrosis. Unfortunately, no current assay fulfills enough of these criteria^[5].

Recently, algorithms of non-ECM-derived serum markers have been proposed as the predictors of fibrosis stage in adult patients with chronic hepatitis C^[29-31]. APO A-I, HPT and A2M are the components of FibroTest^[32] and APO A-I and A2M are the components of PGAA index^[33,34]; they have also been validated as single serum fibrosis markers^[6,35-38].

In the present study, we did not find significantly different levels of examined serum fibrosis markers in children with chronic hepatitis B as compared with the controls. There were also no significant correlation of APO A-I, HPT, and A2M levels with histologically assessed stage of liver fibrosis and inflammation grade. Because liver biopsy is not necessarily a gold standard for assessing liver histology, noninvasive markers will not have complete concordance with histological staging. Only 11.1% children had advanced fibrosis according to Batts and Ludwig^[21] and probably for this reason, we also did not observe statistically significant correlation between the examined markers and fibrosis stage.

These findings confirmed previous observation in children by Selimoglu *et al.*^[35,37] who also demonstrated that mean APO A-I level of the patients with chronic hepatitis B was not different from controls and did not correlate with fibrosis and inflammation scores. To our knowledge, A2M and HPT as potent serum fibrosis markers have not been assessed in children yet.

In adults with chronic hepatitis B, Oberti *et al.*^[6] and Huang and Gong^[36] observed that APO A-I and A2M were significantly correlated with the staging of liver fibrosis. Lu *et al.*^[34] confirmed that A2M but not APO A-I was significantly correlated with fibrosis stage. However, HPT was negatively associated with fibrosis^[38]. These opposing correlation of A2M and HPT with fibrosis staging could be explained by the different roles of hepatocyte growth factor (HGF) and TGF beta 1 in fibrogenesis and acute phase response^[39-41]. As seen in experimental fibrosis, transduction with HGF gene suppresses increase of TGF beta 1^[39] and the factor stimulates synthesis of A2M^[40] and reduces synthesis of haptoglobin^[40,41].

Recently, ROC analysis has been recommended to calculate the power of the assays to detect advanced liver fibrosis^[42-46]. In this study, the ability of examined biochemical markers to differentiate children with advanced liver fibrosis from those with mild fibrosis was not significant except APO A-I. The high negative predictive value of this marker could potentially be useful in the selection of patients without significant fibrosis and in avoiding invasive liver biopsy in this group of children. Our study is not consistent with the findings of Selimoglu *et al.*^[35], because they did not find diagnostic performance of APO A-I for discriminating patients with advanced liver

disease. Usefulness of APO A-I as well as HPT and A2M as potent serum fibrosis markers in patients with chronic liver disease needs to be evaluated in large controlled studies.

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