Assessment of liver fibrosis by aspartate aminotransferase-to-platelet ratio index(APRI) in children with biliary atresia

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ABSTRACT

Assessment of liver fibrosis by aspartate aminotransferase to platelet ratio index(APRI) in children with biliary atresia

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Background: Liver fibrosis occurs in many children with biliary atresia. Although liver biopsy is the golden standard to evaluate liver fibrosis, it is invasive and may result in life-threatening complications. Therefore, there is a need to develop and validate noninvasive methods that can accurately reflect the hepatic fibrosis in biliary atresia. A promising tool with widespread availability, the aspartate aminotransferase to platelet ratio index (APRI) is a rapid and noninvasive method to detect liver fibrosis. The aim of this study was to evaluate the diagnostic accuracy of APRI in diagnosing liver fibrosis in children with biliary atresia.

Materials and Methods: Twenty-six children with biliary atresia who received Kasai operation between March 2006 and June 2008 were

included in the study. children with biliary atresia were included in the study. All children (12 male, median age 1.9 months) underwent several laboratory tests in addition to APRI. METAVIR scores were obtained by pathologic examination of liver biopsy specimens at the time of Kasai operation.

Results: On histologic examination, METAVIR score was F1 in 0, F2 in 5, F3 in 9, F4 in 12 cases. The correlation of APRI and METAVIR scores with biochemical parameters did not reach statistical significance. Significant areas under the ROC curves for $F \geq 3$, and $F = 4$ (0.75 and 0.78 for all participants, respectively), distinct cutoff values (0.56 and 1.35) with high total sensitivity and specificity, and high likelihood ratios suggest that APRI is a reliable method for the diagnosis of cirrhosis ($F = 4$) and extensive fibrosis ($F \geq 3$).

Conclusion: APRI is a useful method for assessing liver fibrosis in children with biliary atresia.

Key words: aspartate aminotransferase-to-platelet ratio index, METAVIR, biliary atresia, liver fibrosis

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I. INTRODUCTION

Hepatic fibrosis is the end result of various types of liver injury; it is a wound healing process similar to that observed in other organs.[1] One of the most remarkable aspects of the healing process in the liver is enhanced extracellular matrix production, or fibrogenesis. Furthermore, injury-induced fibrogenesis is characterized by a multifold increase in interstitial collagens such as type I and type III, in addition to many other extracellular matrix constituents.[2] Liver fibrosis occurs in many patients with chronic liver disease. Early treatment of the underlying etiology can limit the progression, but cannot always prevent continuation to the advanced stage, known as cirrhosis.[3] In children, biliary atresia is the most common cause of liver fibrosis, even after surgical intervention. All patients with advanced liver fibrosis will eventually require liver transplantation.[4]

Although liver biopsy is the golden standard to evaluate liver fibrosis, it is invasive and may result in life-threatening complications in both adults and children.[5-7] In addition, it has many drawbacks that include sampling error, inter and intra-observer variability in interpretation, and inability to evaluate progression and regression of fibrosis. Liver biopsy is also associated with complications such as abdominal pain, hypotension, hemobilia, and intraperitoneal hemorrhage, that last of which has an associated mortality rate of up to 0.5%.[8] Furthermore, it is not well accepted by patients, especially when repeated examinations are needed.[9] Therefore, there is a need to develop noninvasive methods to accurately diagnose hepatic fibrosis, cirrhosis, and disease progression in liver diseases.[10]

A promising tool with widespread availability, the aspartate aminotransferase to platelet ratio index (APRI) is a rapid and noninvasive method to detect liver fibrosis.[3] APRI is calculated from two routine laboratory tests such as serum AST level and platelet count. The aim of this study was to evaluate the diagnostic accuracy of APRI for the determination of liver fibrosis in children with biliary atresia.

II. MATERIALS AND METHODS

1. Subjects

Twenty-six consecutive children with biliary atresia who received Kasai operation at Severance Children's Hospital, Seoul, Korea between March 2006 and June 2008 were enrolled in the study. Children with ascites were excluded from this study. The study protocol was in accordance with the Helsinki Declaration.

2. Characteristics of subjects

For all subjects, the following parameters were determined at the time of liver biopsy. Demographic information included gender and age in month at the time of Kasai operation. Biochemical parameters included serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total bilirubin, direct bilirubin, *r*-glutamyl-transpeptidase, alkaline phosphatase, platelet count, prothrombin time. APRI was calculated as serum AST level (U/L)/upper normal x 100/platelet count (10^9/L).[3]

3. Liver histology and quantification of liver fibrosis

Liver biopsy specimens were obtained at the time of Kasai operation, fixed in formalin and embedded in paraffin. Four-micrometer-thick sections were stained with hematoxylin-eosin-safran and picrosirius red. All specimens were

analyzed twice by an experienced liver pathologist blinded to the results of clinical data. Liver fibrosis and necroinflammatory activity were evaluated semi-quantitatively according to the METAVIR scoring system.[4, 11] The METAVIR scoring system consists of 5 stages according to the architectural features of the portal fibrosis: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis and few septa, F3 = numerous septa with cirrhosis, F4 = cirrhosis. Activity was graded as: A0 = none; A1 = mild; A2 = moderate; A3 = severe.

4. Statistical analysis

The diagnostic performance of APRI was assessed by using receiver operating characteristics (ROC) curves. All cutoff values are associated with probability of a true positive (sensitivity) and a true negative (specificity). The ROC curve is a plot of sensitivity versus 1-specificity for all possible cutoff values. The most commonly used accuracy index is the area under the ROC curve; values near 1.0 indicate high diagnostic accuracy. ROC curves were thus constructed for the detection of subjects with METAVIR fibrosis stage 3 or greater cirrhosis. Optimal cutoff values for APRI were chosen either to obtain a 95% sensitivity, to maximize the sum of sensitivity and specificity, to optimize the diagnostic performance (sum of true positives and true negatives over the total number of subjects), or to obtain a 95% specificity according to the diagnostic question.[12]

Spearman correlation coefficients and their associated probability were used to

evaluate the relationship between parameters. All of the data management and statistical calculations were performed with SPSS version 13.0 statistical package (Chicago SPSS Inc., Chicago, IL, USA).

III. RESULTS

1. Subjects

A total of 26 children with biliary atresia were enrolled. Their characteristics at the time of Kasai operation are summarized in table 1. There were 12 male and 14 female; their median age at the time of Kasai operation was 1.90 months.

Table 1 Clinical characteristics of all patients (n = 26)

	All children Median (range)
Age (month) at operation	1.90 (0.5-7.9)
Male (%)	12 (46.2)
AST*(IU/L)	211.7 (42-1889)
ALT†(IU/L)	147.5 (22-1024)
Albumin(g/dL)	3.7 (2.5-4.7)
Total bilirubin(mg/dL)	8.6 (3.4-14.6)
Direct bilirubin(mg/dL)	7.1 (2.8-11.6)
r-glutamyl-transpeptidase(IU/L)	454.5 (38-1403)
Alkaline phosphatase(IU/L)	564.5 (220-2989)
Platelet count(10^3/μL)	377.5 (210-936)
PT-INR‡	1.01 (0.89-1.54)
APRI§	1.35 (0.24-15.15)

*AST means aspartate aminotransferase
†ALT means alanine aminotransferase
‡PT-INR means prothrombin time international normalized ratio
§APRI means aspartate aminotransferase to platelets ratio index

2. Histology

Table 2 summarizes the distribution for METAVIR fibrosis stage, and activity grade. The fibrosis stages were as follows: F1, n = 0; F2, n = 5 (19.2%); F3, n = 9 (34.6%); F4, n = 12 (46.2%). The activity grade distribution was as follows: A1, n = 3 (11.5%); A2, n = 1 (3.8%); A3, n = 22 (84.6%). The reproducibility of METAVIR scoring was good ($\kappa = 0.66$).

Table 2 Patients distribution for METAVIR fibrosis stage and necroinflammatory activity grade

Fibrosis		Activity	
Stage	Number (%)	Grade	Number (%)
0	0	0	0
1	0	1	3 (11.5)
2	5 (19.2)	2	1 (3.8)
3	9 (34.6)	3	22 (84.6)
4	12 (46.2)		

3. Relationship of METAVIR fibrosis stage with biochemical parameters

The Spearman correlation coefficients of the degree of METAVIR fibrosis and APRI for clinical and biochemical parameters are presented in table 3. There is no significant gender difference. The degree of METAVIR fibrosis was significantly correlated with AST, ALT, total bilirubin, direct bilirubin and

alkaline phosphatase (P<0.05 for each).

Table 3 Spearman correlations of METAVIR and APRI value versus clinical and biochemical parameters

	METAVIR	APRI
	r (*P*)	*r* (*P*)
Age (month) at operation	0.61 (0.001)	0.57 (0.003)
Aspartate aminotransferase	0.40 (0.041)	0.91 (<0.001)
Alanine aminotransferase	0.43 (0.030)	0.71 (<0.001)
Albumin	0.13 (0.528)	–0.25 (0.226)
Total bilirubin	0.60 (0.001)	0.64 (<0.001)
Direct bilirubin	0.61 (0.001)	0.67 (<0.001)
r-glutamyl-transpeptidase	0.12 (0.566)	–0.25 (0.226)
Alkaline phosphatase	0.67 (<0.001)	0.58 (0.002)
Platelet count	–0.12 (0.549)	–0.51 (0.008)
PT-INR[†]	0.46 (0.018)	0.38 (0.052)

[†]PT-INR means prothrombin time international normalized ratio
[‡]NS means not significant

4. Diagnostic accuracy of APRI for the determination of liver fibrosis and cirrhosis

Figure 1 and 2 show the ROC curve for all subjects according to two different thresholds of fibrosis stage with APRI: F0, F1, and F2 patients versus F3 and F4 patients (F ≥ 3); and F0, F1, F2, and F3 patients versus F4 patients (F = 4). The

areas under the ROC curves (95% CI) were 0.75 for F ≥ 3 and 0.78 for F = 4.

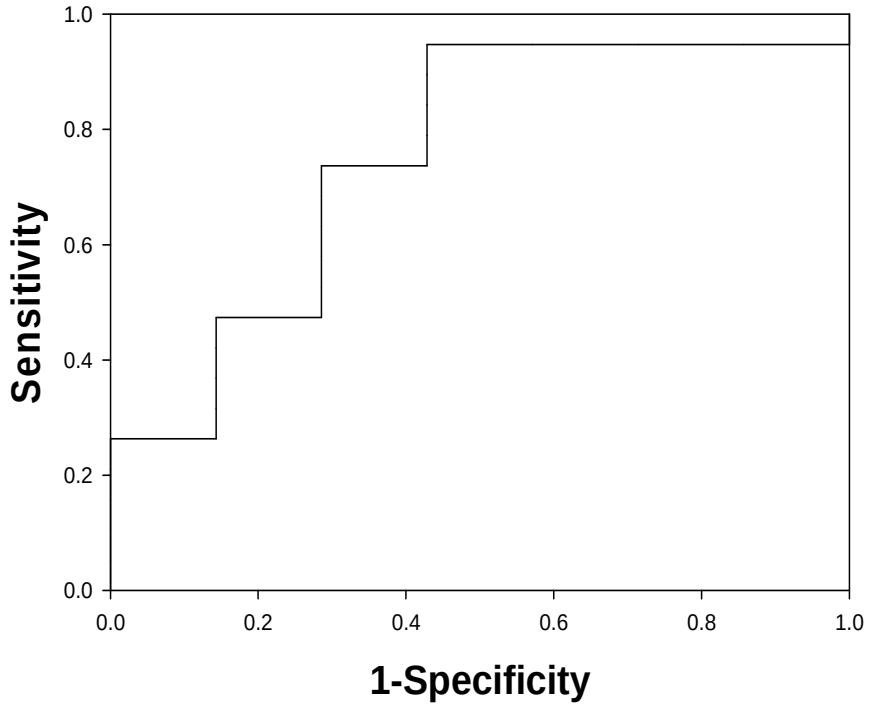

Fig. 1. Receiver operator characteristics (ROC) curve for APRI for the determination of METAVIR F ≥ 3. The area under ROC curve was 0.75 (95% CI).

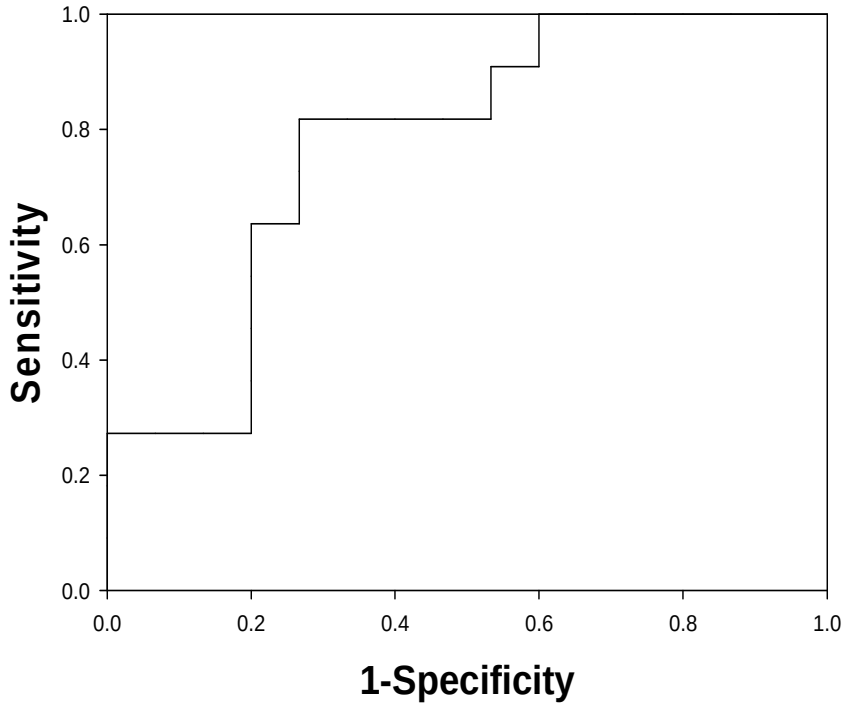

Fig. 2. Receiver operator characteristics (ROC) curve for APRI for the determination of METAVIR F = 4. The area under ROC curve was 0.78 (95% CI).

5. Determination of APRI cutoff values

Table 4 shows the optimal APRI cutoff values obtained for all subjects as well as corresponding sensitivity, specificity, and likelihood ratio. The cutoff value of APRI for F ≥ 3 was 0.56 with a great total sensitivity and specificity of 1.52,

and the clear cutoff value (1.35) was obtained for F = 4 with a total sensitivity and specificity of 1.55.

Table 4 APRI cutoff values for the determination of METAVIR fibrosis score F ≥ 3 and F = 4

	Severe (F ≥ 3)	Cirrhosis (F = 4)
Optimal cutoff*	0.56	1.35
Sensitivity	0.95	0.82
Specificity	0.57	0.73
Likelihood ratio	2.21	3.15
Positive predictive value	0.91	0.75
Negative predictive value	0.73	0.80

*The optimal cutoff values are those giving the highest sum of sensitivity and specificity

IV. DISCUSSIONS

The prognosis of chronic cholestatic diseases depends in part on the extent of fibrosis in liver.[13, 14] Although liver biopsy is the golden standard method for the determination of liver fibrosis, it is invasive and has many serious limitations. First of all, liver biopsy is not well accepted by adults or children because of pain and complications. Saadeh at al. [15] and Poynard et al. [16] reported that liver biopsy results in pain in 24.6% of patients, and have a risk of severe complications of 3.1 per 1,000. A French survey recently showed that approximately half of patients with hepatitis C refuse to be referred to hepatologists for fear of liver biopsy.[9]

Secondly, the procedure is associated with significant sampling error. Histological staging is based on a biopsy specimen that represents at most 1/50,000 of the total liver mass.[8] In addition, the distribution of fibrosis in liver parenchyma is heterogeneous. Bedossa et al.[17] recently reported that, by using the METAVIR scoring system, only 75% of 25 mm biopsy specimens were classified correctly in terms of fibrosis stage. Using the Batts and Ludwig classification,[18] Regev et al.[19] showed a difference of at least 1 fibrosis stage between the right and left lobes in 33% of 124 patients, while Siddique et al.[20] found that 45% of patients had a difference of at least one fibrosis stage between two specimens taken at the same puncture site.

Due to these limitations, the APRI was developed as alternative. Wai et al.[3]

reported an APRI derived and validated in a cohort of 270 patients with chronic HCV, the area under ROC curves for significant fibrosis and cirrhosis in the training and validation cohort were 0.80 to 0.88 and 0.89 to 0.94, respectively. Shaheen and Myers[21] demonstrated that based on their recent bivariant meta-analysis of APRI diagnostic accuracy, the 0.5 threshold was 81% sensitive and 50% specific. Assuming a 47% prevalence of significant fibrosis, [21] this translated into an estimated positive predictive value of 59% and negative predictive value of 75%. Although these predictive values appear suboptimal, the negative predictive value was more acceptable in lower prevalence settings such community-based cohorts.[22] APRI has the advantage of requiring only two routine laboratory tests. For the identification of significant fibrosis, scores less than 0.5 (on a scale from 0 to 10) had a negative predictive value of 86%, whereas scores greater than 1.5 had a positive predictive value of 88%. Based on these high predictive values, the authors concluded that APRI could replace biopsy in approximately half of patients. Subsequently, numerous studies have attempted to externally validate these findings, but results have been controversial.[23, 24] In 2008, Cales et al.[25] documented comparison of the overall accuracy of several tests such as FibroMeter, Fibrotest, Fib-4, APRI, and Hepascore in patients with chronic hepatitis C. The area under ROC (F0-1 vs F2-4) were as follows: FibroMeter: 0.853, Fibrotest: 0.811, Fib-4: 0.799, APRI: 0.786 and Hepascore: 0.784. Finally, de Lédinghen et al.[4] showed that APRI was significantly correlated to METAVIR fibrosis scores in children with

chronic liver diseases.[4]

Our study examines the accuracy and reliability of APRI in assessing fibrosis and histological stage in children with biliary atresia. Our results showed a significant positive correlation between APRI and fibrosis stages. Significant areas under the ROC curves for $F \geq 3$, and $F = 4$ were 0.75 and 0.78, respectively. These results, along with high likelihood ratios and distinct cutoff values (0.56 and 1.35) with high total sensitivity and specificity, suggest that APRI is a reliable method to diagnose cirrhosis ($F = 4$) and extensive fibrosis ($F \geq 3$) in our patients.

V. CONCLUSION

This results of our study support APRI as a useful noninvasive method to assess liver fibrosis in children with biliary atresia.

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ABSTRACT(IN KOREAN)

담도 폐쇄증 환아에서 aspartate aminotransferase/혈소판 비(APRI)를 이용한 간 섬유화의 평가

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고 홍

목적 : 간 섬유화는 담도 폐쇄증을 가진 소아에서 쉽게 관찰되며, 간 섬유화를 평가하기 위해서는 간생검이 표준 검사법으로 받아들여져 왔다. 그러나 간생검은 침습적인 방법으로 통증을 수반하고 생명을 위협할 수 있는 합병증을 유발할 수 있다. 따라서 만성 간질환 환아에서 간 섬유화를 측정할 수 있는 비침습적 검사법의 개발이 필요하다. C형간염 성인 환자에서는 간 섬유화를 빠르고 비침습적으로 평가할 수 있는 검사법으로 aspartate aminotransferase/혈소판 비(APRI)가 이용되고 있다. 그러나 소아에서는 APRI를 이용하여 만성 간 질환에 대한 섬유화 정도를 평가한 논문은 드물다. 이에 본 연구에서는 담도 폐쇄증을 가진 환아의 간 섬유화를 평가하는데 있어서 APRI의 진단적 유용성을 알아

보고자 한다.

대상 및 방법 : 2006년 3월부터 2008년 6월까지 세브란스 어린이병원에서 담도 폐쇄증으로 수술을 받은 총 26명의 환아(남아 12명, 평균 연령 1.9개월)를 대상으로 혈청 AST와 혈소판치를 측정한 후 APRI를 구하였다. 간 섬유화 진단의 표준 검사법으로 수술 당시 시행하여 얻은 간생검 조직에 대한 조직학적 검사와 METAVIR fibrosis scoring system을 이용하였다.

결과 : 전체 대상 환아의 조직학적 검사에서 METAVIR fibrosis scoring system에 따른 간 섬유화는 F1이 0례, F2가 5례, F3가 9례, F4가 12례 였고, 일부 혈액검사는 APRI 및 METAVIR fibrosis score와 통계학적으로 유의한 상관관계를 나타내었다. 본 연구에서 중증의 간 섬유화 (F ≥3)는 AUROC가 0.75로 이에 대한 APRI의 진단분리점은 0.56으로 민감도는 95%, 특이도 57%, 양성 예측률 91%, 음성 예측률 73%였다. 간경변(F=4)은 AUROC는 0.78로 이에 대한 APRI의 진단분리점은 1.35로 민감도는 82%, 특이도 73%, 양성 예측률 75%, 음성 예측률 80%였다.

결론 : APRI는 비침습적으로 담도 폐쇄증을 가진 소아에서 간 섬유화의 정도를 평가할 수 있는 유용한 검사법이다.

핵심되는 말 : aspartate aminotransferase 혈소판 비, METAVIR, 담도 폐쇄증, 간 섬유화