ATA Index: A novel score for predicting fibrosis stage in chronic viral hepatitis

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Abstract

Background and Aim: This study was designed to predict the fibrosis stage with a clinical scoring system that may reduce the need for liver biopsy.

Materials and Methods: The study cohort included the treatment of 430 chronic hepatitis B (CHB) and 170 chronic hepatitis C (CHC) of naive patients. The patients were divided into two groups as mild to moderate and severe fibrosis. After an index obtained in the study cohort, the index was tested in a validation cohort and compared with the FIB-4 Index.

Results: The AUC of CHC index was found of 0.89 the sensitivity of 0.91 the specificity of 0.74, the positive predictive value (PPV) of 0.54 and the negative predictive value (NPV) of 0.96. The FIB-4 Index was applied to the CHC study cohort and the ATA Index Hepatitis C was found to be superior in terms of AUC (0.89–0.82), sensitivity (0.91–0.76) and NPV (0.96–0.86). The AUC of CHB Index was determined of 0.92, the sensitivity of 0.90, the specificity of 0.84, the PPV of 0.53 and the NPV of 0.98. Compared to the FIB-4 Index in CHB study cohort, the ATA Index Hepatitis B was predominant in terms of AUC (0.92–0.88), sensitivity (0.90–0.75), NPV (0.98–0.94) and PPV (0.53–0.49).

Conclusion: ATA Indexes can predict the non-existence of severe fibrosis with an accuracy similar to FIB-4 Index and may reduce the need for liver biopsy.

Keywords: Fibrosis; FIB-4; non-invasive; viral hepatitis.

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are two of the most serious health problems all over the world. In terms of HBV, about one-third of the world's population has serologically old or new evidence of infection. HBV is the most common cause of acute and chronic liver disease worldwide. HBV-associated end-stage liver disease and hepatocellular carcinoma (HCC) cause approximately one million deaths per year. In terms of HCV, approximately 3–4 million newly infected patients have been reported every year and, 170 million chronic hepatitis C (CHC) patients worldwide are at risk of cirrhosis and HCC.[1] The World Health Organization estimated that around 399.000 people died from HCV-related causes in 2016.

Liver fibrosis is one of the most important characteristics of chronic hepatitis B (CHB) and CHC, which usually progresses to cirrhosis and HCC. Evaluation of liver fibrosis is a crucial step to predict progression rate of the disease, respond to treatment, and choose the optimal treatment timing. Liver biopsy is still the gold standard method for the evaluation of liver fibrosis. However, there are some technical limitations of this invasive method (sampling errors and intra-observer and inter-observer variations) and risks such as intra-abdominal bleeding, severe abdominal pain, or death.[2,3] These disadvantages have led researchers to look for non-invasive methods. Several advanced imaging methods (transient elastography, magnetic resonance imaging elastography, acoustic radiation force impulse elastography),[4,5] and combination biomarker panels (e.g. fibrotest, hepascore) are costly non-invasive ways of assessing liver fibrosis.[6,7] These methods are not accessible to all clinicians everywhere, and price is a limiting factor. On the other hand, in clinical practice we use laboratory tests evaluating liver functions as platelet count, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), prothrombin time (PT), and albumin. Additionally, there are laboratory-based panel tests to help predicting liver fibrosis including, AST/ALT ratio (AAR),[8] cirrhosis discriminant score (CDS),[9] age-platelet index (AP index),[10] Pohl Score,[11] Forns Index,[12] AST Platelet Ratio Index,[13] and FIB-4 Index.[14]

In this study, CHB and CHC patients who underwent liver biopsy, and those diagnosed with non-biopsy-compensated cirrhosis by clinical, ultrasonographical, endoscopic, and laboratory findings were included. This study aimed to examine the relationship between laboratory parameters and liver fibrosis in untreated patients and, to formulate laboratory parameters predicting fibrosis. In this way, the aim was to reduce the number of liver biopsies.

Materials and Methods

Patients

This study included 170 CHC and 430 CHB patients diagnosed and followed-up in the hepatology outpatient clinic between 2000 and 2013. Patients who underwent liver biopsy for HBV or HCV, whose pre-biopsy laboratory tests performed no later than a month ago, and diagnosed with non-biopsy-compensated cirrhosis by clinical, ultrasonographical, endoscopic and laboratory findings were included. Exclusion criteria were antiviral treatment before the liver biopsy, decompensated cirrhosis, alcohol consumption over 40 g/d, HIV infection, previous hepatocellular cancer or non-liver cancer history, other chronic liver diseases (autoimmune, toxic, alcoholic etc.), CHB and delta co-infection or superinfection, CHC and HBV co-infection and inadequate biopsy specimen for grading.

Histological Grading and Laboratory Parameters

Metavir scoring system was used for fibrosis staging. Pathology specimens of 412 CHB and 154 CHC patients meeting research criteria were evaluated retrospectively by the same pathologist (E.E). Patients were divided into five groups according to m etavir fibrosis stages as F0, F1, F2, F3 and, F4. Patients with F0, F1 or F2 fibrosis were then classified as ''mild to moderate fibrosis'' and patients with F3 or F4 fibrosis were classified as ''severe fibrosis''.

Five out of 412 CHB patients have been excluded due to inadequate tissue samples. 23 CHB patients diagnosed with non-biopsy-compensated cirrhosis by clinical, ultrasonographical, endoscopic and laboratory findings were included in the study as Metavir fibrosis stage F4. A total of 430 CHB patient data were evaluated.

Of the 154 CHC patients, 3 of the tissue samples were left out of study because they could not be accessed. 19 CHC patients diagnosed with non-biopsy-compensated cirrhosis by clinical, ultrasonographical, endoscopic, and laboratory findings were included in the study as Metavir fibrosis stage F4. A total of 170 CHC patients' data were evaluated.

The upper limit of normal (ULN) for some laboratory parameters (AST, ALT, ALP, GGT) was different in certain years due to the use of different devices in the laboratory. To ensure standardization, the ratio of available parameters to ULN was used.

Validation Cohort

After obtaining formulas to predict mild to moderate and severe fibrosis in CHB and CHC patients in the study cohort, the formulas were tested in validation cohorts. Validation cohorts included 200 patients (100 CHC and 100 CHB) followed-up in another hepatology outpatient clinic.

Statistical Analysis

Statistical analyses were performed using the SPSS software version 20.0. The univariate analysis to identify variables associated with fibrosis severity was investigated using chi-square, t-test or Mann-Whitney U tests, where appropriate. The possible factors identified with univariate analysis were also entered into logistic regression analysis. Binary logistic regression analysis was used to determine which clinical parameters were risk factors in the fibrosis stage. Probability formulas were created from variables modeled as a result of logistic regression for CHB and CHC patient groups.

To define define accuracy and the optimal cut-off for the predicted probability of each formula in distinguishing mild to moderate and severe fibrosis were evaluated using the receiver-operating-characteristic (ROC) curve analysis and Youden Index. For optimal cut-off, sensitivity, specificity, positive and negative predictive values (NPV) were calculated. The Type-I error rate was taken as 0.05 to test the statistical hypotheses.

Results

Chronic Hepatitis C Patient Group

This study included 170 CHC patients [65 men (38%) and 105 women (62%)] followed-up in the hepatology outpatient clinic. Among 170 patients, 19 CHC patients diagnosed with non-biopsy-compensated cirrhosis classified as Metavir fibrosis stage F4. The distribution of patients according to Metavir scoring was determined as: 62 patients F0 (36.5%), 50 patients F1 (29.4%), 15 patients F2 (8.8%), 17 patients F3 (10%), and 26 patients F4 (15.3%). When patients were classified as mild to moderate and severe fibrosis: 127 patients (74.7%) had mild to moderate, 43 patients (25.3%) had severe fibrosis.

Evaluating the the laboratory parameters evaluated according to fibrosis stages, (F0, F1, F2, F3, F4) it was found that: ALT (p=0.005), AST (p<0.001), PT (p=0.003), hemoglobin (p=0.006), albumin (p<0.001), total bilirubin (p<0.001), direct bilirubin (p<0.001), GGT (p<0.001), white blood cell (WBC) (p=0.003), platelet count (p<0.001), glucose (p=0.043), Mean Platelet Volume (MPV) (p=0.019), ALT/ULN (p=0.001), AST/ULN (p<0.001), GGT/ULN (p<0.001) and age (p<0.001) were significantly different between the groups in terms of univariate analysis. ALP/ULN (p=0.349), total cholesterol (p=0.53), triglyceride (p=0.52), and HCV RNA (p=0.701) were not found to be statistically different between groups.

When evaluated according to mild to moderate and severe fibrosis groups: the laboratory parameters of age, ALT, AST, PT, albumin, total bilirubin, direct bilirubin, GGT, hemoglobin, WBC, platelet count, glucose, AST/ULN, ALT/ULN and GGT/ULN were found to be significantly different between groups in terms of univariate analysis (Table 1). Logistic regression analysis showed that age (p=0.001), platelet (p=0.014), AST/ULN (p=0.009) and PT (p=0.01) were the most significant parameters to distinguish mild to moderate and severe fibrosis groups (Table 1). These variables made the most significant contribution to the model.

Formula:

$$P \text{ (Severe fibrosis)} \quad \frac{e^{-11.757+0.085 \times Age - 0.009 \times Platelet + 0.604 \times AST/ULN + 0.546 \times PT}}{1 + e^{-11.757+0.085 \times Age - 0.009 \times Platelet + 0.604 \times AST/ULN + 0.546 \times PT}}$$

With this formula, the probability of having severe fibrosis was found. Overall accuracy was assessed using a ROC curve (Fig. 1). The best cut-off point for the probability of distinguishing mild to moderate and severe fibrosis was found 0.16 with Youden Index. The area under the ROC curve (AUC) of CHC Index was 0.89, sensitivity 0.91, specificity 0.74, positive predictive value (PPV) 0.54 and, NPV 0.96, using the best cut-off probability (less than 0.16). The formula was named as 'ATA Index Hepatitis C' (Table 2).
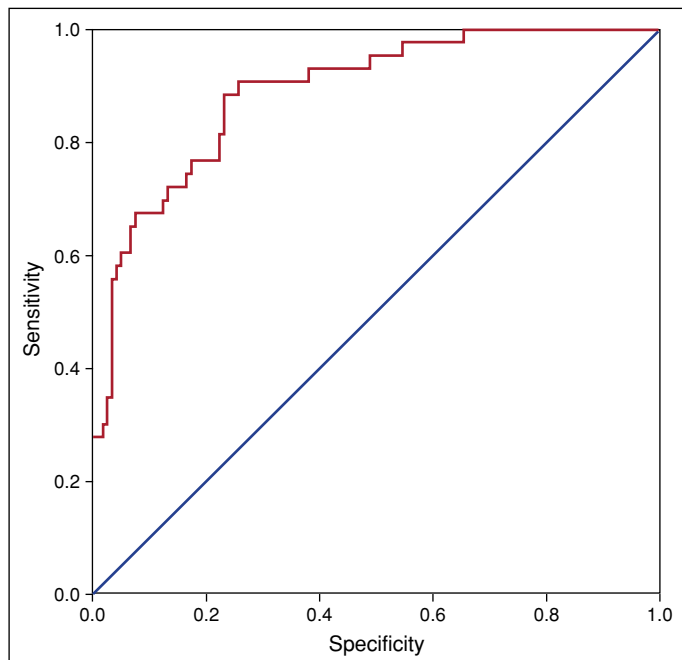
Validation Cohort

The 'ATA Index Hepatitis C' was applied to 100 CHC patients from another Hepatology outpatient clinic as a validation cohort. In the val-

Table 1. Demographics and patient characteristics of chronic hepatitis C patients

| | Fibrosis stage | | | |
	Mild to moderate	Severe	p[a]	Odds ratio[b] (95% CI)
Age, mean (min–max), years	48 (20–72)	57 (40–75)	<0.001	1.089 (1.034–1.147)
Male, n, (%)	51 (%79)	14 (%21)	>0.05	
ALT, mean (min–max), U/L	47 (11–234)	61 (21–301)	0.002	
AST, mean (min–max), U/L	35 (14–176)	70 (22–245)	<0.001	
ALP, mean (min–max), U/L	100 (28–513)	99 (40–394)	0.792	
GGT, mean (min–max), U/L	34 (7–629)	47.5 (22–187)	0.001	
Total bilirubin, mean (min–max), mg/dl	0.72 (0.1–4.5)	0.96 (0.3–5.9)	<0.001	
Direct bilirubin, mean (min–max), mg/dl	0.17 (0–1)	0.3 (0–1)	<0.001	
Albumin, mean (min–max), g/dl	4.3 (2.6–5.4)	4 (2.9–5.3)	<0.001	
Glucose, mean (min–max), mg/dl	94 (58–347)	102 (80–316)	0.011	
Total cholesterol, mean (min–max), mg/dl	165 (56–273)	165 (76–300)	0.319	
Triglyceride, mean (min–max), mg/dl	104 (14–324)	90 (36–366)	0.204	
PT, mean (min–max), second	12 (9.9–15.9)	13 (10.7–19)	<0.001	1.727 (1.137–2.624)
Hemoglobin, mean (min–max), g/dl	14.4 (9–19)	13.4 (9–14)	0.001	
WBC, mean (min–max), 10^9/L	6.6 (2.8–24)	5.5 (2.2–10.9)	0.001	
Platelet count, mean (min–max), 10^9/L	231 (76–474)	158 (44–401)	<0.001	0.991 (0.984–0.998)
MPV, mean (min–max), fL	8.4 (6.8–12.5)	8.9 (7.3–12.7)	0.058	
HCV RNA, mean (min–max)	9.9x105 (0–1.8x107)	7.2x105 (0–4.6x107)	0.745	
ALT/ULN, mean (min–max)	1.17 (0.31–5.6)	1.64 (0.51–6.69	<0.001	
AST/ULN, mean (min–max)	1.03 (0.45–5.68)	2.02 (0.68–7)	<0.001	1.829 (1.165–2.872)
ALP/ULN, mean (min–max)	0.85 (0.27–3.98)	0.83 (0.36–3.28)	0.98	
GGT/ULN, mean (min–max)	0.74 (0.18–10.3)	1.15 (0.38–4.92)	<0.001	

Baseline demographics and laboratory parameters of chronic hepatitis C patients according to mild to moderate and severe fibrosis classification (n=170). a: P values of univariate analysis; b: Odds ratios of multivariate analysis; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyltransferase; HCV RNA: Hepatitis C virus ribonucleic acid; MPV: Mean platelet volume; n: Number; PT: Prothrombin time; ULN: Upper limit of normal; WBC: White blood cell.

Figure 1. ROC curve for hepatitis C patient group.

idation cohort, 34 patients (34%) were in the severe fibrosis group, 66 patients (66%) were in the mild to moderate fibrosis group. In the validation cohort; sensitivity, specificity, positive and NPV of the ATA Index were 0.35, 0.88, 0.60 and 0.72, respectively (Table 2).

Assessment of FIB-4 Index in Study Cohort

The FIB-4 Index was tested in our CHC study cohort (n=170) to assess the value of distinguishing mild to moderate and severe fibrosis. The AUC of FIB-4 Index was 0.82, sensitivity 0.76, specificity 0.78, PPV 0.64 and NPV 0.86 (Table 2).

Chronic Hepatitis B Patient Group

430 CHB patients [275 men (64.1%) and 155 women (35.9%)] followed up in the hepatology department were included to this study. Of the 430 patients, 23 CHB patients diagnosed with non-biopsy-compensated cirrhosis were classified as Metavir fibrosis stage F4. The distribution of patients according to Metavir scoring was: 200 patients (46.5%) F0, 130 patients (30.2%) F1, 27 patients (6.3%) F2, 47 patients (11%) F3 and 26 patients had F4 fibrosis. Classifying the patients as mild to moderate (F0-F1-F2) and severe (F3-F4) fibrosis, it was observed that 357 patients (83) were in the mild to moderate fibrosis and 73 patients (17%) were in the severe fibrosis groups.

Table 2. The characteristics of 'ATA Index Hepatitis B', 'ATA Index Hepatitis C'; ATA Index Hepatitis B and ATA Index Hepatitis C in validation cohorts and FIB-4 Index in training cohorts and in original study

	AUC	Sensitivity	Specificity	PPV	NPV
ATA Index Hepatitis B	0.92	0.90	0.84	0.53	0.98
ATA Index Hepatitis C	0.89	0.91	0.74	0.54	0.96
ATA Index Hepatitis B[a]	0.85	0.31	0.97	0.61	0.91
ATA Index Hepatitis C[b]	0.62	0.35	0.88	0.60	0.72
FIB-4 IndexHepatitis B[c]	0.88	0.75	0.84	0.49	0.94
FIB-4 Index Hepatitis C[d]	0.82	0.76	0.78	0.64	0.86
FIB-4 Index (cut off 1.45)[e]	0.85	0.74	0.80		0.95[e]

a: Results of 'ATA Index Hepatitis B'applied to the validation cohort of 100 patients with chronic hepatitis B; b: Results of 'ATA Index Hepatitis C' applied to the validation cohort of 100 patients with chronic hepatitis C; c: Results of FIB-4 Index applied to the hepatitis B training cohort; d: Results of FIB-4 Index applied to the hepatitis C training cohort; e: Results of FIB-4 Index (cut off 1.45) from original study;[8] AUC: Area under the ROC curve; NPV: Negative predictive value; PPV: Positive predictive value.

Of the 430 CHB patients, 356 (82.8%) had hepatitis B e antigen (HBeAg) negative and 74 patients (17%) had HBeAg positive. Of the HBeAg positive patients, 56 (75.7%) patients had mild to moderate fibrosis, 18 (24.3%) patients had severe fibrosis. Among HBeAg negative patients, 301 (84.6%) patients were in the mild to moderate fibrosis group and 55 (15.4%) patients were in the severe fibrosis group. The patients were divided into two groups according to their HBV DNA levels as $\leq 10^5$ and $>10^5$ copy/ mL. HBV DNA data were not found for 9 patients. Among 421 CHB patients, 271 patients' (64.4%) had HBV DNA level of $\leq 10^5$. Of these 271 patients, 245 (90.4%) were in the mild to moderate fibrosis group, 26 patients (9.6%) were in the severe fibrosis group. 150 patients (35.6%) had more than 10^5 BV DNA level 106 (70.7%) of which had mild to moderate fibrosis whereas 44 (29.3%) had severe fibrosis. According to HBV DNA level, there was a significant difference between mild to moderate and severe fibrosis groups (p<0.001). Patients with who had severe fibrosis had a higher level of $>10^5$ HBV DNA than patients with had mild to moderate fibrosis.

When laboratory parameters were assessed according to fibrosis staged (F0, F1, F2, F3, F4): ALT (p<0.001), AST (p<0.001), PT (p<0.001), Hemoglobin (p=0.037), albumin (p<0.001), total bilirubin (p<0.001), direct bilirubin (p<0.001), ALP (p=0.043), GGT (p<0.001), WBC (p=0.026), platelet (p<0.001), ALT/ULN (p<0.001), AST/ULN (p<0.001), GGT/ULN (p<0.001) and age (p<0.001) were significantly different between groups in terms of univariate analysis. Sex, HBV DNA level (p<0.001) and HBeAg (p=0.02) were also statistically significant between groups.

When laboratory parameters were assessed according to mild to moderate and severe fibrosis gropus: age, sex, HBV DNA, ALT, AST, PT, albumin, total bilirubin, direct bilirubin, GGT, platelet, glucose, AST/ULN, ALT/ULN and GGT/ULN were found to be significantly different between groups in terms of univariate analysis (Table 3). Logistic regression analysis showed that the age (p<0.001), platelet (p<0.001), albumin (p<0.001), PT (p=0.008) and AST/ULN (p=0.007) were the most significant parameters to distinguish mild to moderate and severe fibrosis groups (Table 3).

Formula:

$$P \text{ (Severe fibrosis)} \quad \frac{e^{5.325+0.283 \times AST/ULN+0.066 \times Age+0.364 \times PT-2.847 \times Albumin-0.016 \times Platelet}}{1+e^{5.325+0.283 \times AST/ULN+0.066 \times Age+0.364 \times PT-2.847 \times Albumin-0.016 \times Platelet}}$$

The probability of having severe fibrosis was found with this formula and the overall accuracy was assessed using the ROC curve (Fig. 2). This
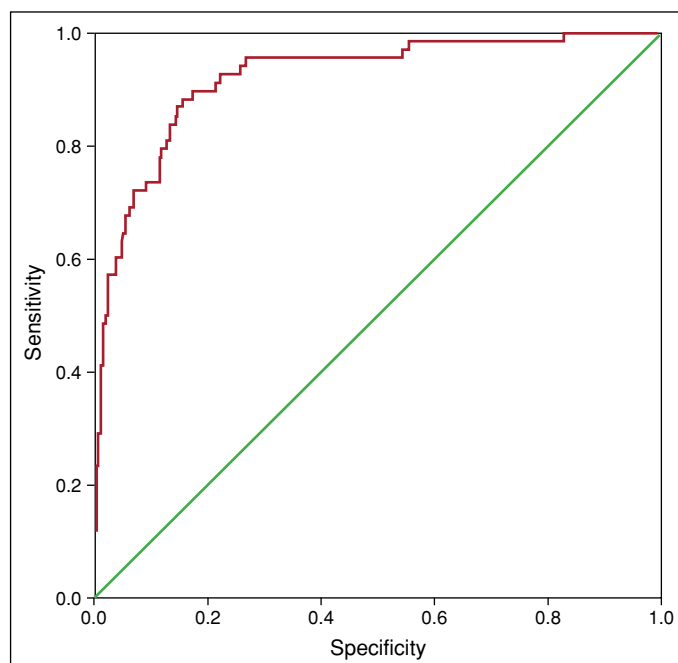
Figure 2. ROC curve for hepatitis B patient group.

formula was named 'ATA Index Hepatitis B'. The best cut-off point for the probability of distinguishing between mild to moderate and severe fibrosis was found 0.17 with the Youden Index. Using the best probability cut-off (less than 0.17), the AUC of 'ATA Index Hepatitis B' was 0.92, sensitivity 0.90, specificity 0.84, PPV 0.53 and, NPV 0.98 (Table 2).

Validation Cohort

'ATA Index Hepatitis B' was applied to 100 CHB patients from other Hepatology clinic as a validation cohort. In the validation cohort, 13 patients (13%) were in the severe fibrosis group, 87 patients (87%) were in the mild to moderate fibrosis group. In the validation cohort; sensitivity was 0.31, specificity was 0.97, positive and NPV of the 'ATA Index Hepatitis B' were 0.61 and 0.91 respectively (Table 2).

Evaluation of FIB-4 Index in Training Cohort

The FIB-4 Index was tested in our CHB study cohort (n=430) to assess-

Table 3. Demographics and patient characteristics of chronic hepatitis B patients

| | Fibrosis stage | | | |
	Mild to moderate	Severe	p[a]	Odds ratio[b] (95% CI)
Age, mean (min–max), years	40 (15–75)	50 (19–74)	<0.001	1.069 (1.034–1.104)
Male n, (%)	216 (%78.5)	59 (%21.5)	0.001	
ALT, mean (min–max), U/L	41 (2–581)	55 (16–946)	0.005	
AST, mean (min–max), U/L	29 (12–447)	53 (22–498)	<0.001	
ALP, mean (min–max), U/L	77 (33–320)	87 (42–338)	0.058	
GGT, mean (min–max), U/L	24 (3–352)	49 (11–438)	<0.001	
Total bilirubin, mean (min–max), mg/dl	0.68 (0.1–7.13)	0.9 (0.3–3.7)	<0.001	
Direct bilirubin, mean (min–max), mg/dl	0.14 (0.01–13.6)	0.2 (0–1.5)	<0.001	
Albumin, mean (min–max), g/dl	4.5 (3–5.4)	3.9 (1.3–4.7)	<0.001	0.058 (0.020–0.171)
Glucose, mean (min–max), mg/dl	87 (59–276)	90 (58–360)	0.001	
Total cholesterol, mean (min–max), mg/dl	177 (92–1128)	172 (106–262)	0.248	
Triglyceride, mean (min–max), mg/dl	102 (20–692)	100 (30–282)	0.767	
PT, mean (min–max), second	12.1 (9.9–16.9)	13.2 (10.4–20.2)	<0.001	1.439 (1.098–1.887)
Hemoglobin, mean (min–max), g/dl	14.9 (9.6–19)	14.7 (9.9–17)	0.292	
WBC, mean (min–max), 10⁹/L	6.6 (3–8.2)	6.1 (2–12)	0.072	
Platelet count, mean (min–max), 10⁹/L	220 (68–586)	165 (34–309)	<0.001	0.984 (0.977–0.992)
MPV, mean (min–max), fL	8.5 (6.3–11.8)	8.7 (6.5–11.4)	0.259	
ALT/ULN, mean (min–max)	1 (0.05–18.7)	1.35 (0.47–21)	<0.001	
AST/ULN, mean (min–max)	0.8 (0.3–12)	1.4 (0.6–12.1)	<0.001	1.327 (1.081–1.630)
ALP/ULN, mean (min–max)	0.6 (0.3–2.7)	0.7 (0.3–2.6)	0.223	
GGT/ULN, mean (min–max)	0.5 (0.08–6.4)	1 (0.2–7.18)	<0.001	

Baseline demographics and laboratory parameters of chronic hepatitis B patients according to mild to moderate and severe fibrosis classification (n=430). a: P values of univariate analysis; b: Odds ratios of multivariate analysis; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CI: Confidence interval; GGT: Gamma-glutamyltransferase; MPV: Mean platelet volume; n: Number; PT: Prothrombin time; ULN: Upper limit of normal; WBC: White blood cell.

es the value of distinguishing mild to moderate and severe fibrosis. The AUC of the FIB-4 Index was 0.88, sensitivity 0.75, specificity 0.84, PPV 0.49 and NPV 0.94 (Table 2).

Discussion

In this study, we aimed to create formulas with laboratory parameters to predict liver fibrosis naive CHB and CHC patients who did not undergo biopsy in the treatment. We intended to distinguish between mild to moderate (F0-F1-F2) and severe (F3-F4) fibrosis groups. Our CHC formula contains age, platelet, PT, AST/ULN and the formula for CHB contains albumin in addition to these parameters. ATA Index Hepatitis B and ATA Index Hepatitis C can predict the non-existence of severe fibrosis with a similar accuracy to FIB-4 Index. ATA Indexes may reduce the need for liver biopsies.

It is not surprising that one of the most important variables in the models was age. Hepatitis C model studies showed that the progression of fibrosis does not follow a linear course over time, progression is slower at younger ages, the fastest is between the ages of 45–50, years of age and the progression accelerates within 10–15 years of time periods.[15] For hepatitis B, the younger the infection occurs, the higher the rate of chronic infection, indicating the importance of age and duration of exposure to the virus. Increased environmental exposure (especially oxidative stress), decreased hepatic blood flow, impaired immune function and dysfunction in hepatic macrophages and stellate cells by aging are

proposed mechanisms.[16] It also includes the Forns Index, FIB-4 Index and API score age.[12,14,17]

Platelet was also one of the most significant variables in both formulas. The three main mechanisms of thrombocytopenia are, the reduction of thrombopoietin, platelet sequestration in the spleen due to splenomegaly as a result of portal hypertension, the increased platelet destruction, and the shortened life expectancy of platelets in liver diseases. [18] Bone marrow suppression and autoimmunity may also contribute to thrombocytopenia in CHC patients. APRI, Forns, FIB-4, API, Pohl, CDS indexes also use the platelet count.[11–14,17,19] There was no statistically significant difference between mild to moderate and severe fibrosis groups in MPV for CHC and CHB patients. There are several studies on MPV elevation in thrombocytopenia and MPV variation in different diseases. However, there are also studies reporting the anticoagulant and time dependency of MPV measurement.[20,21] Lance et al.[20] suggests the storage of blood in EDTA and that the measurement time should not exceed 120 minutes after venepuncture. All of our all blood samples were collected in EDTA but the duration between the MPV measurement and venepuncture was remained unknown. Considering the lack of standardization in terms of MPV measurement due to the retrospective study design, it is not possible for us to comment on fibrosis effect on MPV.

Non-invasive tests are useful in early and advanced fibrosis evaluation, but less useful in the evaluation of moderate fibrosis. Imaging analysis of the fibrosis and Metavir score shows that the fibrous tissue

growth is not linear. A gradual increase in Metavir is associated with a progressive increase in the area of fibrosis For example, F1 has 1.7 times more fibrosis than the F0 stage; F2, F3 and F4 are, 3, 7 and 12 times F0,[22] respectively. The APRI and Forns indexes group patients into F0-F1 (non-significant fibrosis) and F2 to F4 (significant fibrosis). [12,13] Metavir F1 and F2 are close to each other and difficult to distinguish by non-invasive methods. Vallet et al.[14] showed the difficulty in distinguish from F0 to F1 or F2 and F1 from F2 in the study tested FIB-4 Index in CHC patients. Therefore, we grouped patients as mild to moderate (F0-F1-F2) and severe (F3-F4) fibrosis and intended to distinguish these groups. The FIB-4 Index is derived from the Apricot (AIDS Pegasys Ribavirin International Coinfection Trial) database, a pivotal trial evaluating the efficacy of pegylated interferon and ribavirin in patients co-infected with HIV and HCV.[23] The formula for the FIB-4 Index is: age (years) X AST [U/L]/ (platelets [109/L] X (ALT [U/L]) 1/2). Vallet et al.[14] showed that the FIB-4 Index can be used to exclude of severe fibrosis in CHC patients with high selectivity (0.80) and high NPV (0.95).

We classified patients into mild to moderate and severe fibrosis groups as like Vallet et al.[14] Severe fibrosis was detected in 25% of CHC patients and in 17%. of CHB patients. When both groups (CHB and CHC) were compared, the rate of severe fibrosis was higher in the CHC patient group. The characteristics of ATA Index Hepatitis C and ATA Index Hepatitis B were; AUC 0.89 and 0.92; sensitivity 0.91 and 0.90; specificity 0.74 and 0.84; PPV 0.54 and 0.53; NPV 0.96 and 0.98, respectively. ATA Index Hepatitis B was found to be superior to ATA Index Hepatitis C in terms of NPV, specificity and AUC. These results were attributed to the higher number of patients in the CHB group.

ATA Indexes (Hepatitis B and hepatitis C) were tested in validation cohorts of 200 patients (100 CHB and 100 CHC) from another Hepatology center. When we applied 'ATA Index Hepatitis B' to the validation cohort of 100 CHB patients, AUC was 0.85; sensitivity 0.31; specificity 0.97; PPV 0.61 and NPV 0.91. And the results of 'ATA Index Hepatitis C' tested in validation cohort including 100 CHC patients were as AUC: 0.62; sensitivity: 0.35; specificity: 0.88; PPV: 0.60 and NPV: 0.72. In our study, AUC, sensitivity and NPVs of ATA Indexes were lower in validation cohort than training cohort. This can be due to the difference of prevalence. In addition, the use of internal validation cohort limits to generalize the results. Therefore, we selected the validation cohort from a different center. But our indexes need to be tested in other cohorts.

As Vallet et al.[14] divided CHC patients into as mild to moderate and severe fibrosis groups similar to our study, we compared the 'ATA Index Hepatitis C' and FIB-4 Index. The FIB-4 Index has two cutoff values as 1.45 (<1.45 low risk for severe fibrosis) and 3.25 (>3.25 high risk for severe fibrosis). The authors recommend using a lower cut-off to rule out severe fibrosis. 'ATA Index Hepatitis C' has one cut off with a high NPV, so we can compare lower the cut off properties of the FIB-4 Index with our 'ATA Index Hepatitis C'. Comparing the characteristics of indexes in the original studies, the 'ATA Index Hepatitis C' appears to be superior to the FIB-4 Index according to AUC (0.89–0.85), sensitivity (0.91–0.74) and NPV (0.96–0.95) (Table 2). When FIB-4 Index was tested in our study cohort, the 'ATA Index Hepatitis C' appears to be superior with regard to AUC (0.89–0.82), sensitivity (0.91–0.76) and NPV (0.96–0.86) to FIB-4 Index (Table 2). In several studies the diagnostic performance of the the FIB-4 Index was tested to determine various stages of fibrosis using different cut-offs, among patients with hepatitis C infection. In the FIB-4 Index validation study in 2304 patients with CHC, AUC was reported to distinguish mild to moderate

and severe fibrosis as 0.83 (NPV: 0.90) using the cut off 1.21.[24] Cheng et al.[25] tested FIB-4 for the prediction of severe fibrosis and cirrhosis in 113 patients with CHC. They reported the AUC:0.852 with a cut-off 1.799 to distinguishing severe fibrosis.[25] Ragazzo et al.[26] also analyzed the FIB-4 Index in 104 patients with CHC. They set the cut-off as 1.22 for differentiation of severe fibrosis and reported the AUC as 0.778. In a study involving 1716 HCV patients from Germany APRI and FIB-4 were tested and the AUC was reported as 0.73 using the cutoff 2.9 for differentiation of severe fibrosis.[27] These results show that, although the diagnostic performance of the FIB-4 Index is tried to be increased by determining different cut-offs, the AUC ranges between 0.73 to 0.85. The difference of cut-off values can be explained by the differences in patient population, including patient distribution in different the fibrosis groups and the reference ranges used for AST and ALT levels. However, there are also FIB-4 validation studies using the original cut off (1.45) to rule out severe fibrosis. In a study in which 575 CHC patients were tested with regard to the performance of a combination of APRI and FIB-4 Index as an alternative to transient elastography, the original cut-off (1.45) was used for FIB-4 in distinguishing severe fibrosis. They reported that the FIB-4 Index had an AUC of 0.854 with a sensitivity 81.5%, specificity 79%, PPV 85% and NPV 71%.[28] Comparing FIB-4 results of the recent studies with the 'ATA Index Hepatitis C', the 'ATA Index Hepatitis C' results appear to be superior to the FIB-4 Index for AUC in terms of sensitivity and NPV to r exclude severe fibrosis.

We tested the FIB-4 Index in the CHB patients and compared it with 'ATA Index Hepatitis B'. The 'ATA Index Hepatitis B' was found to be superior to the FIB-4 Index in the CHB study cohort in the differentiation of mild to moderate and severe fibrosis (Table 2). It can also be suggested that the FIB-4 Index is functional in ruling out severe fibrosis in CHB patients with high selectivity and NPV. Although the FIB-4 Index was from the data of patients with HCV, several studies have also validated the FIB-4 Index for patients with CHB. Kim et al.[29] tested FIB-4 in 668 CHB patients and reported the AUC of 0.91 to distinguish severe fibrosis with a new cut-off 1.6. An FIB-4 Index validation study in 284 CHB patients reported the AUC of 0.86 with a cut off 1.58 set for to distinguis severe fibrosis.[24] A meta-analysis of six studies involving 1473 patients reported an AUC of 0.79 with a sensitivity of 0.76 and specificity of 0.74 to predict severe fibrosis by cut-off values ranging 1 to 3.25.[30] Another meta-analysis, involving 22 studies of 6338 patients tested FIB-4 Index reported that the mean AUC of the FIB-4 Index for severe fibrosis was 0.81 (ranging 0.74–0.91) with different cut off values.[31] These studies show that the AUC of FIB-4 ranges between 0.74 to 0.91to distinguish severe fibrosis in patients with HBV. The 'ATA Index Hepatitis B' study cohort had AUC of FIB-4 in our study is 0.88 and also in the range. 'ATA Index Hepatitis B' had an AUC 0.92 in study cohort and decreased to 0.85 in validation cohort and showed a good diagnostic performance, especially excluding severe fibrosis with high sensitivity (0.90) and NPV (0.98). Recently Ayed et al.[32] designed a study to assess liver fibrosis in CHB patients with a new combined prediction model using APRI and FIB-4 scores. In this study including 179 CHB patients, the FIB-4 Index results were AUC: 0.76, sensitivity: 0.74, specificity 0.73, PPV 32.3, NPV 0.94.[32] In our study, the 'ATA Index Hepatitis B' appears to be more specific (0.84 in study cohort and 0.97 in the validation cohort) and similar in excluding severe fibrosis (NPV: 0.98 in study cohort and 0.91 in the validation cohort). However, FIB-4 and 'ATA Index Hepatitis B' show good performance levels (AUC>0.80) to rule out severe fibrosis in our CHB cohort.

Our study has some limitations. First, we included patients who had laboratory data within one month of biopsy, we had not simultaneous blood samples which can provide more accurate prediction. Second, ATA Indexes have one cut-off point results in suboptimal sensitivity and specificity. FIB-4 Index uses two cut-off points for diagnosing specific fibrosis stages. The high cut-off with high specificity (i.e. fewer false-positive results) provides to diagnose higher stages of fibrosis, and a low cut-off with high sensitivity (i.e. fewer false-negative results) provides to rule out the presence of severe fibrosis. So we could compare low cut-off results of FIB-4 with ATA Indexes to rule out the presence of severe fibrosis. Third, we prevented inter-observer variability by evaluating all pathology samples by one pathologist but we could not prevent intra-observer variability. Forth, ATA Indexes are not simple formulas and needs calculator or excel program for calculation.

In conclusion, 'ATA Index Hepatitis B' and 'ATA Index Hepatitis C' can predict the absence of severe fibrosis with a similar accuracy to FIB-4 Index. Although the indexes tested in an external cohort, our results need to be validated by different cohorts to be generalized.

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