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The predictive value of noninvasive serum markers of liver fibrosis in patients with chronic hepatitis C

LIVER

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Original Article

ABSTRACT

Background/Aims: This study aims to show the predictive value of noninvasive serum markers on the hepatic fibrosis level.

Materials and Methods: This cross sectional study involves 120 patients with chronic hepatitis C. The noninvasive markers used were as follows: age-platelet index (AP index), cirrhosis discriminant score (CDS), aspartate aminotransferase (AST)-alanine aminotransferase (ALT) ratio (AAR), fibrosis-4 (FIB-4) index, AST-platelet ratio index (APRI), Goteborg University Cirrhosis Index (GUCI), FibroQ, King's score, platelet count. Concurrent liver biopsies were evaluated using the modified Ishak and Knodell scoring systems. In accordance with the Knodell scores, F3-F4 scores were defined as "severe fibrosis," and the modified Ishak scores stage of ≥3 (F3–F6) were defined as "clinically significant fibrosis." Receiver Operating Characteristic (ROC) curve analyses were carried out to compare the noninvasive markers with hepatic fibrosis level.

Results: Mean age of the patients was 51.7±11.6. A total of 10 patients (8.3%) with Knodell scores and 24 patients (20%) with modified Ishak scores were evaluated to have ≥F3 hepatic fibrosis. ROC analyses with the Knodell and modified Ishak scores were as follows: AP index=0.61–0.57, CDS=0.66–0.55, AAR=0.60–0.49, FIB-4=0.70–0.68, APRI=0.67–0.72, GUCI=0.66–0.72, FibroQ=0.64–0.54, King's score=0.68–0.54, platelet count=0.61–0.55.

Conclusion: We found that APRI, FIB-4, King's score, and GUCI can be used to determination patients with mild fibrosis with a high negative predictive value and in the differentiation of severe/significant fibrosis from mild to moderate fibrosis.

Keywords: Liver fibrosis, liver fibrosis prediction, serum markers, liver biopsy, chronic hepatitis C

INTRODUCTION

The course of hepatitis C infection varies. The best indicator of disease progression is the degree of liver fibrosis (1). The risk of progression to severe fibrosis or cirrhosis within 10–20 years is low in patients without fibrosis or inflammation and in those with minimal fibrosis (2). Bridging fibrosis is associated with a high risk of progression to cirrhosis (1).

It is important to determine the degree of liver fibrosis to identify patients with a high risk of disease progression. Although liver biopsy is accepted as a reference to histologically examine the liver, there are disadvantages to the procedure, such as sampling errors, variability in assessment, and complications such as bleeding, pain, and infection (3,4). With the recent introduction of antifibrotic therapy, which has fewer adverse effects and a high treatment success, and considering that biopsy is an invasive diagnostic procedure, the serum fibrosis marker tests, which are indicative of the reserve, structure, and function of the liver, will replace biopsy to obtain information regarding the prognosis of patients with hepatitis C virus (HCV) in the years ahead. However, although these drugs are not currently licensed in Turkey, they will be administered only to selected patients, primarily because of their high cost. It is important to determine the patients' degree of liver fibrosis to provide a more rational treatment with antiviral drugs that are currently in use for patients with advanced-stage fibrosis and cirrhosis. The ideal test is one that

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determines the degree of liver fibrosis accurately and easily, without the potential complications associated with liver biopsy. Many direct and indirect serum markers of fibrosis have been found to estimate the degree of liver fibrosis at this stage. Several algorithms to assess fibrosis that combine different fibrosis tests have been proposed to improve the accuracy of noninvasive diagnosis of liver fibrosis in patients with chronic hepatitis C. These serum markers may be a suitable alternative to liver biopsy because they are safe, cheap, and noninvasive. These noninvasive tests aim to accurately identify liver fibrosis, and thus, reduce the use of liver biopsy in indeterminate cases. Standardization studies are required before the tests are implemented, although they appear to be a suitable alternative to liver biopsy. The objective of the present study was to compare the scores of nine serum fibrosis markers with that of liver biopsy (as the gold standard diagnostic technique) in accurately determining the level of fibrosis in patients with chronic hepatitis C.

MATERIALS AND METHODS

Patients with chronic hepatitis C admitted to the Department of Hepatology-Gastroenterology of Türkiye Yüksek İhtisas Research and Training Hospital between January 2006 and March 2014 were included in the present study. The study protocol was approved by the Ankara Yüksek İhtisas Research and Training Hospital local ethics committee. Patients were included only after signing the informed consent form approved by Türkiye Yüksek İhtisas Research and Training Hospital Local Ethics Committee. The data of patients who had not previously received HCV treatment, who had a positive serum HCV ribonucleic acid (RNA) test (>50 IU/mL) according to real-time polymerase chain reaction (PCR) analysis (COBAS Amplicor HCV Test, version 2.0, Roche Molecular Systems, Indianapolis, USA), who were infected with HCV genotype 1a, and who had a liver biopsy performed were retrospectively examined. Patients with decompensated chronic liver disease, inadequate liver histopathology and laboratory data, use of alcohol (>40 g/day), coinfection with hepatitis B, hepatitis C, or hepatitis D, or concomitant autoimmune and metabolic disorders were excluded.

Liver histology

Histopathological evaluation of liver biopsies, which were obtained prior to the treatment of hepatitis C, was performed retrospectively and independently by two different pathologists. Staging and grading of liver histopathology were performed according to the modified Ishak and Knodell protocols (5,6). Clinically significant fibrosis was defined as modified Ishak stage of 3 or more fibrosis (F3–F6), and no/mild fibrosis was defined as modified Ishak stage F0–F2. In accordance with Knodell scores, patients with F3 and F4 scores were defined as severe fibrosis, and patients with F0–F2 were defined as mild to moderate fibrosis.

Noninvasive liver fibrosis tests

The following data (previously examined in blood samples simultaneously taken with liver biopsy) were collected: white blood cell and platelet counts; levels of hemoglobin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, and albumin; and prothrombin- international normalized ratio (INR). Platelet count (7), AST-ALT ratio (AAR) (8), AST-platelet ratio index (APRI) (9), fibrosis-4 (FIB-4) index (10), cirrhosis discriminant score (CDS) (11), age-platelet index (AP index) (12), Goteborg University Cirrhosis Index (GUCI) (13), King's score (14), and FibroQ (15) were calculated according to the published or patented formulae. The platelet count cut-off value was considered to be $<150\times10^3$ μ/L (7). The performance of a noninvasive diagnostic method was evaluated by calculating the area under the receiver curve (AUROC), taking liver biopsy as the reference standard (16). The AUROC value ranges from 0.5 (lack of discrimination) to 1.0 (perfect discrimination). Values of ≥0.9 were considered excellent, 0.8–0.9 good, 0.7–0.8 fair, and <0.7 poor (16). Our own cut-off value for sensitivity and specificity, false (–), false (+), positive predictive value (PPV), negative predictive value (NPV), accuracy rate, likelihood ratios (LR) (+), and LR (–) values were obtained for indices with significant p values in the receiver operating characteristic (ROC) analysis. Furthermore, the sensitivity, specificity, false (–), false (+), PPV, NPV, accuracy rate, LR (+), and LR (–) values were calculated using the cut-off values to distinguish significant fibrosis from mild fibrosis, as reported in the literature regarding each index.

Statistical analysis

We utilized frequencies (percent) for categorical variables and mean±standard deviation [or median (minimum-maximum)] for metric variables The area under the ROC curve gives an estimate of the overall accuracy of each technique. An area of 0.50 implies that the variable adds no information. The areas under the ROC curves with their standard error (SE) estimates for all variables were calculated in the manner described by Hanley and McNeil (16). The sensitivity, specificity, false positive rate, false negative rate, PPV, NPV, LR (+), LR (–), diagnostic accuracy with their 95% confidence intervals were calculated for diagnostic performance of noninvasive liver fibrosis tests; p values less than 0.05 were considered as statistically significant. To determine the best compromise between sensitivity and specificity, we evaluated the cut-off value with maximum Youden index which equals to the sum of sensitivity and specificity minus 1. All (J=Sensitivity+Specificity–1) statistical analyses were carried out using a commercial software Statistical Package For Social Sciences (SPSS) version 15.0 (IBM Corporation; Chicago, IL, USA).

RESULTS

A total of 120 patients (57.5% men; mean age 51.7±11.6 years) were included in the present study. Demographic data of the patients are presented in Table 1. With regard to the results of the histopathological evaluation, according to the Knodell classification, the average fibrosis score was 1.35±0.68, and the

Table 1. Baseline characteristics of chronic hepatitis C patients

Patients	Median (range) or (n, %)
Age at biopsy (years)	53 (23–88)
Sex	
Male	69 (57.5)
Female	51(42.5)
Hemoglobin (g/dL)	13.6 (7.6–19)
White blood cell (µL)	6226 (600–13500)
Platelet count (103/µL)	219.5 (44–470)
INR	1.00 (0.8–1.4)
AST (U/L)	12 (11–566)
ALT (U/L)	48.5 (11–451)
Total bilirubin (mg/dL)	0.72 (0.16–1.9)
Albumin (g/dL)	4.4 (3.1–5.6)
HCV RNA (IU/mL)	3.9×10^5 ($15–7.7 \times 10^7$)
AP index	4 (0–9)
CDS	4 (0–7)
AAR	0.79 (0.38–1.94)
FIB4	1.29 (0.37–13.09)
APRI	0.43 (0.16–11.2)
GUCI	0.44 (0.17–10.8)
FibroQ	1.91 (0.47–7.79)
King	8.97 (2.3–263)
Platelet count (103/µL)	219.5 (44–470)

INR: international normalized ratio; AST: aspartate aminotransferase; ALT: alanine aminotransferase; HCV RNA: hepatitis C virus ribonucleic acid; AP index: age-platelet index; CDS: cirrhosis discriminant score; AAR: aspartate aminotransferase-alanine aminotransferase ratio; FIB-4: Fibrosis-4 index; APRI: AST-platelet ratio index; GUCI: Göteborg University Cirrhosis Index

histology activity index (HAI) was 6.78±2.99; according to the modified Ishak classification, the average fibrosis score was 1.65±1.01, and the HAI was 6.43±2.67. Twenty-four (20%) patients had F3–6 fibrosis according to the Ishak score. The number of patients with F5–F6 fibrosis according to the Ishak score was low (two patients; 1.6%). Ten patients (8.3%) had F3-F4 fibrosis according to the Knodell score (Table 2).

The AUROCs values used for distinguishing severe fibrosis (F3-F4) from mild to moderate fibrosis (F0–F2) according to the Knodell fibrosis score were as follows: 0.613 [95% confidence interval (CI), SE=0.09, p=0.237] for the AP index; 0.66 (95% CI, SE=0.09, p=0.102) for the CDS index, 0.60 (95% CI, SE=0.08, p=0.316) for the AAR index, 0.70 (95% CI, SE=0.09, p=0.033) for FIB-4, 0.67 (95% CI, SE=0.07, p=0.067) for APRI, 0.66 (95% CI, SE=0.08, p=0.097) for GUCI, 0.64 (95% CI, SE=0.09, p=0.136) for FibroQ, 0.68 (95% CI, SE=0.09, p=0.064) for the King's score, and 0.61 (95% CI, SE=0.07, p=0.241) for platelet count (Table 3). The FIB-4 score was associated with a slightly higher AUROC value

Table 2. Histopathological characteristics of the chronic hepatitis C patients

	Knodell fibrosis score Mean±SD or (n, %)	Ishak fibrosis score Mean±SD or (n, %)
Fibrosis	1.35±0.68	1.65±1.01
F0–F2	110 (91.7)	96 (80)
F3-F4 (Knodell)–F3–F6 (Ishak)	10 (8.3)	24(20)
HAI	6.78±2.99	6.43±2.67
>Grade 7–14	57 (47.5)	51 (42.5)

SD: standard deviation; HAI: histological activity index

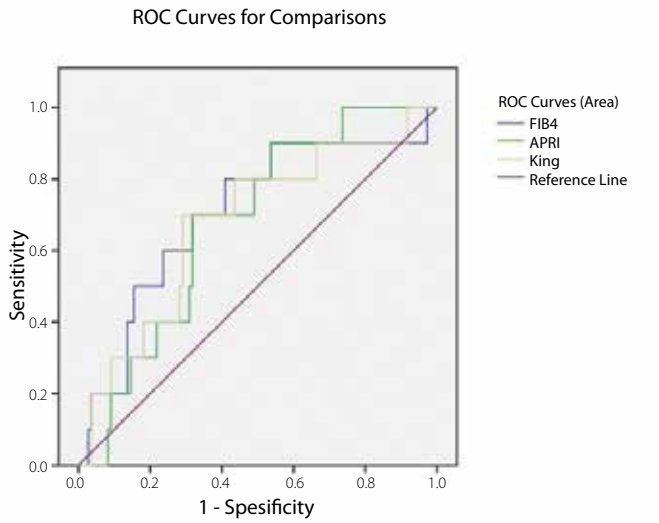

Figure 1. Receiver operating characteristic curve comparisons of the noninvasive tests for the prediction of severe fibrosis according to the Knodell score. FIB-4: fibrosis-4 index; APRI: aspartate aminotransferase-platelet ratio index

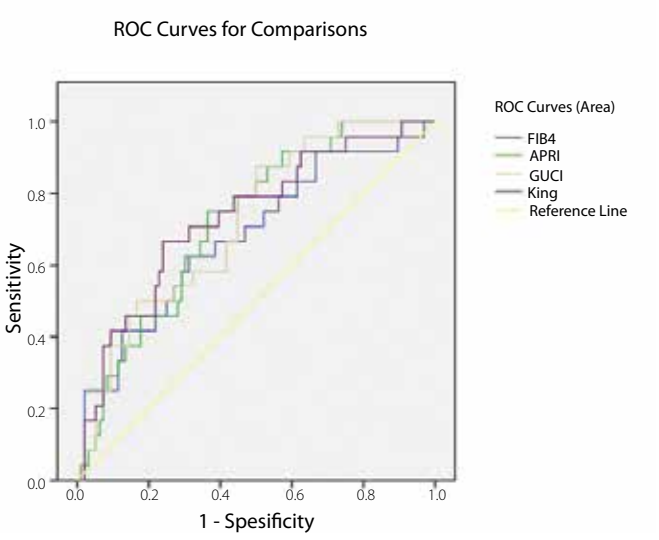

Figure 2. Receiver operating characteristic curve comparisons of the noninvasive tests for the prediction of significant fibrosis according to the İshak score. FIB-4: fibrosis-4 index; APRI: aspartate aminotransferase-platelet ratio index; GUCI: Göteborg University Cirrhosis Index

(although this was not statistically significant) than the APRI and King's score for fibrosis according to the Knodell score [p=0.68 (FIB-4 vs. APRI), p=0.62 (FIB-4 vs. King), p=0.98 (APRI vs. King)].

Table 3. Performance of studies investigating of noninvasive tests according to the Knodell fibrosis score

	Cutoff values sensitivity and specificity	AUC (SE)	p	Sensitivity (95% CI) Median (range)	Specificity (95% CI) Median (range)	False (–)	False (+)	PPV (95% CI) Median (range)	NPV (95% CI) Median (range)	Diagnostic accuracy	LR+ Median	LR– Median
AP index		0.613 (0.09)	0.237									
	≥4			0.80 (0.49–0.94)	0.45 (0.36–0.55)	0.20	0.54	0.12 (0.07–0.19)	0.96 (0.90–0.98)	0.48	1.47	0.44
	≥6			0.30 (0.11–0.60)	0.77 (0.69–0.84)	0.70	0.23	0.11 (0.06–0.18)	0.92 (0.86–0.96)	0.73	1.32	0.91
CDS		0.66 (0.09)	0.102									
AAR		0.60 (0.08)	0.316									
	>1			0.20 (0.06–0.51)	0.77 (0.69–0.84)	0.80	0.23	0.07 (0.04–0.14)	0.91 (0.84–0.95)	0.72	0.88	1.04
FIB4	***1.6**	0.70 (0.09)	0.033	**0.70 (0.40–0.89)**	**0.68 (0.60–0.76)**	**0.30**	**0.32**	**0.17 (0.11–0.25)**	**0.96 (0.90–0.98)**	**0.68**	**2.20**	**0.44**
	≥1.45			0.70 (0.40–0.89)	0.59 (0.50–0.68)	0.30	0.41	0.13 (0.08–0.21)	0.95 (0.90–0.98)	0.60	1.71	0.51
	>3.25			0.20 (0.06–0.51)	0.95 (0.90–0.98)	0.80	0.05	0.29 (0.21–0.38)	0.93 (0.86–0.97)	0.89	4.40	0.84
APRI	***0.55**	0.67 (0.07)	0.067	**0.70 (0.40–0.89)**	**0.68 (0.59–0.76)**	**0.30**	**0.32**	**0.17 (0.11–0.25)**	**0.96 (0.90–0.99)**	**0.68**	**2.20**	**0.44**
	≥0.5			0.70 (0.40–0.89)	0.64 (0.55–0.73)	0.30	0.35	0.15 (0.09–0.23)	0.96 (0.90–0.98)	0.65	1.97	0.46
	>1.5			0.10 (0.02–0.40)	0.92 (0.85–0.96)	0.90	0.08	0.10 (0.05–0.17)	0.92(0.85–0.96)	0.85	1.22	0.98
GUCI		0.66 (0.08)	0.097									
	≥0.2			1.00 (0.72–1.00)	0.06 (0.03–0.13)	0	0.94	0.09 (0.05–0.16)	1 (0.96–0.99)	0.14	1.07	0
	≥1			0.20 (0.06–0.51)	0.89 (0.82–0.94)	0.80	0.11	0.14 (0.09–0.22)	0.92 (0.86–0.96)	0.83	1.83	0.9
FibroQ		0.64 (0.09)	0.136									
	>0.6			1.00 (0.72–1.00)	0.02 (0.00–0.06	0	0.98	0.08 (0.04–0.15)	1 (0.96–0.99)	0.10	1.02	0
	>1.6			0.90 (0.60–0.98)	0.36 (0.28–0.46)	0.1	0.64	0.11 (0.07–0.19)	0.98 (0.92–0.99)	0.41	1.41	0.28
	>2.6			0.50 (0.24–0.76)	0.75 (0.67–0.82)	0.50	0.24	0.16 (0.10–0.24)	0.94 (0.88–0.97)	0.73	2.04	0.66
King	***11.85**	0.68 (0.09)	0.064	**0.70 (0.40–0.89)**	**0.71 (0.62–0.78)**	**0.30**	**0.29**	**0.17 (0.11–0.26)**	**0.96 (0.91–0.99)**	**0.71**	**2.41**	**0.42**
	≥12.3			0.50 (0.24–0.76)	0.72 (0.63–0.79)	0.50	0.28	0.14 (0.08–0.22)	0.94 (0.88–0.97)	0.70	1.77	0.70
	≥16.7			0.30 (0.11–0.60)	0.82 (0.74–0.88)	0.70	0.18	0.13 (0.08–0.21)	0.93 (0.86–0.96)	0.77	1.65	0.86
Platelet count		0.61 (0.07)	0.241									
	<150x10^{3}µL			0.20 (0.06–0.51)	0.86 (0.79–0.91)	0.80	0.14	0.12 (0.07–0.19)	0.92 (0.85–0.96)	0.81	1.47	0.93

*our cut-off value

AUC (SE): area under the curve (standard error); CI: confidence interval; AP index: age-platelet index; CDS: cirrhosis discriminant score; AAR: aspartate aminotransferase-alanine aminotransferase ratio; FIB-4: fibrosis-4 index; APRI: aspartate aminotransferase-platelet ratio index; GUCI: Göteborg University Cirrhosis Index; PPV: positive predictive value; NPV: negative predictive value; +LR: positive likelihood ratio; –LR: negative likelihood ratio

The AUROCs values used for distinguishing significant fibrosis (F3–F6) from no-mild fibrosis (F0–F2) according to the Ishak fibrosis score were as follows: 0.57 (95% CI, SE=0.06, p=0.263) for the AP index; 0.55 (95% CI, SE=0.07, p=0.445) for the CDS index; 0.49 (95% CI, SE=0.07, p=0.896) for the AAR index; 0.68 (95% CI, SE=0.06, p=0.007) for FIB-4; 0.72 (95% CI, 0.05, p=0.001) for APRI; 0.72 (95% CI, SE=0.05, p=0.001) for GUCI; 0.54 (95% CI, SE=0.07, p=0.525) for FibroQ; 0.73 (95% CI, SE=0.06, p<0.001) for the King's score; and 0.55 (95% CI, SE=0.06, p=0.453) for platelet count (Table 4). The King's score was associated with a slightly higher AUROC value (although this was not statistically significant) than the APRI, GUCI, and FIB-4 test scores for fibrosis according to the Ishak score [p=0.38 (FIB-4 vs. APRI), p=0.17 (FIB-4 vs. King), p=0.65 (APRI vs. King), p=0.41 (FIB-4 vs. GUCI), p=0.95(APRI vs. GUCI), p=0.66 (King vs. GUCI)]. The median AUROC value of ≥0.8 was not detected in any of the scoring systems (Table 3, 4).

ROC curves of serum fibrosis markers were constructed and superimposed to determine which score would have the most clinical utility to predict significant fibrosis (Figure 1, 2).

For fibrosis markers with significant p values or values close to 0.05 on the AUROC, we calculated the most appropriate cut-off values in our study group. The optimal cut-off value according to the Knodell fibrosis score was 1.6 for FIB-4 (sensitivity 0.70, specificity 0. 68, PPV 0.17, and NPV 0.96), 0.55 for APRI (sensitivity 0.70, specificity 0.68, PPV 0.17, and NPV 0.96), and 11.85 for the King's score (sensitivity 0.70, specificity 0.71, PPV 0.17, and NPV 0.96) (Table 3). The optimal cut-off values according to the Ishak fibrosis score were 1.38 for FIB-4 (sensitivity 0.67, specificity 0.61, PPV 0.30, and NPV 0.88), 0.46 for APRI (sensitivity 0.75, specificity 0.63, PPV 0.34, and NPV 0.91), 0.46 for GUCI (sensitivity 0.67, specificity 0.58, PPV 0.28, and NPV 0.87), and 10.76 for King's score (sensitivity 0.71, specificity 0.69, PPV 0.36, and NPV 0.90) (Table 4). FIB-4>1.6, APRI≥0.55, King's score≥11.85 were associated with a NPV of 0.96 according to the Knodell fibrosis score; FIB-4>1.38, APRI≥0.46, GUCI>0.2, and King's score≥10.76 were associated with NPVs ranging from 0.88 to 1.00 according to the Ishak fibrosis score. When the modified Ishak and Knodell scores were used as reference values, the

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Table 4. Performance of studies investigating noninvasive tests according to the Ishak fibrosis score

	Cutoff values sensitivity and specificity	AUC (SE)	p	Sensitivity (95% CI) Median (range)	Specificity (95% CI) Median (range)	False (–)	False (+)	PPV (95% CI) Median (range)	NPV (95% CI) Median (range)	Diagnostic accuracy	LR+ Median	LR– Median
AP index		0.57 (0.06)	0.263									
	≥4			0.71 (0.51–0.86)	0.47 (0.37–0.57)	0.29	0.53	0.25 (0.18–0.34)	0.86 (0.79–0.92)	0.52	1.33	0.62
	≥6			0.25 (0.12–0.45)	0.77 (0.68–0.84)	0.75	0.23	0.21 (0.15–0.30)	0.80 (0.72–0.87)	0.67	1.09	0.97
CDS		0.55 (0.07)	0.445									
AAR		0.49 (0.07)	0.896									
	>1			0.21 (0.09–0.40)	0.77 (0.68–0.84)	0.79	0.23	0.18 (0.12–0.27)	0.79 (0.71–0.86)	0.66	0.91	1.03
FIB4	***1.38**	0.68 (0.06)	.007	**0.67 (0.47–0.82)**	**0.61 (0.51–0.70)**	**0.33**	**0.38**	**0.30 (0.22–0.39)**	**0.88 (0.80–0.93)**	**0.62**	**1.73**	**0.54**
	≥1.45			0.62 (0.43–0.79)	0.61 (0.51–0.70)	0.37	0.38	0.29 (0.21–0.38)	0.87 (0.79–0.92)	0.62	1.62	0.61
	>3.25			0.21 (0.09–0.40)	0.98 (0.93–0.99)	0.79	0.02	0.71 (0.62–0.79)	0.83 (0.75–0.89)	0.82	10	0.81
APRI	***0.46**	0.72 (0.05)	0.001	**0.75 (0.55–0.88)**	**0.63 (0.53–0.72)**	**0.25**	**0.36**	**0.34 (0.26–0.43)**	**0.91 (0.84–0.95)**	**0.66**	**2.06**	**0.39**
	≥0.5			0.62 (0.43–0.79)	0.68 (0.58–0.76)	0.37	0.33	0.33 (0.24–0.42)	0.88 (0.80–0.93)	0.67	1.94	0.55
	>1.5			0.17 (0.07–0.36)	0.94 (0.87–0.97)	0.83	0.06	0.40 (0.31–0.49)	0.82 (0.73–0.88)	0.78	2.67	0.89
GUCI	***0.46**	0.72 (0.05)	0.001	**0.67 (0.47–0.82)**	**0.58 (0.48–0.68)**	**0.33**	**0.42**	**0.28 (0.21–0.38)**	**0.87 (0.80–0.93)**	**0.60**	**1.60**	**0.57**
	≥0.2			1.00 (0.86–1.00)	0.07 (0.04–0.14)	0	0.93	0.21 (0.15–0.30)	1 (0.96–0.99)	0.26	1.08	0
	≥1			0.25 (0.12–0.45)	0.92 (0.84–0.96)	0.75	0.08	0.43 (0.34–0.52)	0.83 (0.75–0.89)	0.78	3	0.82
FibroQ		0.54 (0.07)	0.525									
	>0.6			1.00 (0.86–1.00)	0.02 (0.00–0.07)	0	0.98	0.20 (0.14–0.29)	1 (0.96–0.99)	0.22	1.02	0
	>1.6			0.71 (0.51–0.85)	0.35 (0.27–0.45)	0.29	0.65	0.22 (0.15–0.30)	0.83 (0.75–0.89)	0.43	1.10	0.82
	>2.6			0.33 (0.18–0.53)	0.75 (0.65–0.82)	0.67	0.25	0.25 (0.18–0.34)	0.82 (0.73–0.88)	0.67	1.33	0.89
King	***10.76**	0.73 (0.06)	<0.001	**0.71 (0.51–0.85)**	**0.69 (0.59–0.77)**	**0.29**	**0.31**	**0.36 (0.28–0.45)**	**0.90 (0.83–0.95)**	**0.69**	**2.27**	**0.42**
	≥12.3			0.58 (0.39–0.75)	0.77 (0.68–0.84)	0.42	0.23	0.39 (0.30–0.48)	0.88 (0.81–0.93)	0.73	2.55	0.54
	≥16.7			0.42 (0.24–0.61)	0.86 (0.78–0.92)	0.58	0.13	0.43 (0.34–0.53)	0.86 (0.78–0.91)	0.77	3.08	0.67
Platelet count	<150x10^{3}µL	0.55 (0.06)	0.453	0.17 (0.07–0.36)	0.86 (0.78–0.92)	0.83	0.13	0.23 (0.16–0.32)	0.80 (0.72–0.87)	0.72	1.23	0.96

*our cut-off value

SE: standard error; AP index: age-platelet index; CDS: cirrhosis discriminant score; AAR: aspartate aminotransferase-alanine aminotransferase ratio; FIB-4: fibrosis-4 index; APRI: aspartate aminotransferase-platelet ratio index; GUCI: Göteborg University Cirrhosis Index; PPV: positive predictive value; NPV: negative predictive value; +LR: positive likelihood ratio; –LR: negative likelihood ratio

NPV results that we calculated according to our own cut-off values were higher than the NPV results calculated according to cut-off values defined originally in the literature.

The sensitivity, specificity, false (–), false (+), PPV, NPV, accuracy rate, LR (+), and LR (–) values were calculated by applying the optimized cut-off values that distinguish severe fibrosis from mild to moderate fibrosis according to the modified Ishak scores and significant fibrosis from no-mild fibrosis according to the previously established Knodell scores, with significant p values or values close to 0.05 on the AUROC (14,7,8,12,9,10,11,13,15) (Table 3, 4).

According to the Knodell score, a FIB-4 index of <1.6 (which was our own cut-off value) (n=78) had a NPV of 0.96 to exclude severe fibrosis (F3-4), and a FIB-4 index of >3.25 (n=7) had a PPV of 0.29 to confirm the existence of severe fibrosis. Using these cut-offs, 90.4% of the 85 biopsies with FIB-4 values outside of the 1.6–3.25 range (70.8% of total liver biopsies) were correctly classified.

According to the Knodell score, an APRI score of <0.55 (which was our own cut-off value) (n=75) had a NPV of 0.96 to exclude severe fibrosis (F3-4), and an APRI score of >1.5 (n=10) had a PPV of 0.10 to confirm the existence of severe fibrosis. Using these cut-offs, 85.9% of the 85 biopsies with APRI values outside of the 0.55–1.5 range (70.8% of total liver biopsies) were correctly classified.

According to the Knodell score, a King's score of <11.85 (which was our own cut-off value) (n=81) had a NPV of 0.96 to exclude severe fibrosis (F3-F4), and a King's score of >16.7 (n=23) had a PPV of 0.13 to confirm severe fibrosis. Using these cut-offs, 81.6% of the 104 biopsies with King's score values outside of the 11.85–16.7 range (86.6% of total liver biopsies) were correctly classified.

According to the Ishak score, a FIB-4 index of <1.38 (which was our own cut-off value) (n=67) had a NPV of 0.88 to exclude extensive fibrosis (F3-F4), and a FIB-4 index of >3.25 (n=7) had a PPV of 0.71 to confirm significant fibrosis. Using these cut-offs, 86.3% of the 74 biopsies with FIB-4 values outside of the 1.45–3.25 range (61.6% of total liver biopsies) were correctly classified.

According to the Ishak score, an APRI value of <0.46 (which was our own cut-off value) (n=67) had a NPV of 0.91 to exclude significant fibrosis (F3–6), and an APRI value of >1.5 (n=10) had a PPV of 0.40 to confirm significant fibrosis. Using these cut-offs, 84.2% of the 77 biopsies with APRI values outside of the 0.46–1.5 range (64.1% of total liver biopsies) were correctly classified.

According to the Ishak score, GUCI values of <0.46 (which was our own cut-off value) (n=64) had a NPV of 0.87 to exclude significant fibrosis, a GUCI value of >1 (n=14) had a PPV of 0.43 to confirm significant fibrosis. Using these cut-offs, 79% of the 78 biopsies with GUCI values outside of the 0.46–1 range (64.1% of total liver biopsies) were correctly classified.

According to the Ishak score, a King's score of <12.3 (which was our own cut-off value) (n=84) had a NPV of 0.88 to exclude significant fibrosis (F3–6), and a King's score of >16.7 (n=23) had a PPV of 0.43 to confirm significant fibrosis. Using these cut-offs, 78.3% of the 107 biopsies with King's score values outside of the 12.3–16.7 range (89.1% of total liver biopsies) were correctly classified.

DISCUSSION

In the present study, FIB-4, APRI, and King's score were shown to be useful in differentiating severe fibrosis from mild to moderate fibrosis according to the Knodell classification system, and FIB-4, GUCI, APRI, and King's score were useful in differentiating significant fibrosis from no to mild fibrosis according to the Ishak classification system. Our results indicate that the high NPV of these scores enable us to identify patients with mild fibrosis, providing prognostic data without the need for a liver biopsy.

Currently, many direct and indirect serum markers for fibrosis are used to estimate the degree of liver fibrosis. The ideal noninvasive fibrosis marker should be simple, accessible, cheap, accurate, and reliable (17). Noninvasive tests are very useful to distinguish between very advanced fibrosis and minimal to no fibrosis; the sensitivity and specificity of a diagnostic test needs to be higher. However, the accuracy of noninvasive tests decreases when diagnosing intermediate fibrosis (18). Most of the non-invasive tests had ROC curve values around 0.80 in showing significant fibrosis. This is of great magnitude, considering the best test has an AUROC value around 0.90 The utility of noninvasive serum tests in the determination of the degree of fibrosis in patients with chronic hepatitis C remains unclear because of the absence of gold standard tests that demonstrate accuracy and because current tests are insufficient to identify the intermediate stage of fibrosis (20). However, considering the low treatment success of the antiviral therapies currently in use, it is important to determine the degree of fibrosis in these patients to obtain information about the progression of the disease and decide on a treatment course. In prevalence studies conducted in Turkey, anti-HCV serological positivity was 0.95% among the population (21). Thus, approximately 700,000–1,000,000 individuals are currently infected with the virus, and there is concern that this number will increase further. The determination of fibrosis using serum fibrosis markers, which are noninvasive and cheaper than liver biopsy, may be considered to be a more cost-effective method for the assessment of the risk of clinical deterioration and the degree of the fibrosis. Although the results of these tests do not directly correlate with the scar tissue in the liver, they are useful in obtaining information on the amount of fibrosis and risk of progression of the disease (18). Till date, many serum fibrosis markers have been studied; however, which marker should be used for a particular cut-off value remains unclear.

The ability of APRI and FIB-4 tests to determine mild fibrosis (F0–F2) has been found to be low; however, they have been useful in the determination of advanced fibrosis (F3-F4) with high AUROC curves in patients with chronic hepatitis C (10). APRI was first described in the literature in 2003, and AUROC values have been found to be (0.8–0.88) for significant fibrosis and (0.89–0.94) for cirrhosis. NPV and PPV have been found to be 86%–88% for the detection of significant fibrosis and cirrhosis (9). However, in later studies, a wide range of sensitivity, specificity, PPV, NPV, and AUROCs values were found: 71%–97%, 13%–74%, 34%–84%, 43%–92%, and 0.6%–0.86, respectively (9,10,22-29). The occurrence of different results may be associated with the number of patients with significant fibrosis and AST. In our study, according to the Ishak and Knodell classifications, the mean AUROC values in the diagnosis of significant/severe fibrosis were -0.72 and -0.67, respectively, and sensitivity, specificity, PPV, and NPV were found to be 75%–70%, 63%–68%, 34%–17%, and 91%–96%, respectively. These results were believed to be effective in identifying patients without significant/severe fibrosis with high NPV results. In addition, liver biopsy can be avoided in 70.8% and 64.1% of patients according to the Knodell and Ishak scores respectively, results that are higher than the 54.2% reported by Petersen et al. (23) (when cut-off values are set as ≤0.42 and ≥1.2) and 51% reported originally by Wai et al. (9).

The mean AUROC values were found to be 0.71–0.83; sensitivity, specificity, PPV, and NPV were found to be 62%–74%, 60%–77%, 37%–83%, and 76%–94.7%, respectively, according to various cut-offs in the studies performed with FIB-4 (10,22,24-26,28,30,31). In our study, according to the Ishak and Knodell classifications, the mean AUROC values in the diagnosis of significant/severe fibrosis were –0.72 and –0.67, respectively, and sensitivity, specificity, PPV, and NPV were found to be 67%–70%, 61%–68%, 17%–30%, and 88–96%, respectively. As with the APRI score, it was believed to be useful in the detection of patients without significant/severe fibrosis with high NPV results. Using FIB-4 according to the Knodell and modified Ishak scores liver biopsy could be avoided in 70.8 and 61.6% of the total liver biopsies, respectively. These results are comparable to the values of 71% reported by Sterling et al. (30) and 72.8% reported by Vallet-Pichard et al. (10) and are higher than the value of 65.4% reported by Yu Hsieh et al. (32).

GUCI was first used by Kandemir et al. (33), who showed that this index may be used in distinguishing patients with severe fibrosis (stages 3-4) from those with mild to moderate fibrosis (stages 0–2). In a study conducted by İslam et al. (13), AUROC value was found to be 0.85, and when the cut-off value was taken as 1, the sensitivity, specificity, PPV, and NPV were 80, 78, 31, and 97%, respectively. In a study conducted by Fouad et al. (34), when cut-off values were set as 1.56, cirrhosis was identified with a sensitivity, specificity, PPV, and NPV of 60, 88.7, 89.8, and 80.5%, respectively. In a study conducted by Ehsan et al. (35), when the cut-off value was taken to be ≥1.5, cirrhosis was identified with a sensitivity of 74% and specificity of 89%. In our study, according to the Ishak classification, the AUROC value was 0.72; sensitivity, specificity, PPV, and NPV were 67, 78, 28, and 87%, respectively; and 64.1% of total liver biopsies were correctly classified.

The AUROC value was found to be 0.91 and 0.79 in the detection of significant fibrosis and cirrhosis with the King's score (14). A King's score of ≥16.7 predicted cirrhosis in 34% of patients with a sensitivity, specificity, and NPV of 86, 80, and 96%, respectively; a score of ≥12.3 had a sensitivity, specificity, NPV, and PPV of 70, 85, 77, and 81% (14). In a study by Cross et al. (36), the AUROC value was 0.82 for the diagnosis of significant fibrosis and 0.89 for the diagnosis of cirrhosis. The AUROC value was 0.73–0.68 according to the Ishak and Knodell classifications; sensitivity, specificity, PPV, and NPV were found to be 71%–70%, 69%–71%, 36%–17%, and 90–96%, respectively, in our study. Using the King's score liver biopsy could be avoided in 86.6% and 89.1% of total liver biopsies according to the Knodell and Ishak score, respectively.

The sensitivity and PPV values were found to be low because of the small number of patients with significant/severe fibrosis (20% of the patients according to the Ishak score and 8.3% according to the Knodell score). Therefore, the effectiveness of these serum markers was decreased in identifying patients with significant/severe fibrosis. The sensitivity was increased in the context of an increasing number of patients with severe fibrosis or cirrhosis.

The results of noninvasive liver function test scores show considerable disparity among studies due to the variation in parameters such as cut-off values, fibrosis severity, number of patients, age, sex, and muscle mass of patients. Thus, the use of noninvasive liver function tests in the determination of liver fibrosis is controversial because of the absence of gold-standard studies and the presence of moderate accuracy results with these markers in patients with intermediate stages of fibrosis (20,37). In clinical practice, the determination of fibrosis stage does not need to be exact as a pathological scoring system; the absolute stage is less important than determining whether patients have mild or advanced liver disease (18). Therefore, these tests may be applied to determine whether the patients have severe fibrosis and cirrhosis and to identify specific treatment courses and potential complications in this respect. When the high NPV (80%–91%) values according to the modified Ishak and Knodell classification of APRI, FIB-4, King's scores, and GUCI tests were taken into account, they were observed to be effective in identifying patients without significant/severe fibrosis in our study; this indicated that less aggressive approaches could be exhibited with regard to follow-up and treatment in these patients. Liver biopsy should be re-performed in suspected cases and in situations that require precise biopsy; liver biopsy and noninvasive methods should be used as an integrated system to enable a more efficient evaluation of patients with chronic hepatitis C (38).

These are several strengths of the present study: the inclusion of many patients, who formed a homogeneous group that did not include treatment-experienced patients with chronic hepatitis C; the comparison among nine different fibrosis tests; and the assessment of the increase in accuracy with two different histopathological correlations (Ishak and Knodell scoring systems). The most important limitation of our study, is the small number of patients with significant/severe fibrosis, which is believed to have led to the low PPV and sensitivity similar to some previous studies. Better results could be obtained using a combination of indirect serum markers, which we studied, and direct markers showing deposition or removal of extracellular matrix in the liver or liver elasticity-based imaging techniques. Because these tests are expensive and have lower applicability, their feasibility in clinical practice may be difficult. However, APRI, FIB-4, King's scores, and GUCI tests that are cheap, easy to calculate, and readily available were shown to be useful in clinical practice.

Our results indicate that high NPV of APRI, FIB-4, GUCI, and King's scores enable us to identify patients with mild fibrosis, providing prognostic data without the need for a liver biopsy. On the other hand, sensitivity and PPV were found to be low, probably due to the small number of patients with significant/severe fibrosis in our study. Therefore, we suggest further studies that utilize serum markers with higher sensitivity and PPV to combine with our results for obtaining a marker panel that has both high PPV and NPV for evaluating liver fibrosis.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Türkiye Yüksek İhtisas Research and Training Hospital.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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