Diagnostic value of combined serum biomarkers for the evaluation of liver fibrosis in chronic hepatitis C infection: A multicenter, noninterventional, observational study

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ABSTRACT

Background/Aims: *The hepatitis C virus (HCV) infection is important cause of chronic hepatitis. Liver biopsy is considered the gold standard for assessment of fibrosis but this procedure is an invasive procedure. We aimed to evaluate the diagnostic efficiency of non-invasive serum biomarkers, separately and in combinations, on liver fibrosis in treatment-naive chronic hepatitis C (CHC) patients.*
Materials and Methods: *Two hundred and sixteen treatment-naive CHC patients were enrolled from 32 locations across Turkey in this open-labelled, non-interventional prospective observational study. FibroTest®, aspartate aminotransferase-to-platelet ratio index(A-PRI), aspartate aminotransferase and alanine aminotransferase ratio (AAR), fibrosis index based on four factors (FIB-4), Age-plate-let(AP) index and Forns index were measured and compared with Metavir scores got from liver biopsies.*
Results: *Data from 182 patients with baseline liver biopsy were suitable for analysis. One hundred and twenty patients (65.9%) had F0-F1 fibrosis and 62 patients (34.1%) had F2-F4 fibrosis. APRI 0.732 area under the curve(AUC) indicated advanced fibrosis with 69% sensitivity and 77% specificity. FIB-4 0.732 AUC and FibroTest 0.715 AUC indicated advanced fibrosis with 69% and 78.4% sensitivity, and 75% and 71.4% specificity, respectively. The combined use of tests also led to an increase in AUC and specificity. Combinations of FibroTest with APRI and/or FIB-4, and FIB-4 with APRI were optimal for the evaluation of liver fibrosis.*
Conclusion: *Fibrotest, FIB-4, APRI, AP index and Forns index exhibit good diagnostic performance for determining liver fibrosis in CHC patients, and the use of at least two tests together will increase their diagnostic value still further.*
Keywords: *Chronic hepatitis C infection, liver fibrosis, FibroTest, APRI, Forns index, Fib-4, non-invasive serum biomarkers*

INTRODUCTION

Hepatitis C virus (HCV) infection is a disease that affects approximately 2.8% of the world population and 1% of the Turkish population (1,2). The determination of hepatic fibrosis plays a significant role in treatment decisions and follow-up of chronic hepatitis C (CHC) patients (3). Liver

biopsy is the gold standard in the determination of hepatic fibrosis; however, it is an invasive procedure, can result in serious complications, cannot be performed on every patient, and is difficult to repeat. Sampling errors, as well as differences of interpretation among pathologists, may occur (4-6). Currently, there are several noninvasive methods, such as serum biomarkers and imaging methods, for the assessment of liver fibrosis in CHC infection (7). Ultrasonography, magnetic resonance imaging, computed tomography, and transient elastography (TE-FibroScan) are examples of imaging methods. TE is the most recommended diagnostic imaging method that measures liver stiffness; however, it is too expensive and is used only in a few hospitals in Turkey. Even more, the reimbursement agency does not pay the TE fee in Turkey. However, serum biomarkers have numerous advantages, including the absence of risks, objective interpretation, patient acceptance, cost effectiveness, and the fact that they are easily applicable and repeatable tests (8). These biomarkers can be direct (collagen, hyaluronic acid, laminin, and YKL-40) and indirect [FibroTest (FT), ActiTest (AT), aspartate aminotransferase and alanine aminotransferase ratio (AAR), aspartate aminotransferase-to-platelet ratio index (APRI), four factors (FIB-4), age-platelet (AP) index, and Forns index]. Direct biomarkers are liver matrix components, the routine clinical use of which is limited. In contrast, indirect biomarkers are easy to perform serum-based tests, which include biochemical parameters routinely measured in the peripheral blood of CHC patients (9-12). These serum biomarkers have successfully identified hepatic fibrosis in CHC patients, but noninvasive serum biomarkers have some limitations, such as low accuracy of discrimination between intermediate stages of fibrosis. The diagnostic performance of serum biomarkers can be increased when these are used in combination in the determination of hepatic fibrosis (3).

In this study, the diagnostic performances of serum biomarkers such as FT, APRI, AAR, FIB-4, the AP index, and the Forns index were analyzed separately and in combination in treatment-naïve CHC patients. The results were compared with those from liver biopsy in terms of predicting fibrosis.

MATERIALS AND METHODS

In this noninterventional, observational study, 216 treatment-naïve CHC patients aged between 18 and 65 years from 32 locations in Turkey were included. The study patients were anti-HCV positive for at least 6 months and had detectable levels of HCV-RNA by PCR for CHC. The patient recruitment began on November 1, 2008, and

it continued for 24 months after approval of the ethics committee. Informed consent was obtained from all patients before starting any procedure.

Patients co-infected with HIV or hepatitis B virus, patients with suspected hepatocellular carcinoma or acquired/inherited liver disease, patients with a serious concomitant disease (renal failure, hypertension, DM, etc.), patients using immunosuppressive drugs during the previous 6 months, patients with abnormal hematological parameters that contraindicated liver biopsy (prolonged prothrombin time, low platelet count, etc.), and patients who used alcohol regardless of duration and who abused drugs were excluded from the study. Demographic, clinical, histological, and laboratory data were recorded.

Percutaneous liver biopsy was performed using a disposable Hepafix suction needle (B. Braun, Melsungen, Germany). The same pathologist examined all liver biopsy samples. The stage of fibrosis was determined using the METAVIR scoring system (13). Two groups were established based on fibrosis scores: Group 1, F0-F1 (no fibrosis) and Group 2, F2-F4 (significant/advanced fibrosis). Blood samples for serum biomarkers were collected simultaneously with the liver biopsy procedure and stored at −80°C until analysis.

Table 1. Noninvasive serum biomarker methods

Serum Biomarkers	Methods
Fibrotest® (FT)	(Biopredictive, Paris, France) Patented formula combining α-2-macroglobulin, γGT, apolipoprotein A1, haptoglobin, total bilirubin, age, and gender
Forns Index	$7.811 - 3.131 \times$ (platelet count) $+ 0.781 \times$ (GGT) $+ 3.467 \times$ (age) $- 0.014 \times$ (cholesterol)
AST to Platelet Ratio (APRI)	AST (Upper Limit of Normal) (IU/L)/ platelet (10^9/L) $\times 100$
FIB-4	[Age (year) $\times$ AST (IU/L)]/[platelet (10^9/L) $\times \sqrt{}$ ALT (IU/L)]
Age-platelet (AP) index	Age (year) (<30=0; 30-39=1; 40-49=2; 50-59=3; 60-69=4; ≥70=5) + platelet (10^9/L) (≥225=0; 200-224=1; 175-199=2; 150-174=3; 125-149=4; <125=5)
AST/ALT ratio (AAR)	AST (IU/L)/ALT (IU/L)

APRI: aspartate aminotransferase-to-platelet ratio index; FIB-4: fibrosis index based on four factors; AST: aspartate aminotransferase; ALT: alanine aminotransferase

Noninvasive serum biomarkers, FT, APRI, AAR, FIB-4, the AP index, and the Forns index were calculated for all patients using laboratory data based on a single center with the formulas shown in Table 1 (8,9,11,14-20).

The following combinations were calculated simultaneously: FibroTest+APRI, FibroTest+APRI+FIB-4, FibroTest+FIB-4, FibroTest+AP index, FibroTest+Forns index, FibroTest+AAR, APRI+FIB-4, APRI+AP index, APRI+AAR, APRI+Forns index, FIB-4+AP index, FIB-4+AAR, FIB-4+ Forns index, AP index+AAR, AP index+-Forns index, and AAR+Forns index.

Statistical analysis

Descriptive statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) 13.0 software (SPSS Inc.; Chicago, IL, USA). Data were expressed as mean±standard deviation (SD), percentage (%), or median (min-max), as appropriate. p-values of <0.05 were considered statistically significant. Categorical data were analyzed using the Chi-squared and Fisher's tests. Data obtained in measurements of normal distribution were analyzed using the Kolmogorov-Smirnov test. Data conforming to normal distribution were analyzed using the Student's-t test, and non-conforming data were analyzed using the Mann-Whitney U test. Correlation analysis was performed using the Spearman's correlation test. The receiver operating charac-

teristic (ROC) analysis was performed to calculate the sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of the noninvasive serum biomarkers for the prediction of hepatic fibrosis.

RESULTS

Of the 216 patients recorded in this study, 182 (84.3%) were eligible for assessment. Thirty-four patients were excluded from the analysis due to insufficient liver biopsy samples, absence of one or more laboratory parameters, or missing data entry.

The mean (SD) age was 49.9±11.4 years, and 59.9% of the 182 patients were female. One hundred and twenty (65.9%) patients were classified as having METAVIR fibrosis stage F0-F1 (no fibrosis; Group 1) and 62 (34.1%) patients as having F2-F4 (significant/advanced fibrosis; Group 2). Patients with higher fibrosis scores were observed to have a higher average age, indicating that advanced age was correlated with higher fibrosis (p=0.003). No statistical significance was observed between gender and fibrosis. The patients with advanced fibrosis had higher mean ALT and AST values (p<0.005). Although platelet counts were lower in patients with advanced fibrosis, no statistically significant difference was observed compared with patients with no fibrosis (p=0.053). There was a significant difference in terms of APRI, FIB-4, the

Table 2. Demographic features and laboratory parameters of the 182 patients

Serum Biomarkers Methods				
Mean age		48.4±12.2	53.1±9.0	0.003
Gender, n (M/F)		45/75	30/32	0.193
Mean AST, U/L		43.2±32.5	65.7±48.2	0.000
Mean ALT, U/L		60.2±55.6	77.5±62.6	0.004
Mean platelet count (×10³/microL)		235.4±57.7	215.9±71.0	0.053
Protein		7.6±0.5	7.5±0.5	0.327
Albumin		4.3±0.6	4.3±0.5	0.654
APRI		0.51±0.5	0.95±1.2	0.000
AAR		0.85±0.3	1.03±0.6	0.060
FIB-4		1.30±0.9	2.35±2.1	0.000
AP index		4.59±1.7	5.41±1.7	0.002
Forns index		4.37±1.6	5.28±2.0	0.034
	F0	31	9	
FibroTest	F1-F2	40	20	0.003
	F3	15	9	
	F4	5	13	

AST: aspartate aminotransferase; ALT: alanine aminotransferase; APRI: aspartate aminotransferase-to-platelet ratio index; AAR: AST/ALT ratio; FIB-4: fibrosis index based on four factors; AP index: age-platelet index

Table 3. Sensitivity, specificity, and predictive values of serum biomarkers either alone or combined

	Cutoff	p	AUC	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
APRI	>0.5	0.0001	0.732	69.0	77.0	81.1	63.5
AAR	>1.0	0.059	0.589	37.3	79.8	69.2	51.2
FIB-4	>1.5	0.0001	0.732	69.0	75.0	80.6	61.5
AP index	≥6	0.002	0.639	54.2	70.7	75.2	48.5
Forns index	>5	0.045	0.629	56.7	68.3	75.9	47.2
Fibrotest	≥F2	0.0001	0.715	78.4	71.4	85.5	60.6
Fibrotest + APRI	≥F2 >0.5	0.0001	0.729	56.4	89.5	81.0	72.1
Fibrotest + APRI + FIB-4	≥F3 >0.5 >1.5	0.0001	0.724	54.9	89.9	79.5	73.7
Fibrotest + FIB-4	≥F2 >1.5	0.0001	0.719	62.5	81.3	81.2	62.5
Fibrotest + AP index	≥F2 >6	0.018	0.616	43.1	80.0	74.3	51.2
Fibrotest + Forns index	≥F2 >5	0.132	0.597	37.9	81.4	76.0	45.8
Fibrotest + AAR	≥F2 >1.0	0.148	0.571	26.4	87.9	69.0	53.8
APRI + FIB-4	>0.5 >1.5	0.0001	0.740	62.1	86.0	79.6	72.0
APRI + AP index	>0.5 >6	0.0001	0.689	47.5	90.3	76.7	71.8
APRI + AAR	>0.5 >1.0	0.0094	0.621	27.1	97.1	69.9	84.2
APRI + Forns index	>0.5 >5	0.0364	0.599	25.9	94.0	68.6	71.4
FIB-4 + AP index	>1.5 >6	0.0001	0.687	54.2	83.2	77.7	62.7
FIB-4 + AAR	>1.5 >1.0	0.0119	0.617	32.2	91.3	70.1	67.9
FIB-4 + Forns index	>1.5 >5	0.0055	0.658	44.4	87.2	77.3	61.5
AP index + AAR	>6 >1.0	0.0317	0.599	28.3	91.4	71.1	63.0
AP index + Forns index	>6 >5	0.0446	0.606	34.9	86.3	75.9	51.7
AAR + Forns index	>1.0 >5	0.238	0.570	22.2	91.8	70.5	57.1

NPV: negative predictive value; PPV: positive predictive value; APRI: aspartate aminotransferase-to-platelet ratio index; AAR: AST/ALT ratio; FIB-4: fibrosis index based on four factors; AP index: age-platelet index

AP index, and the Forns index between the two groups (p<0.05). This finding shows that APRI, FIB-4, the AP index, and the Forns index are important serum biomarkers in the determination of liver fibrosis (Table 2). AAR was not significant in the determination of the stage of fibrosis. The correlation between the METAVIR fibrosis scores and FibroTest results (r=0.321; p<0.001) increased with the stage of fibrosis.

METAVIR fibrosis scores and FibroTest (r=0.321; p<0.001), APRI (r=0.302; p<0.001), the Forns index (r=0.339; p=0.001), and the AP index (r=0.254; p=0.001) results were also positively correlated.

The ROC analysis of noninvasive tests revealed that the area under the curve (AUC) exceeded the reference line in the majority of tests (Figure 1). The APRI 0.732 AUC indicated advanced fibrosis with 69% sensitivity and 77% specificity. The FIB-4 0.732 AUC and FibroTest 0.715 AUC indicated advanced fibrosis with 69% and 78.4% sensitivity and 75% and 71.4% specificity, respectively. The combined use of tests also led to an increase in AUC and specificity. A combination of FibroTest with APRI and/or FIB-4 and of APRI with FIB-4 was identified to be optimal for the assessment of fibrosis (Table 3). The error bar graph analysis of noninvasive tests showed that FibroTest, APRI, and FIB-4 could be used to differentiate patients with advanced fibrosis from patients with minimal fibrosis (Figure 2-7).

DISCUSSION
Liver biopsy is the gold standard method for the determination of hepatic fibrosis, but it has many disadvantages, such as side effects, patient reluctance, inadequate sample, evaluation errors, and high cost (21,22). These limitations have led to the development of noninvasive alternative methods for the evaluation of liver fibrosis. These methods can be summarized as serum biomarkers and imaging methods for the assessment of liver fibrosis in CHC infection (7). TE is the most recommended diagnostic imaging method that measures liver stiffness, as mentioned in the introductory section. In many studies comparing the performance of TE and serum biomarkers mostly in viral hepatitis, it has been found that TE and serum biomarkers have equal performance for detecting significant fibrosis (20). Although the combination of TE and serum biomarkers is more effective than the combination of two serum biomarkers for a significant fibrosis finding, it is more expensive when compared with serum biomarkers (20). However, serum biomarkers have emerged as a leading methodology, as these tests are repeatable, noninvasive, and cost-effective. The costs of
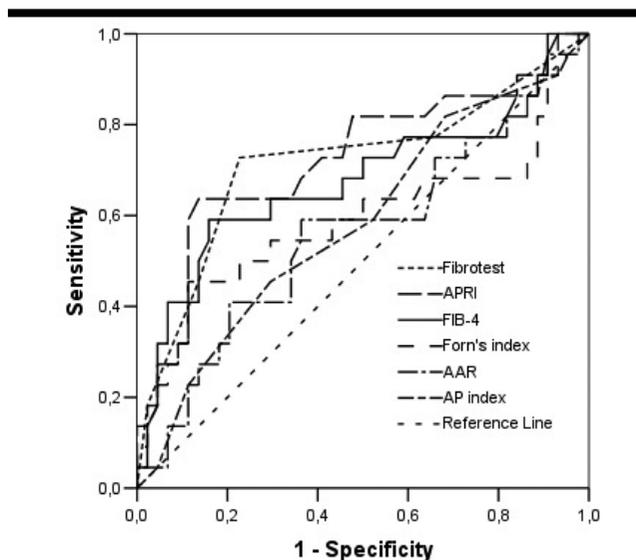

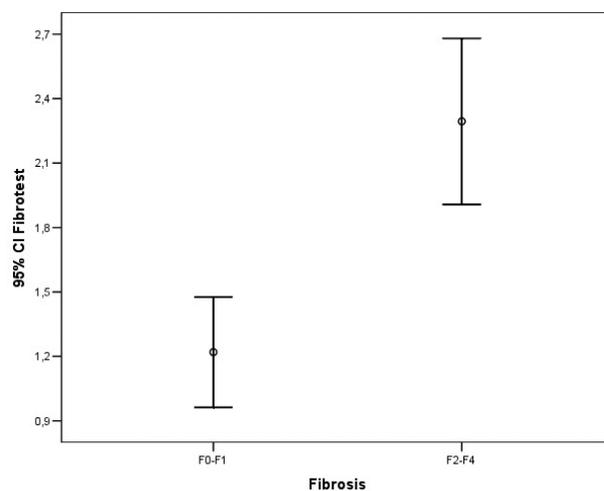**Figure 1.** ROC curve analysis of noninvasive tests for diagnosis of liver fibrosis

Figure 2. Error bar graphic images of FibroTest for diagnosis of liver fibrosis

these tests vary, FibroTest being the most expensive. The others can be easily calculated using equations involving the patient's routine biochemical tests. Serum biomarkers are recommended by the American Association for the Study of Liver Diseases, the European Association for the Study of the Liver, and the World Health Organization for determining stage of fibrosis in patients with HCV infection in the light of these advantages (23-25). However, these tests may exhibit low accuracy in differentiating intermediate or low stages of fibrosis from increased stages of fibrosis. In addition, interpreting the results may be difficult since
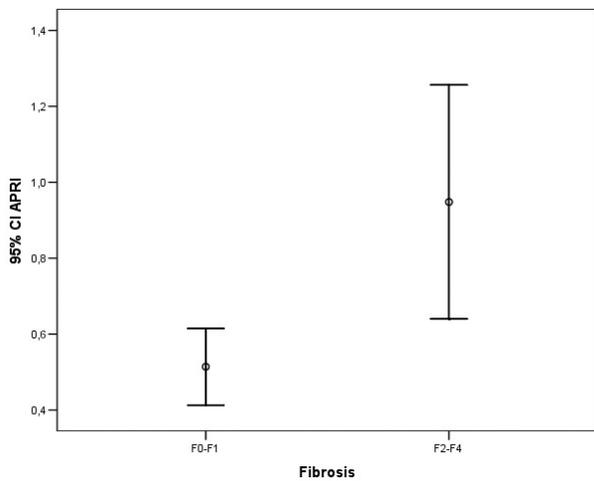
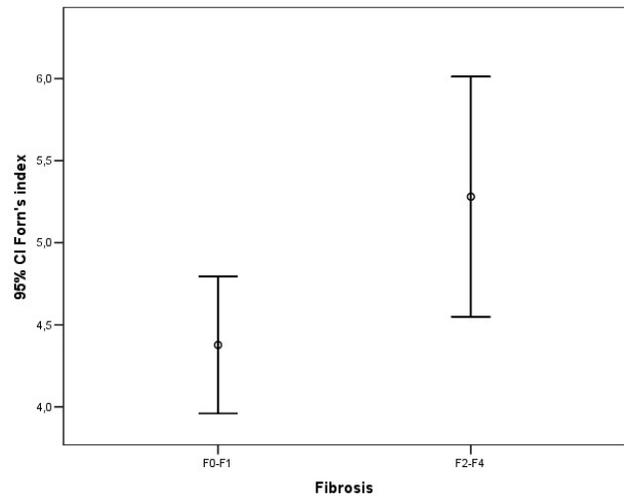**Figure 3.** Error bar graphic images of APRI for diagnosis of liver fibrosis

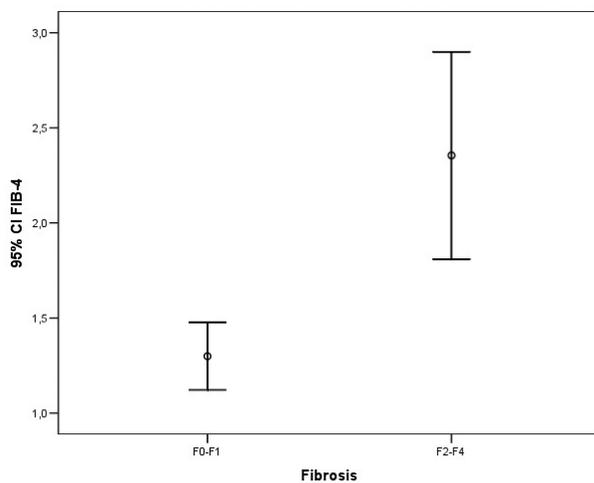**Figure 5.** Error bar graphic images of the Forns index for diagnosis of liver fibrosis

Figure 4. Error bar graphic images of FIB-4 for diagnosis of liver fibrosis

Figure 6. Error bar graphic images of the AP index for diagnosis of liver fibrosis

various stages of fibrosis have different cutoff values. The validation of serum biomarkers for evaluating fibrosis is of critical importance for the routine clinical use of these tests. When applied individually in cases of significant fibrosis, the diagnostic value of these tests does not exceed 80%. For this reason, considering more than one serum biomarker can increase the susceptibility of the tests.

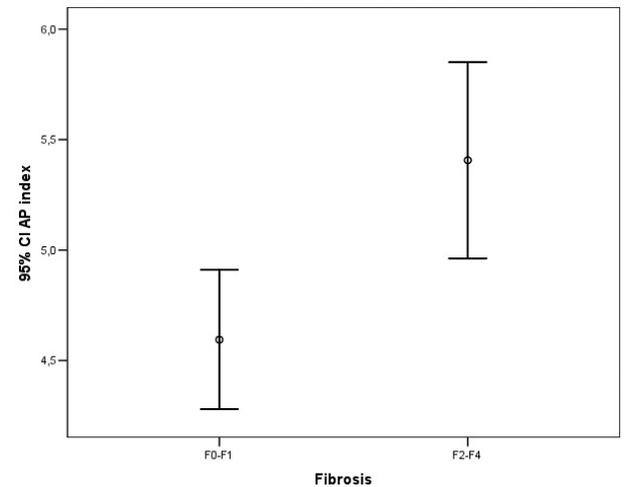The data collected from the 182 enrolled patients clearly showed that fibrosis scores increase with age (p=0.003). This may be attributed to infection with HCV at an early age that remained untreated for many years due to a lack of patient awareness. The advanced age may thus have affected the efficacy of noninvasive tests, particularly FIB-4, the AP index, and the Forns index, in the determination of fibrosis (Table 2). Significant differences were observed in the APRI, FIB-4, AP index, and Forns index values between the fibrosis groups (p<0.05), indicating that APRI, FIB-4, the AP index, and the Forns index are important serum biomarkers for the determination of significant fibrosis. A positive correlation was observed between APRI, FIB-4, the AP index, and the Forns index
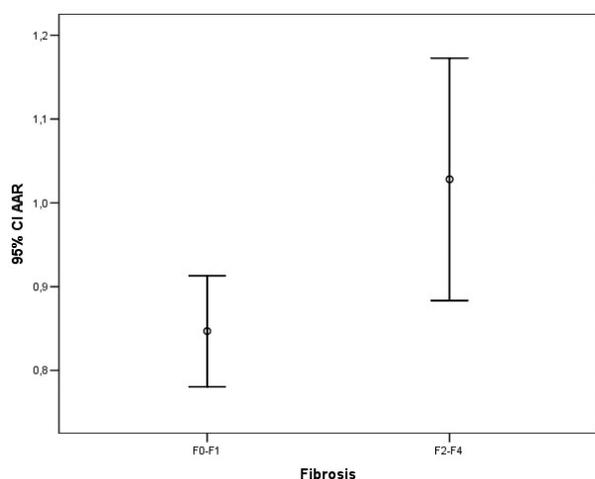
Figure 7. Error bar graphic images of AAR for diagnosis of liver fibrosis

and the METAVIR fibrosis scores. There was also a positive correlation between FibroTest and fibrosis. Sensitivity analysis showed that the FibroTest demonstrated the highest sensitivity, 78.4%, followed by APRI, FIB-4, the Forns index, and the AP index. AAR exhibited the lowest sensitivity, although no statistically significant difference was observed between the groups. The specificity values of all tests were close to one another. NPV was higher than PPV in all tests in this study. The AUC values were similar, the highest values being observed in the APRI, FIB-4, and FibroTest tests. The AUC, sensitivity, specificity, NPV, and PPV from noninvasive tests were similar to or lower than those reported in the original publications for those tests (9,17,26-31). Wai et al. (9) reported that APRI PPV for the determination of significant fibrosis was higher than NPV. AUC was reported at 0.80. However, the opposite was observed in the present study, in which NPV was higher than PPV. This difference may be attributable to the use of the Ishak score by Wai et al. (9) for fibrosis scoring, as well as the greater number of patients with cirrhosis and advanced fibrosis in that study. In the meta-analysis by Lin et al. (11), for the prediction of significant fibrosis, the estimated PPV and NPV of the 0.5 cutoffs were 55% and 69%, respectively. At the 1.5 cutoff, the estimated PPV and NPV were 82% and 63%, respectively. At the 0.7 cutoff, the estimated PPV and NPV were 70% and 79%, respectively. PPV and NPV of APRI for the prediction of significant fibrosis differ at various thresholds. In this meta-analysis, the best cutoff for diagnosing significant fibrosis was 0.7, whereas for diagnosing cirrhosis it was 1. In our study, at the 0.5 cutoff, the estimated PPV and

NPV of APRI were 63.5% and 81.1%, respectively. In other studies, for investigating the diagnostic performance of APRI, different results have been reported for the estimated significant fibrosis and cirrhosis (28-30). In the study of Leroy et al. (32), when >0.5 cutoff was used for APRI in the diagnosis of significant fibrosis (F2-F4), the specificity was found to be only 26.8%; however, when >1.5 cutoff was used, the specificity was found to be 87.8%. At the same time, the concomitant presence of FibroTest>0.59 and APRI>2 improved PPV for significant fibrosis to 96.7% and for extensive fibrosis to 92.2%. The fact that our results are different from study results of Leroy et al. (32) may be due to the low number of patients with advanced fibrosis. Our study concluded that APRI had better performance for mild fibrosis.

While the FIB-4 AUC and sensitivity values in this study were parallel to those reported by Sterling et al. (27), the NPV value was higher. However, it should be noted that Sterling et al. (27) investigated patients co-infected with HCV/HIV and used the Ishak score for fibrosis scoring. In a study by Vallet-Pichard et al. (26), the FIB-4 index enabled the correct identification of patients with severe fibrosis (METAVIR F3-F4) with a 0.85 AUC. An FIB-4 index lower than 1.45 had an NPV of 94.7% to exclude any extensive fibrosis (F3-F4) with a sensitivity of 74.3% and a specificity of 80.1%. A higher FIB-4 index (>3.25) was recommended to confirm the existence of significant fibrosis. In our study, an FIB-4 index higher than 1.5 had PPV and NPV of 61.5% and 80.6%, respectively, with a sensitivity of 69% and a specificity of 80.1%.

Noninvasive tests exhibited improved diagnostic performance in patients with advanced fibrosis (17,29,31). The poor performance of noninvasive tests in this research compared with that of the original studies may be attributed to the fact that only 34% of patients had serious fibrosis and that the number of patients with cirrhosis was low. Recently, several studies have shown that the diagnostic performance of serum biomarker tests increases significantly when these are used in combination (33-36). Mummadi et al. (10) showed that there was a significant correlation between change in the stage of fibrosis and change in APRI and FIB-4. In this study, APRI had a positive predictive value (PPV) of 67% and a negative predictive value of 67% (NPV) for progression of fibrosis. As for FIB-4, PPV and NPV were 75%. The results of our study are similar, with a specificity of >80% being observed when the tests were used in combination. The combination of FibroTest with APRI and/or FIB-4 and APRI with FIB-4 exhibited good diagnostic performance

for determining significant fibrosis. The FibroTest is more expensive than other serum biomarkers, and the use of APRI or FIB-4 is therefore recommended for the detection of hepatic fibrosis, particularly when resources are limited (25). However, none of these tests are of equal value in the assessment of both fibrosis and cirrhosis. For instance, APRI has been successfully validated for the diagnosis of both significant fibrosis and cirrhosis, whereas FIB-4 is useful only for the diagnosis of significant fibrosis (METAVIR stage≥F2) (25). One of the greatest advantages of combining tests is the resulting decrease in diagnostic differences. This decrease in diagnostic differences with the use of serum biomarker tests is clearly demonstrated in the present study. Our results show that the specificity of tests increases when these are used together.

In conclusion, there is a consistent and growing need for easily reproducible tests for the effective diagnosis of advanced hepatic fibrosis. Studies comparing the efficacies of liver biopsy and noninvasive tests provide guidance for the development and introduction of new techniques. This study concludes that APRI, FIB-4, AP index, Forns index, and FibroTest, as well as the combined use of these tests, can help identify CHC patients with significant/advanced fibrosis. However, none of the parameters according to cutoff points have a good diagnostic performance. The concurrent use of at least two of these tests will be useful for the evaluation and follow-up of CHC patients with fibrosis. In particular, the use of FIB-4 and APRI together may be indicative of significant/advanced fibrosis.

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