ORIGINAL ARTICLE

Validation of Three Non-Invasive Markers in Assessing the Severity of Liver Fibrosis in Chronic Hepatitis C

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ABSTRACT

Objective: To compare various biochemical markers i.e. APRI (AST to platelet ratio index), aspartate aminotransferase (AST) alanine aminotransferase (ALT) ratio, FIB-4 (AST, platelet, AST and age) with biopsy for assessing the severity of hepatic fibrosis in patients with hepatitis C.

Study Design: Descriptive study.

Place and Duration of Study: Medical Department, Liaquat University of Medical and Health Sciences, Jamshoro, from July 2005 to March 2007.

Methodology: Consecutive hepatitis C virus RNA positive and previously untreated patients were studied. Liver biopsy with histological evaluation and AST/ALT ratio, AST to platelet ratio index and FIB-4 were assessed in all patients. Receiver operative curves were developed.

Results: There were 158 patients (109 males, 49 females). On histological grounds non-advanced fibrosis (F0-1) was present in 74 (46.5%) of cases, whereas 84 (53.5%) patients had advanced (F2-4) fibrosis. The area under the receiver operating characteristic curves of APRI < 0.05-1 and FIB-4 < 1.45 were 0.7 and 0.74 respectively, which means that APRI < 1 and FIB-4 < 1.45 will exclude advanced fibrosis in 70% and 74% of patients respectively. An APRI of > 1 and FIB-4 will predict advanced fibrosis in 87% and 98% of patients respectively. AST/ALT ratio was inferior to both of these biomarkers.

Conclusion: Both APRI and FIB-4 not only exclude minimal fibrosis but can predict advanced fibrosis in the majority of the patients. The simultaneous use of several indirect markers of liver fibrosis does not improve their diagnostic accuracy.

Key words: *Hepatitis C. Biomarkers. Liver fibrosis. Alanine aminotransferase. Aspartate aminotransferase.*

INTRODUCTION

According to recent epidemiological data, Chronic Hepatitis C (CHC) remains a major health problem with around 170 million individuals affected worldwide.[1] Although, numerous randomized-controlled trials demonstrate that antiviral treatment results in higher sustained viral responses than placebo or older antiviral therapies, limited data exist regarding the long-term benefit from viral eradication or antiviral treatment.[2] Histologic examination of the liver is an integral part of the evaluation of patients with Chronic Hepatitis C (CHC) as the knowledge of the stage of liver fibrosis is essential for prognostication and decisions on antiviral treatment.[3] CHC patients with no or minimal fibrosis at presentation appear to progress slowly and treatment possibly could be delayed or withheld to prevent cirrhosis.[4] On the other hand, patients with significant fibrosis (i.e., septal or bridging fibrosis) progress almost invariably to cirrhosis over a 10-20-year period so antiviral treatment should be strongly considered.[5]

In CHC, liver biopsy represents the Gold standard for evaluating presence, type and stage of liver fibrosis and to characterize necroinflammation. However, it is an invasive test with the potential for serious, albeit rare, complications requiring hospital admission or prolonged hospital stay, apart from possible sampling error and interobserver variability.[6] Because of this, a marker that could be used as a surrogate and reduce the number of liver biopsies would be of a great benefit. Such a marker could also be useful in patients who do not initially receive treatment for reasons such as mild fibrosis, comorbidity, or social/psychiatric issues, and for those who fail treatment.

Although a simple blood test would be ideal, it is more likely that a combination of blood tests (such as an index) will be needed to correctly estimate the presence or absence of significant fibrosis. Many of these non-invasive markers have been described and tested in patients with CHC, some being more simple and economical, such as AST-to-platelet ratio (APRI), FIB-4 and AST (aspartate aminotransferase)-to-alanine aminotransferase (ALT) ratio (AAR) and others, such as Fibro test, being more sophisticated.[7]

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AST-to-platelet ratio (APRI) is a simple index, made up of easily available routine laboratory tests. With worsening fibrosis and increasing portal pressures, there is reduced thrombopoietin production and increased platelet sequestration in the spleen.[8] Advancing liver fibrosis is also associated with reduced clearance of AST.[9] The APRI test can thus potentially differentiate between those with and without significant fibrosis or cirrhosis appearing most useful for excluding significant fibrosis in HCV.[10] AST-to-ALT ratio (AAR), which is the ratio of AST to ALT, tends to increase with advancing stages of fibrosis from the level of approximately 0.8 in normal subjects. A value of greater than 1 suggests the diagnosis of cirrhosis. The FIB-4 combines biochemical variables (platelet count, AST, and ALT) with age. It had reasonably good accuracy for predicting advanced fibrosis in patients with chronic HCV.[11]

The aim of this study was to compare these biochemical markers such as APRI, AST, ALT ratio and FIB-4 with the histological staging of the patients with hepatitis C.

METHODOLOGY

This descriptive study included consecutive anti-HCV and HCV RNA positive, untreated patients with chronic hepatitis C, inducted between July 2005 and March 2007, at the Department of Medicine, Liaquat University of Medical and Health Sciences, Hyderabad. Liver biopsy was done in all patients. A written consent for biopsy was taken from the patients before the biopsy. Clinical data were collected at the time of liver biopsy, and blood samples for LFT, prothrombin time, protein profile and blood glucose and blood complete picture with platelet count were collected before the biopsy. Criteria for exclusion from the study were co-infection with the hepatitis B virus, regular alcohol intake, previous interferon treatment, and clinical or radiological evidence of cirrhosis (gastroesophageal varices, ascites, and hepatic encephalopathy). The upper limits of normal (ULN) of alanine aminotransferase (ALT) was taken as 41 U/L for men and 31 U/L for women. For AST, the ULN was 38 U/L for men and 32 U/L for women.

Liver biopsy was performed under local anaesthesia by a trained person. A core biopsy needle (14-gauge) was used and the procedure was conducted under ultrasound guidance. An adequate biopsy sample, defined as specimen size greater than 10 mm and more than 5 portal tracts was obtained in all patients.[12] No major complications such as requirement of blood transfusion, hypotension or biliary peritonitis were observed. A single qualified histopathologist, who was unaware about the clinical data, assessed the biopsy slides. Fibrosis stage was determined according to the METAVIR group scoring system and was classified as F0 = no fibrosis; F1 = portal fibrosis without septa; F2= few septa; F3 = numerous septa without cirrhosis; or F4 = cirrhosis.[13] Non-advance fibrosis was defined as the presence of F0 and F1 and advanced fibrosis comprises F2 to F4 according to Metavir Cooperative Study.[13]

Patients were divided into two groups–one with AST/ALT ratio < 1 and the other with AST/ALT ratio > 1.[14] The APRI was determined as AST level (UNL)/ platelets counts (10^9/L) x 100.[10] Patients were divided into APRI <0.5 to 1 and other group with APRI > 1. FIB-4 was calculated as age (years) x AST (U/L)/platelet count (10^9/L) x (ALT (U/L)) 1/2.17. Patients with value of FIB-4 upto 1.45 were separated from those with FIB-4 > 1.45.

Continuous variables such as age, platelet count, ALT (SGPT), AST (SGOT) and bilirubin were expressed as mean with standard deviation, and categorical variables such as APRI < 0.5-1, APRI > 1, FIB-4 < 1.45, FIB-4 > 1.45, AST/ALT ratio < 1, AST/ALT ratio > 1, unadvanced fibrosis (F0 + F1) and advanced fibrosis (F2, F3, F4) as count with percentage. The predictive accuracy of APRI, FIB-4 and AST/ALT ratio was tested by measuring the areas under curves receiver operating characteristic (AUCROC). Based on the ROC, the best cutoff points to predict the absence or presence of significant fibrosis was chosen. Diagnostic accuracy was evaluated by calculating the sensitivity, specificity, positive and negative predictive values (PPV and NPV respectively). Statistical analysis was performed by SPSS software version 16.0. ROC curve comparison was performed which uses calculation of the area under the curve and 95% confidence intervals (CIs) by the technique described by Hanley and McNeil.[15] The main endpoint was discriminating patients with advanced fibrosis (F2, F3, F4) from those with non-advanced fibrosis (F0, F1) using a combination of relevant biochemical variables. P-value of 0.05 or less was considered statistically significant.

RESULTS

In this descriptive study, 158 patients were enrolled consisting of 109 (69%) males and 49 (31%) females. Mean age of the patients were 36.7 $\pm$ 10 years. Patients with Diabetes mellitus were 26 (17%). Mean BMI was 26.1$\pm$4.2, mean platelet count was 368$\pm$82.2 10^9/L, mean ALT (SGPT) was 77$\pm$60.8 IU/L, mean ALT (SGOT) was 56$\pm$56.8 IU/L, mean AST/platelet ratio was 0.8$\pm$0.436, mean AST/ALT ratio was 0.8$\pm$0.43 and mean FIB-4 value was 1.4$\pm$0.63. Non-advanced fibrosis was present in 74 (46.5%) cases, which comprised no fibrosis (FO) in 24 (15%) and mild fibrosis (F1) in 50 (31.5%) cases. Advanced fibrosis was present in 84 (63.5%) cases which included moderate fibrosis (F2) in 50 (31.5%), bridging fibrosis (F3) in 28 (18%) and cirrhosis (F4) in 6 (4%) cases. With APRI from < 0.5 to

Table I: Baseline characteristics of patients.

Quantitative variables	Mean	± STD deviation	95% Confidence interval of the difference
Age (years)	36.7025	10.03212	35.1261-38.2790
BMI	26.1329	4.20639	25.4719-26.7939
INR	1.0326	0.06842	1.0218-1.0433
Platelet count 10^9/L	2.3682E2	82.22091	223.8965-249.7365
SGPT IU/L	77.7563	60.86619	68.1920-87.3207
SGOT IU/L	56.0570	38.84974	49.9522-62.1617
BILIRUBIN mg/dL	0.9717	0.66161	0.8677-1.0757
Biochemical markers quantitative variables	Non-advanced fibrosis (F0+F1) Frequency (%)	Advanced fibrosis (F2+F3+F4) Frequency (%)	
APRI < 0.5-1	70/158 (44.3%)	63/158 (40%)	
APRI > 1	4/158 (02.5%)	21/158 (13.2%)	
FIB-4 < 1.45	59/158 (37.3%)	43/158 (27.2%)	
FIB-4 > 1.45	15/158 (09.4%)	41/158 (26%)	
AST/ALT Ratio < 1	61/158 (38.6%)	59/158 (37.3%)	
AST/ALT Ratio > 1	13/158 (08.2%)	25/158 (16%)	

ALT = alanine aminotransferase; AST = aspartate aminotransferase; APRI = AST to Platelet ratio index; FIB-4 = Age, aspartate aminotransferase, platelet count, alanine aminotransferase; AST/ALT ratio = aspartate aminotransferase-alanine aminotransferase; > = more than; < = less than.

1, 70 patients showed non-advanced fibrosis whereas 63 showed advanced fibrosis. In group of APRI > 1, 4 patients had non-advanced fibrosis and 21 had advanced fibrosis. In FIB-4 < 1.45, 59 patients had non-advanced fibrosis and 43 had advanced fibrosis. With FIB-4 > 1.45, 15 patients showed non-advanced fibrosis and 41 had advanced fibrosis. In AST/ALT ratio < 1, 61 patients had non-advanced fibrosis and 59 patients advanced fibrosis. With AST/ALT ratio > 1, 13 showed non-advanced fibrosis and 25 had advanced fibrosis. Table I shows the baseline characteristics of the patients studied.

AUCROC (area under curve receiver operating characteristic) of APRI < 0.5-1 was 0.7 ($p < 0.001$) for non-advanced fibrosis with negative predictive value (NPPV) 67.6% (Figure 1-A), whereas AUCROC of APRI > 1 was 0.87 ($p = 0.002$) with positive predictive value (PPV) of 94.2% (Figure 1-B). AUCROC (Figure 1-C) of

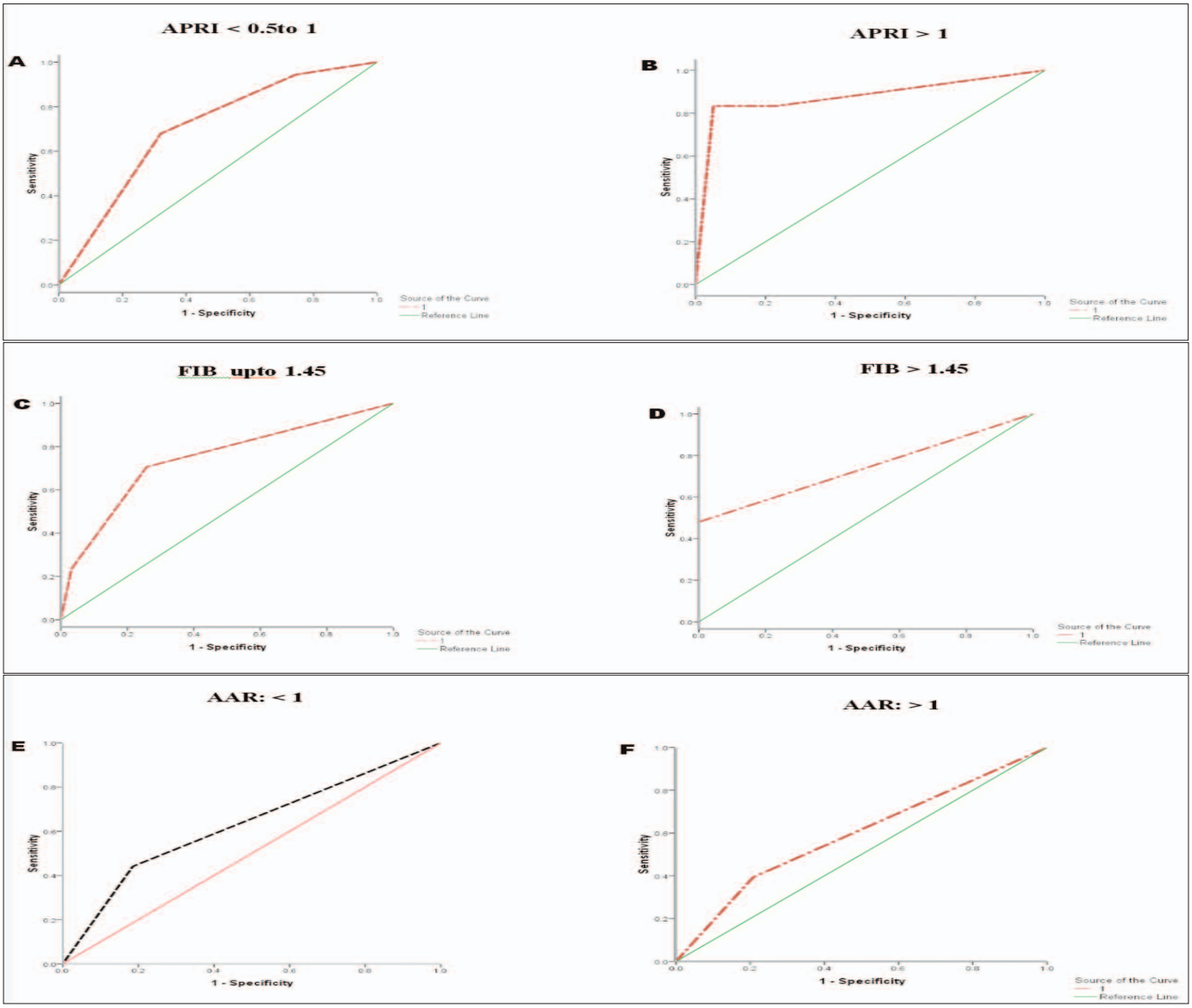

Figure 1 (A-F): ROC of all biochemical markers with stages of fibrosis.

Table II: Results of AUCROC (area under curve receiver operating characteristic) showing sensitivity, specificity, positive and negative predictive values of all biochemical markers comparing with the stage of fibrosis.

Biochemical markers	Fibrosis stage	AUROC (95% CI)	Cut off used for METAVIR stages conversion	Sensitivity (%)	Specificity (%)	Negative predictive value (%)	Positive predictive value (%)	p-vlaue
APRI: < 0.5-1	Non-advanced	0.70 (0.61-0.79)	2.0	67.9	67	67.6	67.9	< 0.001
APRI: > 1	Advanced	0.87 (0.068-0.97)	2.5	83.3	94	84.9	94.2	0.002
FIB-4: upto 1.45	Non-Advanced	0.74 (0.64-0.84)	1.5	70	74	71	73	0.005
FIB-4: > 1.45	Advanced	0.98 (0.95-0.99)	1.5	96	92	95	92	< 0.001
AAR: < 1	Non-advanced	0.62 (0.51-0.74)	0.5	44	81	59	70	.023
AAR: > 1	Advanced	0.59 (0.47-0.71)	0.5	39	79	56	65	0.12 (NS)

Auc = Area under curve; ALT = alanine aminotransferase; AST = aspartate aminotransferase; APRI = AST to Platelet ratio index; FIB-4 = Age, aspartate aminotransferase, platelet count, alanine aminotransferase; AST/ALT ratio = aspartate aminotransferase-alanine aminotransferase; > = more than; < = less than; NS = Non-significant.

FIB-4 < 1.45 was 0.74 (p = 0.005) with NPV of 73% whereas AUCROC of FIB-4 > 1.45 (Figure 1-D) showed 0.98 with PPV of 96% (p < 0.001). AUCROC (Figure 1-E) of AST/ALT ratio < 1 was .62 (p = 0.023) with NPV of 59% whereas AST/ALT ratio > 1 had AUCROC 0.59 (p = 0.12) with PPV of 65% as shown in Figure 1-F. Table II shows AUCROC PPV and NPV of all biochemical markers for non-advanced and advanced fibrosis.

DISCUSSION

Over the past few years, the performance of liver biopsy in accurately staging liver disease has been questioned. The heterogeneity of liver fibrosis in chronic hepatitis C,[11-16] the potential inter- and intra-observer variability in the assessment of fibrosis,[17] and the need for adequately sized specimens[11] are major disadvantages of the procedure. A study on virtual liver biopsy indicated that a specimen length of at least 25 mm would be necessary to correctly evaluate fibrosis.[17] However, this goal is achieved in only 14% of the procedures,[9] increasing the risk of misdiagnosing. Hence, serum markers can theoretically offer a more accurate view of fibrogenic events occurring in the entire liver with the advantage of providing frequent fibrosis assessment without additional risk.

Several indirect markers of liver fibrosis have been proposed in recent years; among them AST/ALT ratio,[8] APRI,[15] FIB-4[11] offer considerable advantage of being non-proprietary test based on analysts almost always measured during the routine evaluation of HCV-infected patients. In this study, in contrast with what occurs in clinical practice,[10] majority of patients i.e. 53.5% had advanced fibrosis with a METAVIR fibrosis score F2, F3 and F4. Non-advanced fibrosis accounted for a METAVIR fibrosis score of F1 and F2 was found in 46.5% patients. In this study, an APRI < 0.5 to 1 was able to predict those patients with mild or no fibrosis with a AUCROC of 0.7 with a negative predictive value of 67.6%, whereas an APRI > 1 predicted significant fibrosis with the AUCROC of 0.87 with a positive predictive value of 94.2%. This means that an AST/platelet ratio index of < 0.5 to 1 had a sensitivity of 67%, whereas an index of > 1 had a specificity of 94% for predicting the presence or absence of bridging fibrosis or cirrhosis in accordance to the study conducted by Sterling *et al.*[9] The importance of APRI is further strengthened by Ned Snyder *et al.* who found the APRI to be reasonably accurate in predicting the mild and significant fibrosis. According to Parise *et al.* APRI shows the 80% sensitivity and 66% specificity for the diagnosis of significant fibrosis at the cut-off value of 0.7.[18]

The FIB-4 combines biochemical variables (platelet count, AST and ALT) with age. In the present study, FIB-4 index of upto 1.45 have AUCROC of 0.74 with a negative predictive value of 71% to exclude severe fibrosis with a sensitivity of 70%, whereas FIB-4 index higher than 1.45 have a positive predictive value to confirm the existence of a significant fibrosis (F2-F4) of 92% with a specificity of 92%. This is in accordance to the study by Vallet-Pichard *et al.*[11] According to Cross *et al.* FIB-4 was found to have an AUC of 0.85 (95% CI 0.82–0.89) for the prediction of severe fibrosis (F3-4) and 0.91 (95% CI 0.86-0.93) for the prediction of cirrhosis.[14]

In this study, AST/ALT ratio < 1 showed a AUCROS value of 0.6 (p= 0.023) with non-predictive value of 59% for non-advanced fibrosis with a sensitivity of 44%, whereas AST/ALT ratio > 1 predicted advanced fibrosis with a positive predictive value of 65%. This is in contrast to Park *et al.* and Giannini *et al.* who observed a significant correlation between the degree of structural hepatic damage and the AST/ALT ratio.[19,20] According to Praise *et al.*, AST/ALT ratio was found to have low sensitivity for prediction of advanced fibrosis.[18] In this study, the results of APRI and FIB-4 was far better in predicting non-advanced and advanced fibrosis than AST/ALT ratio. Lackner *et al.* suggested that AST/platelet ratio to be more accurate than AST/ALT ratio for diagnosing fibrosis.[21]

CONCLUSION

The APRI and FIB-4 are simple novel indices composed of readily available routine laboratory tests that can accurately differentiate mild to moderate fibrosis from bridging fibrosis and cirrhosis in patients with hepatitis C. AST/ALT ratio has shown less prediction ability for

fibrosis. In addition, its applicability to the majority of patients can reduce the need for liver biopsy in the majority of individuals with an overall good accuracy. If additional studies in patients with chronic HCV support these findings, either the FIB-4 or APRI can be used to accurately identify patients with significant fibrosis who might benefit from anti-HCV therapy and just as importantly in patients with mild disease in whom therapy could be deferred.

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