Original Article

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Use of Simple Routine Markers for Non-invasive Assessment of Liver Fibrosis in Iraqi Patients with Chronic Viral Hepatitis

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ABSTRACT

Background: Detecting the presence and degree of hepatic fibrosis is essential in order to make therapeutic decisions and predict clinical outcomes. Currently, the place of liver biopsy as a reference standard for assessing liver fibrosis has been challenged by the increasing awareness of drawbacks related to its use (invasiveness, sampling error, inter-/intra-observer variability). In parallel with this, a rapid growth of noninvasive methods for assessment of liver fibrosis occurred in recent years ranging from serum assays to imaging techniques.**Objectives:** The aim of this study was to verify the usefulness of AST-to-platelets ratio index (APRI) and FIB4 (which depends on the 4 variables of ALT, AST, platelets, and age), two simple, inexpensive, and routinely available test models, to predict significant fibrosis and cirrhosis in Iraqi patients with chronic viral hepatitis.**Patients and Methods:** A retrospective study of the records of 133 treatment-naive patients with chronic viral hepatitis were included in the study, 94 (71%) patients with chronic hepatitis C (CHC) and 39 (29%) patients with chronic hepatitis B (CHB). All patients had a percutaneous liver biopsy interpreted by the hospital pathology department. Transaminase levels and platelet counts were obtained from the results of blood samples taken on the same day of liver biopsy. All testing(hematology, biochemistry and histopathology) was done by the same hospital laboratory.**Results:** The diagnostic performance of APRI and FIB4 scores as determined by area under the receiver operating characteristic curve (AUROC) was as follows: for presence of significant fibrosis (Ishak stage =3, METAVIR =F2) in CHC, 0.61 and 0.69 (p = 0.0833 and 0.0015); and in CHB, 0.59 and 0.54 (p = 0.3599 and 0.6727), respectively. For presence of advanced fibrosis (Ishak stage =4, METAVIR =F3) in CHC, 0.86 and 0.90 (p = <0.0001 for both); and in CHB, 0.70 and 0.54 (p = 0.1032 and 0.7485) , respectively. For the presence of cirrhosis in CHC (Ishak stage 6, METAVIR F4), 0.91 and 0.87 (p<0.0001 for both), respectively. AUROC for diagnosis of cirrhosis in CHB could not be assessed because of inadequate number of patients. **Conclusions and Recommendations:** The APRI and FIB4 scores are only of value for the exclusion of advanced fibrosis and cirrhosis in patients with CHC. They are not useful in the detection of intermediate stages of fibrosis in patients with CHC. Both scores perform significantly better in patients with CHC compared to CHB. The use of APRI or FIB4 scores may decrease the number of staging liver biopsies in only a minority of patients with CHC. Improvement in the performance of serum markers or the use of a combination of a serum marker and a liver stiffness measurement modality such as transient elastography is recommended to avoid liver biopsy in a larger proportion of patients with CHC.

Abbreviations

:ALT
, Alanine aminotransferaseAPRI
, Aspartate aminotransferase-to-platelets ratio indexARFI
, Acoustic radiation force impulseAST
, Aspartate aminotransferaseAUROC
, Area under receiver operating characteristic curveCHB
, Chronic hepatitis BCHC
, Chronic hepatitis CCT
, Computerized tomographyDANA, *D*ifference between the mean stage of *A*dvanced fibrosis minus the mean stage of *N*on-*A*
dvanced fibrosisFIB4
, A liver fibrosis score using 4 variables (age, AST, ALT, platelets)HBV
, Hepatitis B virusHCV
, Hepatitis C virusHVPG
, Hepatic venous pressure gradientINR
, International normalized ratioKpa
, Kilo PascalMETAVIR
, Meta-analysis of Histological Data in Viral HepatitisMR
, Magnetic resonanceMRE
, Magnetic resonance elastographyNAFLD
, Non-alcoholic fatty liver diseaseNPV
, Negative predictive valuePPV
, Positive predictive value ROC
, Receiver operating characteristic curveRTE
, Real-time elastographySD
, Standard deviationTE
, Transient elastographyU/L
, Unit per literχ^2 test
, Chi-squared test

Keywords: Liver Fibrosis, APRI, FIB4, Non-invasive

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Accurate assessment of liver fibrosis has become increasingly important in order to make therapeutic decisions, determine prognosis, and to follow-up disease progression.[1]Historically, liver biopsy has been the gold standard for assessing the degree of liver fibrosis.[1,2] The biopsy specimen allows clinicians to obtain diagnostic information not only on fibrosis but also on inflammation, necrosis, steatosis and hepatic deposits of iron or copper.[3] However, increasing awareness of several drawbacks of liver biopsy has repeatedly questioned its accuracy and value in clinical practice.[4] For these reasons, non-invasive tests for the diagnosis of liver fibrosis have evolved rapidly over the last several years, especially for patients with chronic hepatitis C (CHC).[5]non-invasive methods for the assessment of liver fibrosis are now globally-accepted valid tools in the care of patients with CHC.[6,7]

The large number of non-invasive methods to detect and quantify liver fibrosis developed over the last decadecanbe divided into two main types: serum markers and imaging modalities.[8,9]

Serum Markers:Serum markers are classified as direct (or class I), which represent extracellular matrix components (reflecting the pathophysiology of liver fibrogenesis); and indirect (or class II), which use routine laboratory parameters (reflecting the consequences of the liver damage). Direct and indirect markers may be used alone or, more commonly, in combination to produce composite scores.[10]Indirect serum markersinclude simple routine blood tests such as liver biochemistry and components of complete blood count. Aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio index =1 has shown good specificity (although relatively insensitive) to detect cirrhosis in patients with CHC with reported positive and negative predictive values ranging from 74-100% and 47-53% respectively.[11,12] However, its usefulness was not confirmed when validation was assessed in independent patient cohorts.[13]Due to the poor accuracy of individual serum markers to assess liver fibrosis, indices combining panels of markers have been developed and widely validated, with reportedly "sufficient" diagnostic accuracy.Some commercially available panels utilize expensive direct serum markers and are protected by patents,whereas others include routine blood tests and are freely available.[14]

The APRI scoreis one of the most extensively studied serum markers.[15]In a recent meta-analysis of 40 studies of patients with CHC, investigators concluded that an APRI cut-off of 1.0 had a sensitivity of 76% and specificity of 72% for predicting cirrhosis; similarly, an APRI cut-off of 0.7 had a sensitivity of 77% and specificity of 72% for predicting significant hepatic fibrosis.[14]

APRI has also been assessed in patients with CHB[15], but a recent meta-analysis concluded that APRI has a limited value in identifying CHB-related significant fibrosis and cirrhosis.[16] This might partially be explained by the frequent development of macronodular cirrhosis which contains little fibrous tissue in patients with chronic hepatitis B.

[16]The FIB4 score combines platelet count, ALT, AST and age and was initially developed for use in HCV/HIV co infection where it correctly classified 87%, and avoided biopsy in 71% of the validation set, with an AUROC of 0.77, sensitivity 70% and specificity 97% In a subsequent analysis including a large cohort of HCV-monoinfected patients, FIB4 enabled good discrimination of both severe fibrosis (AUROC 0.85) and cirrhosis (AUROC 0.91).[17] More recently this marker has been assessed in patients with CHB with reported 71% sensitivity and 73% specificity for diagnosing METAVIR=F2fibrosis.[18,19] Moreover, it has been shown to be reliable in the setting of non-alcoholic fatty liver disease (NAFLD); using a cut-off value of 1.3 the sensitivity and specificity for predicting advanced (METAVIRF3-F4) fibrosis were 74-85% and 65-71% respectively, and 34% and 98% when a FIB4 threshold of 2.67 was used.[20]

Although noninvasive, easy to repeat and highly applicable, serum markers have obvious limitations.Their main disadvantage is represented by their low accuracy to detect intermediate stages of fibrosis as compared to cirrhosis.

Another drawback is the potential lack of liver-specificity.

Imaging and Liver Stiffness Measurement techniques Classical imaging techniques, including ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI) are used in clinical practice for the prediction of cirrhosis either directly (by detecting overt morphological changes of the cirrhotic liver) or indirectly by detecting signs of portal hypertension.[1] However, the necessity to accurately identify earlierstages of liver fibrosis has led to the development of novel imaging modalities to measure liver stiffness as a surrogate marker of liver fibrosis.[21] Transient Elastography (TE) (Fibroscan®, Echosens®, Paris, France)[22] is the most widely validatednoninvasivemethod in Europe, for CHC and other liver diseases1.The volume of liver tissue evaluated by TE approximates a cylinder 4x1 cm which is at least 100 times bigger than a liver biopsy.[23] Moreover, TE is painless and rapid (<5 min) and thus highly acceptable for patients.[27]

Thediagnostic performance of TE has been widely addressed.[24,25]For detection cirrhosis, the pooled estimated sensitivity and specificity of TE approaches 90%, whereas for significant fibrosis it is substantially lower with pooled estimates of

Sensitivity and specificity in the range of 70-80%.[11,12] Despite the wide acceptance and incorporation in clinical practice, TE is not free of limitations.[25] Acoustic radiation force impulses (ARFI) has been recently suggested to be a valid method to assess liver fibrosis.[11] This technologypermits evaluation of stiffness in a region of interest involving mechanical excitation of tissue by short-duration acoustic pulses while performing a real-time B-modeultrasound, with the advantage to choose the representative area of interest avoiding large vessels and ribs.[26]

A recent meta-analysis examining the value of ARFI versus TE for the assessment of liver fibrosis concluded that ARFI seems to be a good method for assessing liver fibrosis, Magnetic resonance (MR) elastographyuses a modified phasecontrast method to evaluate the propagation of the shear waves within the liver.[27] Advantages include the potential to analyze the whole parenchyma, as well as the applicability for patients with obesity or ascites.

Sequential combination algorithms:

Recently, research has focused on the sequential use of two or more non-invasive methods in order to increasethe diagnostic accuracy for liver fibrosis.[28]Castera et al showed that a combination of Fibroscan® and Fibrotest® resulted in excellent diagnostic accuracy for detecting both significant fibrosis (AUROC 0.88) and cirrhosis (AUROC 0.95) in CHC.[29]Sebastiani et al proposed and validated a sequential algorithm based on combining APRI and Fibrotest® inCHCpermittingavoidance of 50-80% of liver biopsies.[30]

Patients And Methods:

We retrospectively studied the medical records of patients with chronic viral hepatitis who attended the Gastroenterology and Hepatology Teaching Hospital between January 2011 and December 2013 and had a percutaneous liver biopsy performed. Patients with CHB or CHC who had a conclusive histopathology report of their liver biopsy were eligible for the study.Patients with CHChad PCR assays to confirm the diagnosisbefore having the liver biopsy. All patients with CHC who had a liver biopsy had had genotype 1 (or 4) according to guidelines applying for currently available antiviral therapy (pegylated interferon and ribavirin).[31,32] Patients with CHB also had their liver biopsy performed according to indications of current guidelines.[33,34]Patients with the following conditions were excluded from the study: presence of other causes of liver disease, hepatocellular carcinoma, prior liver transplantation, current immun osuppressive therapy, insufficient liver tissue for staging of fibrosis, and incomplete data on complete blood counts and/or liver biochemistry panel.All patients were antiviral treatment-naïve and had no hematological disorder including anemia , which might affect their liver enzymesand/or platelet levels.Results of liver enzymes levels and platelet counts were for blood samples obtained on the same day of performing the liver biopsy. Biochemical and hematological tests were done in the same hospital laboratory to minimize inter-laboratory variability particularly in the measurement of transaminase levels.Liver biopsies were interpreted by the histopathology department of the hospital. APRI and FIB4scores were calculated as follows:

- APRI[35] = [(AST/ULN) × 100] / platelet count
- FIB4[36] = (Age x AST) / (platelet count x √ALT)
- AST and ALT measured in IU/L, ULN is the upper normal limit of AST, platelet count is measured in 10^9/L.

Performance of APRI and FIB4 scores were measured by calculating the area under the receiver operating characteristic curve (AUROC). Sensitivity, specificity, positive (PPV) and negative predictive values (NPV) were calculated for bothtests for the prediction of significant fibrosis (Ishak =3), advanced fibrosis (Ishak =4) and cirrhosis (Ishak 6).[37]

Statistical Analysis:

Statistical analysis was performed using MedCalc®statistical software for Windows v.12.7 (2013).Demographic, biochemical and histological features were categorized as continuous or categorical variables. D'Agostino-Pearson analysis was usedto test for normal distribution. For normally-distributedvariables, the data were expressed as arithmeticmean ± standard deviation (SD). For variables that were not normally distributed, the datawere expressed as median (25^{th}-75^{th}percentile range). Comparisonsbetween two groups were made using the Student's *t* -test or Mann-Whitney U testas appropriate. Categorical variables were compared using the χ^2 test.The diagnostic performance of theevaluated variables was assessed by calculating AUROC,sensitivity, specificity, positive (PPV) and negative predictive value (NPV). Based on ROC analysis, the best cut-off points to predict or to exclude significant fibrosis and cirrhosis were chosen. All of the reported *p* -values were two-tailed, and those less than 0.05 were considered to bestatistically significant.

Results:

Liver biopsy reports of219 patients were collectedfor the study; 86 patients were excluded because of incomplete hematology or biochemistry data leaving 133 patients to be included in the study. The median age of the patients was 35years (youngest was 14 years old and oldest was 63 years old), 64 (48%) were males and 69 (52%) were females.The distribution of the stages of fibrosisas reported in histopathology reports of the liver biopsies(according to Ishak scheme) was as follows:

no or mild fibrosis (stages 0-2)in81 (61%); significant fibrosis(stages 3-6) in 52 (39%); andcirrhosis (stage6) in 6(5%) patients.For validcomparison to other studies of non-invasive tests of fibrosis, the stage of fibrosis was converted from its original description in the biopsy report in Ishak scheme (0-6) to the corresponding stage in the more widely used METAVIR scheme (F0-F4) according to accepted approaches. Table 1 summarizes the patients'characteristics and Tables 2 compares liver fibrosis staging systems.

Table 1. Characteristics of the patients included in the study (n=133).

Characteristic	Value[a]
Age (years)	35(25-45)
Male, n (%)	64(48%)
Cause of viral hepatitis	
HCV, n (%)	94 (71%)
AST level (U/L)	24 (17-32)
ALT level (U/L)	25 (20-40)
Platelet count ($x10^9$/L)	247 ± 75
Liver Biopsy (fibrosis stage)	
Ishak 0-2 (METAVIR F0-F1)	81 (61%)
Ishak3-6 (METAVIR F2-F4)	52 (39%)
Ishak 6 (METAVIR F4)	6 (5%)

[a]Normally-distributed continuous variables are expressed as mean ± SD. Non-normally- distributed continuous variables are expressed as median (25^{th}-75^{th}percentile range).
Categorical variables are expressed as n (%).

Table 2.Comparison of Liver FibrosisStaging Systems.

Ishak[37]	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
	Nofibrosis	Fibrous expansion of someportaltracts, with or withoutshort septa	Fibrous expansion of most portal tracts, with or without short septa	Fibrous expansion of most portal tracts with occasional porto-portal bridging	Fibrousexpansion of portal tracts with marked portal-portal as well as porto-central bridging	Marked bridging with occasional nodules (incomplete cirrhosis)	Cirrhosis, probable or definite
METAVIR[39]	F0	F1		F2	F3		F4
	Nofibrosis	Portal fibrosis without septa		Portalfibrosis withrare septa	Numerous septa withoutcirrhosis		Cirrhosis

For the purpose of the study, the patients were divided into 3 sets each containing 2 mutually-exclusive groupsaccording to the stage of fibrosis on liver biopsy:patients with significant fibrosis (Ishak =3, METAVIR =F2) versusno or non-significant fibrosis (Ishak 0-2, METAVIR F0-F1); patients with advanced fibrosis (Ishak=4, METAVIR =F3) versus no or non-advanced fibrosis (Ishak 0-3, METAVIR F0-F2); and patients with cirrhosis (Ishak 6, METAVIR F4) versusno or pre-cirrhoticfibrosis (Ishak 0-5, METAVIR F0-F3).Tables 3, 4, and 5 compare the patients' evaluated parameters according to the fibrosis stage.

Table3.Comparison of evaluated parameters according to the presence of significant fibrosis.

Variable[a]	Significant fibrosis (Ishak =3, F=2) n=52	No significant fibrosis (Ishak 0-2, F0-F1) n=81	*P*value
Age (year)	42(28-48)	32 (24-40)	0.0192*
Gender (male n, %)	29 (56%)	35 (43%)	0.2162
AST(U/L)	25 (18-34)	24 (16-31)	0.3326
ALT(U/L)	28 (21-40)	25 (19-40)	0.5485
Platelet count ($x10^9$/L)	233 ± 86	256 ± 66	0.0856
APRI	0.29 (0.21-0.41)	0.26 (0.15-0.31)	0.0500
FIB4	0.84 (0.47-1.16)	0.57 (0.33-0.82)	0.0040*

[a]Normally-distributed continuous variables are expressed as mean ± SD. Non-normally-distributed continuous variables are expressed as median (25^{th}-75^{th}percentile range).
Categorical variables are expressed as n (%).*Statistically significant

Table 4.Comparison of evaluated parameters according to the presence of advanced fibrosis.

Variable [a]	Advancedfibrosis (Ishak =4, F=3) n=18	No advanced fibrosis (Ishak 0-3, F0-F2) n=115	*P* value
Age (year)	45 (27-48)	33 (25-43)	0.1581
Gender (male n, %)	12 (67%)	52 (45%)	0.1499
AST (U/L)	29 (25-49)	23 (16-31)	0.0585
ALT (U/L)	33 (24-44)	25 (20-39)	0.2134
Platelet count ($x10^9$/L)	194 ± 105	255 ± 65	0.0010*
APRI	0.39 (0.31-0.52)	0.26 (15-0.32)	0.0002*
FIB4	1.11 (0.67-1.65)	0.58 (0.35-0.88)	0.0006*

[a] Normally-distributed continuous variables are expressed as mean ± SD. Non-normally-distributed continuous variables are expressed as median (25^{th}-75^{th}percentile range).
Categorical variables are expressed as n (%).
*Statistically significant

Table 5.Comparison of evaluated parameters according to the presence of cirrhosis.

Variable[a]	Cirrhosis Ishak 6, F4) n=6	No cirrhosis (Ishak 0-5, F0-F3) n=127	*P*value
Age (year)	47 (34-56)	33 (25-45)	0.1752
Gender (male n, %)	6 (100%)	58 (46%)	0.0289*
AST (U/L)	45 (25-61)	24 (16-31)	0.0549
ALT (U/L)	33 (25-47)	25 (20-40)	0.2062
Platelet count ($x10^9$/L)	148 ± 80	252 ± 71	0.0007*
APRI	0.49 (0.43-1.06)	0.27 (0.15-0.33)	0.0003*
FIB4	1.87 (1.30-3.46)	0.59 (0.36-0.91)	0.0009*

[a]Normally-distributed continuous variables are expressed as mean ± SD. Non-normally-distributed continuous variables are expressed as median (25^{th}-75^{th} percentile range). Categorical variables are expressed as n (%). *Statisticallysignificant.

Figures 1 and 2 illustrate the distribution of the APRI and FIB4 values according to the stage of liver fibrosis as determined by liver biopsy (converted to METAVIR scheme) The areas under the ROC curves (AUROC) for APRI and FIB4 scores for prediction of various stages of fibrosis were as follows:For significant fibrosis (Ishak stage =3, F=2) in patients with CHC:0.61 and 0.69 with *p*values of0.0833 and 0.0015; and for CHB are 0.59 and 0.54 with *p* values of 0.3599 and 0.6727, respectively.For advanced fibrosis (Ishak=4, F=3) in patients with CHC are 0.86 and 0.90 with *p* values of <0.0001 for both; and for CHB are 0.70 and 0.54 with *p* values of 0.1032 and 0.7485, respectively.For cirrhosis in CHC are 0.91 and 0.87 with *p* Values of <0.0001 for both scores.The performance of the scores in the prediction of cirrhosis in patients with CHB could not be assessed because of inadequate number of patients in the study sample (only one patient had CHB-related cirrhosis).Table 6summarizes the performance of the two scores using best cut-off values and figures 3 to 7 compare the ROC curves of the scores for various stages of fibrosis.

Table 6.Overall performance of scores for diagnosis of fibrosis and cirrhosis.

Setting	Index	AUROC	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	*P* value
Significant fibrosis (CHC)	APRI	0.61	0.34	46	81	61	70	0.0833
	FIB4	0.69	0.86	54	84	69	74	0.0015*
Significant fibrosis (CHB)	APRI	0.59	0.28	53	67	50	70	0.3599
	FIB4	0.54	0.80	53	67	50	70	0.6727
Advanced fibrosis (CHC)	APRI	0.86	0.34	90	77	32	99	<0.0001*
	FIB4	0.90	0.98	90	83	39	99	<0.0001*
Advanced fibrosis (CHB)	APRI	0.70	0.30	63	87	56	90	0.1032
	FIB4	0.54	1.23	25	97	67	83	0.7485
Cirrhosis (CHC only)	APRI	0.91	0.42	100	87	29	100	<0.0001*
	FIB4	0.87	1.24	80	92	36	99	<0.0001*

PPV, NPV, positive and negative predictive value;CHC, CHB, chronic hepatitis C and B; AUROCarea under receiver operating characteristic curve;Significant fibrosis,Ishak=3(=F2); Advanced fibrosis,Ishak =4 (=F3); Cirrhosis, Ishak 6 (F4).
*Statisticallysignificant

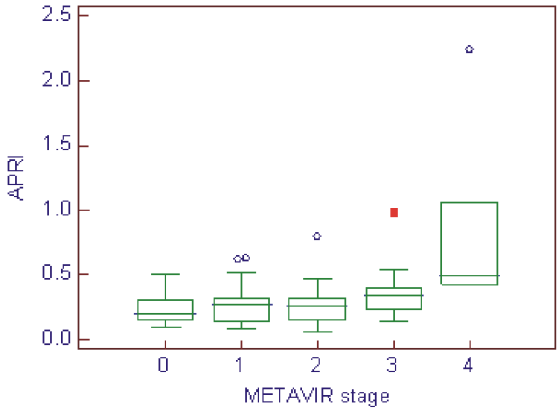

Figure 1.Distribution of APRI values according to METAVIR fibrosis stage, Boxplots depict the median (horizontal line inside the box), the 25th and 75th quartiles (lower and upper edges of the box), and the minimum and maximum values (vertical line).

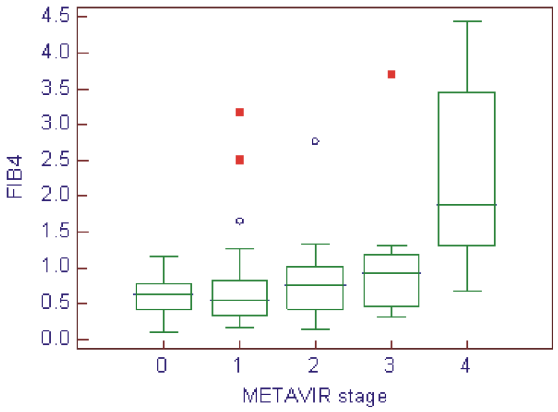

Figure 2.Distribution of FIB4 values according to METAVIR fibrosis stage, Boxplots depict the median (horizontal line inside the box), the 25th and 75th quartiles (lower and upper edges of the box), and the minimum and maximum values (vertical line).

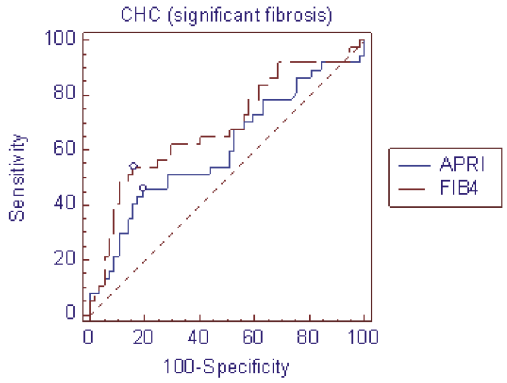

Figure 3. Comparison of ROC curves of APRI and FIB4 for the diagnosis of significant fibrosis (Ishak =3, F=2) in patients with chronic hepatitis C (CHC). AUROC = 0.61 and 0.69, respectively.

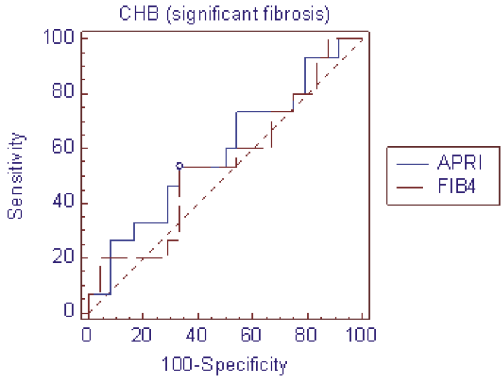

Figure 4.Comparison of ROC curves of APRI and FIB4 for the diagnosis of significant fibrosis (Ishak =3, F=2) in patients with chronic hepatitis B (CHB). AUROC = 0.59 and 0.54, respectively.

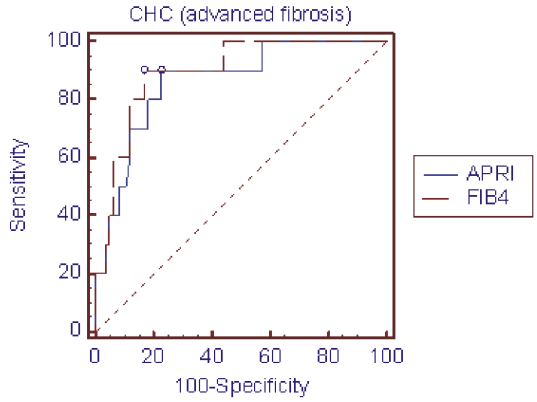

Figure 5. Comparison of ROC curves of APRI and FIB4 for the diagnosis of advanced fibrosis (Ishak =4, F=3) in patients with chronic hepatitis C (CHC). AUROC = 0.86 and 0.90, respectively.

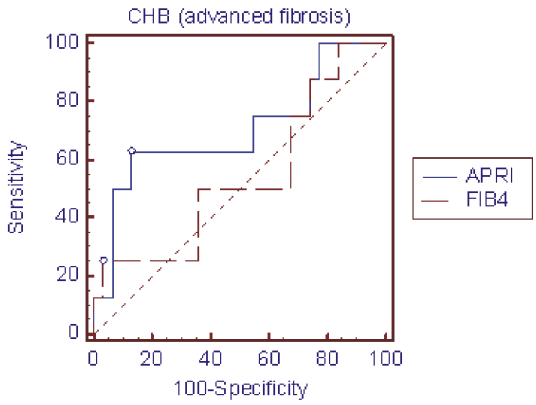

Figure 6.Comparison of ROC curves of APRI and FIB4 for the diagnosis of advanced fibrosis (Ishak =4, F=3) in patients with chronic hepatitis B (CHB). AUROC = 0.70 and 0.54, respectively.

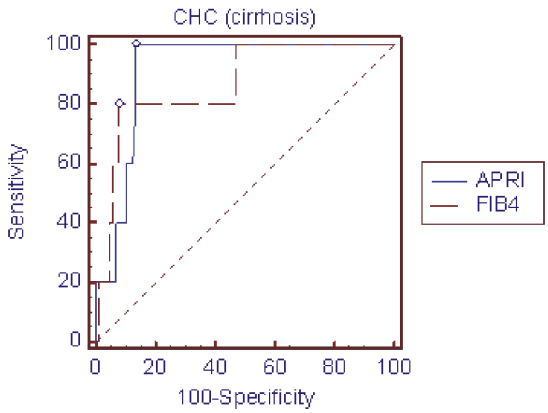

Figure 7. Comparison of ROC curves of APRI and FIB4 for the diagnosis of cirrhosis (Ishak 6, F4) in patients with chronic hepatitis C (CHC). AUROC = 0.91 and 0.87, respectively.

Discussion:

The recently published WHO guideline on management of CHC states the follow in grecommen dation:"In resource-limited settings, it is suggested that APRI or FIB4 be used for the assessment of hepatic fibrosis rather than other noninvasivetests that require more resources such as Fibroscan® orFibrotest®.Conditional recommendation, low quality of evidence".[40]In this study, we examined the performance of APRI and FIB4 scores in patients with CHC and CHB. Although theses scoreswere initially developed forpatients with CHC, bothwere later used in patients with CHB.[41,42]APRI and FIB4 scores have been shown to correlate with significant fibrosis and cirrhosis in patients with CHC[43]and also in CHB.[44] Both are based on routine laboratory tests (AST, ALT, and platelet count) and are, therefore, low cost widely available tools. It is known that platelet counts decrease and AST levels increase with the progression of liver fibrosis.[45] Platelet generation diminishes secondary to a decreasedproduction of thrombopoietin by hepatocytes.[46, 47] Also, platelets are sequestered and destructed in the spleen as liver fibrosis advances and portal hypertension develops.[48]In chronic liver disease including chronic viral hepatitis, an elevated AST/ALT ratio is traditionally considered to be suggestive of advanced fibrosis or cirrhosis and poor long termoutcomes.[49,50]

We used the traditional approach of calculating the AUROC to determine the diagnostic accuracy of APRI and FIB4.[11-13]The reported AUROC is based on sensitivities and specificities across a range of test results and is a measure of discrimination, or the ability of a test to distinguish persons with a condition from those without it.[51] An AUROC of 1.0 indicates perfect discrimination, and an AUROC of 0.5 indicates complete lack of discrimination. Interpretation of values between 0.5 and 1.0 is somewhat arbitrary, but a value of 0.90 or more has been classified as excellent, 0.80 to less than 0.90 as good, 0.70 to less than 0.80 as fair, and less than 0.70 as poor.[52]For the diagnosis of significant fibrosis in CHC, the APRI and FIB4scores had an AUROC of 0.61 and 0.69with corresponding sensitivities and specificities of 46% and 81%, and 54% and 84%,; respectively. For patients with CHB, the AUROC for APRI and FIB4 were 0.59 and 0.54 with corresponding sensitivities and specificities of 53% and 67% for both scores.This poor performance(AUROC <0.70) is consistent with the most recent systematic review[53], showing AUROC of 0.58-0.95 for APRI and 0.61-0.81 for FIB4 in the diagnosis of significant fibrosis (F=2) in CHC.Furthermore, all non-invasive fibrosis tests are known to perform better in patients with CHC compared to CHB.[54]

The relatively low performance of APRI and FIB4for prediction of significant fibrosis in CHC obtained in our study can be explained by several factors:First, most of our patients had transaminase values that are within the normal laboratory range, probably related to the practice of treating patients with significantly elevated liver enzymes empirically without prior staging of fibrosis by liver biopsy. The median value of AST and ALT for our patients with significant fibrosis was 25 and 28 U/L, respectively. It is known that the performance of non-invasive tests that depend on transaminase levels (like APRI and FIB4), decreases in patients with normal transaminase values.[55]Second, the upper limit of normal range for AST has significant inter-laboratory variability contributing to variable calculated APRI scores for the same patient using different laboratory test results, this in turn will lead to variable performance of scores among different studies.[56] Third, the effect of the so called "spectrum bias" which refers to over-representation of extreme stages of fibrosis (F0 and F4) in a study population, is very important.[57] An excess of patients with severe fibrosis will spuriously generate higher sensitivity and specificity values, compared to a population including mostlypatients with lesser and adjacent stages of fibrosis (F1 and F2). This effect has been studied by Poynard et al [58] and measured by the difference between the mean fibrosis stage of patients with significant fibrosis (F =2) minus the mean fibrosis stage of patients withno or mild fibrosis (F0-F1) and called it the DANA score (difference between the mean fibrosis stage of advanced fibrosis minus the mean fibrosis stage of non-advanced fibrosis). In that study,. They found that a difference of 1.0 in DANAtranslates into a difference of 0.1 in AUROC of a non-invasive test (Fibrotest® in their study). If we apply this to our study we get a DANA score of 1.58. This is a low value compared to other studies which yielded higher AUROC for non-invasive fibrosis tests. For example, in 2 published studies of Fibrotest® in CHC, Wilson et al [59] found an AUROC of 0.74, whereas Sene et al [60] found an AUROC of 0.83. This discrepancy in their results was fully explained by the difference in prevalence of fibrosis stages between the two studies, with a DANA of 1.87 for the former and 3.05 for the latterIn other words, the higher the percentage of patients in the middle spectrum of fibrosis (Ishak 1-3, METAVIR F1-F2), the lower the AUROC (and performance) of a non-invasive test. This is not surprising, as we know that all non-invasive markers of fibrosis has higher performance in the diagnosis of cirrhosis (F4) and absence of fibrosis (F0) compared to intermediate stages of fibrosis. In our study, 79% of our patients had an Ishakfibrosis stage of 1-3 (F1-F2), whereas

only 5% had cirrhosis (Ishak6, F4).While affecting the performance of APRI and FIB4 in our study, this disproportionate number of patients with mild fibrosis may have been partly due to a significant number of small-sized liver biopsy specimens (less than 20 mm) in our study which mayhave resulted in the under-estimation of the degree of fibrosis Fourth, the inherent limitations of the reference standard (liver biopsy) are well recognized. Indeed, liver biopsy is prone to sampling errors and to intra-observer and inter-observer variability.[61]For instance, even a 25mm long liver biopsy has a 25% discordance for fibrosis staging.[62]Also, when the specimen size is adequate, the level of experience of the pathologist may even be more important.[63]Therefore, with liver biopsy being an imperfect gold standard, anaccurate non-invasive test will never reach the maximal value of AUROC (1.0).[4] Taking into account a range of accuracies of the biopsy and a range of prevalence of significant fibrosis (that influence the AUROC), Mehta et al have shown that in the most favorable scenario, an AUROC >0.90 for significant fibrosis cannot be achieved even for a perfect marker.[64]

For the diagnosis of advanced fibrosis (Ishak 4-5, F3) and cirrhosis (Ishak 6, F4) in CHC, the APRI and FIB4 scores had a much better overall performance, with AUROCof 0.86 and 0.90 in F3; and 0.91 and 0.87 in F4, respectively. The actual strength of the tests was most clear in exclusion of advanced fibrosis and cirrhosis with a high NPV reaching 100% for APRI in CHC cirrhosis (F4).

Conclusions And Recommendations:

For patients with CHC, APRI and FIB4 scores are only of value for the exclusion of advanced fibrosis or cirrhosis. They are not reliable in the detection of intermediate stages of fibrosis.For patients with CHB, both scores have poor performance and are unreliable for prediction of sign ificant or advanced fibrosis.APRI and FIB4 scores are less accurate in assessment of liver fibrosis in patients with CHC (and CHB) with normal transaminase levels.In CHC, APRI or FIB4 may be used for the assessment of hepatic fibrosis if liver biopsy is not feasible and other more reliable but expensive tests, such as Fibroscan® or Fibrotest®, are not available. APRI and FIB4 need further validation in CHB and CHC with normal ALT.

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