Comparison of FIB-4 and APRI indices as a non-invasive markers for fibrosis in chronic HCV infection

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Abstract: Background and Aim: To assess the value of FIB-4 to both AAR and APRI indexes and their values to differentiate mild to moderate fibrosis from advanced fibrosis in HCV genotype 4-infected Egyptian patients in comparison to liver biopsy. **Methods:** 202 genotype 4 HCV-infected Egyptian patients were included. **Results:** There was a significant relationship between fibrosis stages and serum indices except AAR. A gradual increase in the level of FIB-4, AAR and APRI indices were observed with advancement of the fibrosis stages. The FIB-4 score had the best diagnostic accuracy for advanced fibrosis followed by AAR, and APRI. As the NPV for FIB-4 score is 90% using the lower cut-off, this test may have sufficient accuracy to be used clinically to exclude advanced fibrosis. **Conclusion:** FIB-4 index is a noninvasive test for the assessment of liver fibrosis. It is more sensitive and accurate than both AAR and APRI in defining the degree of fibrosis. It can be used efficiently in cases of chronic HCV mono infection. A score of <1.26 and >2.1 enables the correct identification of patients with HCV infection genotype 4 who have significant fibrosis and could avoid liver biopsy examination in 65.8% of cases. Because the FIB-4 index is readily available, inexpensive, and reproducible, it could replace expensive and/or invasive methods to assess liver fibrosis, especially in developing countries, to detect patients who need antiviral treatment and to monitor liver fibrosis progression or regression.
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Key wards: FIB-4, AAR, APRI, non-invasive fibrosis markers, HCV infection genotype 4.

1.Introduction

Chronic Hepatitis C (CHC) remains a major health problem with around 170 million individuals affected worldwide.[1]. Prevalence of chronic hepatitis C in Egypt is extremely high, affecting 15% to 20% of the population. HCV is the leading cause of liver disease in Egypt and is one of the country's major health problems. Genotype 4 is the predominant genotype of HCV in Egyptian patients (up to 91%). Genotype 4 is prevalent in developing countries in Africa and the Middle East[2].

According to the Egyptian MOH&P guidelines, liver biopsy is mandatory for chronic HCV patients in order to receive free (insured) anti-viral therapy. CHC patients with no or minimal fibrosis at presentation appear to progress slowly and treatment possibly could be delayed or withheld to prevent cirrhosis.[3] On the other hand, patients with significant fibrosis progress to cirrhosis over a 10-20 year period so antiviral treatment should be strongly considered[4]. Because of limited resources, there is a need to allocate the expensive therapy to the sickest patients. If we have a surrogate marker of fibrosis to identify the advanced fibrosis, this will identify patients with high priority for treatment.

The gold standard for assessing hepatic fibrosis is liver histology. Liver biopsy is however limited by its invasive nature[5-7], poor acceptance, especially when repeated measures are required; availability and cost, particularly in developing countries; intra-and inter-observer variability[8,9]and sampling errors, which produce approximately 24% of false negatives for cirrhosis.[10,11]

Poynard *et al*.[12] observed discordances in 29% of patients that were due to marker failure and liver biopsy failure in 2.4% and 18% of cases, respectively. Furthermore, to evaluate diffuse liver diseases in a reliable manner, a specimen sample measuring at least 15 mm is needed.[13] Bedossa *et al*.[14] showed recently that only 65% of biopsies relying on 15-mm samples led to correct diag noses, whereas 75% of biopsies relying on 25-mm samples were correct. Because there were no benefits to taking bigger samples, the investigators suggested that 25-mm samples are necessary to evaluate fibrosis accurately.

Consequently, noninvasive tests to assess hepatic fibrosis have been developed, such AST/ALT ratio (AAR)[15] and the AST-to-platelet ratio index (APRI)[16], which combine several biochemical parameters.

A simple noninvasive test for liver fibrosis known as the FIB-4; a test which produces interesting results using special formula.

This study was designed to assess the value of the FIB-4 index and its threshold values to differentiate mild to moderate fibrosis from advanced fibrosis in HCV-infected patients and compare the FIB-4 index to AAR and APRI indexes in comparison to liver biopsy.

2.Patients and Methods:

Prospectively, 202 HCV-infected consecutive Egyptian patients attending at the Hepatology Department, Theodor Bilharz Research Institute, Egypt, for evaluation of their chronic liver disease. The patients were subjected to thorough clinical examination and were assessed by laboratory investigations; abdominal ultrasonography; and liver biopsy using Menghini needle for histopathologic examination. All patients gave informed consent prior to participation in the study in conformance with the guidelines of the 1975 Declaration of Helsinki as reflected by approval of the institution's human research ethical committee. All procedures, including liver biopsy, were medically indicated for patient management.

All the cases corresponding to the following criteria: (1) anti HCV- and HCV-RNA–positive Genotype 4 (2) liver biopsy prior to any antiviral therapy (3) laboratory assessments allowing FIB-4 calculation (AST, ALT, Bilirubin, Platelet count) performed on the same day as liver biopsy or on the preceding day (4) absence of HIV, HBV infection, alcohol consumption, other liver co-morbidity, including hemochromatosis, Wilson's disease, α1-antitrypsin deficiency, autoimmune hepatitis, and nonalcoholic steatohepatitis and absence of immune suppression.

Laboratory Investigations:

Liver function tests were done using commercially available kits. Hepatitis B markers were tested using enzyme immunoassay kits (Abbott Laboratories; North Chicago, Illinois). CHC was confirmed by HCV infection persisting for longer than 6 months (HCV-RNA positive) and increased ALT values. Circulating anti-HCV antibodies were detected using Murex enzyme immunoassay kit (Murex Diagnostics; Dartford, U K), and the presence of HCV-RNA in patients' sera was detected by PCR using the Amplicor test Roche Diagnostic Systems; Meylan, France).

We used the FIB-4 index for semi-quantitative evaluation of fibrosis in 202 HCV mono-infected patients. We calculate APRI index as AST level (UNL)/platelets counts (109/L) x 100[16], then, compare the results of FIB-4 and APRI with the results of liver biopsy.

Serum AST and ALT levels were routinely measured in our hospital; usual upper normal values were 45 IU/l for men and 40 IU/l for women and 65 IU/l for men and 50 IU/l for women, respectively. Platelet counts were performed in the same hospital; normal values ranged between 150,000 and 400,000/ml^3.

All liver biopsies were analyzed in the pathology department and all interpretations were supervised by a senior expert. The degree of activity and the extent of fibrosis were assessed using the Metavir scoring system.

The FIB-4 values were calculated automatically using the formula: age (years) X AST [U/l]/(platelets [109/l] X(ALT [U/l])$^{1/2}$, in which the age of the patient was the age at the time of the liver biopsy. No financial support had been given. All informations in the study can be shared with others.

Statistical Analysis

The data were analyzed using statistical package SPSS version 18.0 for windows (SPSS Inc., Chicago, IL). Diagnostic results between patients were compared using the non-parametric Wilcoxon-Mann-Whitney U-test while Chi-square (χ2) test was used to compare categorical data. The independently distinguished values of biochemical markers APRI, FIB-4 and AAR for the prediction of significant fibrosis and cirrhosis were evaluated using univariate and multiple regression analysis. Area under the receiver operating characteristic (ROC) curves (AUROCs) was used to compare and deduce the diagnostic accuracies of the selected bio-markers. In ROC curves, the true positive rate (sensitivity) is plotted as a function of the false positive rate (100-specificity) for different cut-off points. To assess the association between non-invasive diagnostics and histology (the golden standard), linear and binary logistic regression analyses were performed.

I. Logistic regression model:

y = exp [_3.858 - (0.0249 X age) - (0.7464 X sex) - (1.0039 X FIA-4 Index) - (0.0302 X platelet) - (0.0691 X bilirubin)]

With age provided in years, male sex = 1, female sex = 0,

3.Results:

The data collection takes 12 months with no drop outs. Non of the patients received anti-viral treatments before undergoing the test.

The demographic and clinico-laboratory results of the 202 patients (male/female 162/40; 80.2%/19.2%) with anti-HCV and HCV-RNA–PCR positive without serologic evidence of co-infection with HBV and HIV are showed in Table 1.

Concerning liver function tests serum ALT levels ranged from 4.1 to 757 IU/L (mean±SE, 93.64±6.36) and that of AST from 7 to 308 IU/L (mean ±SE, 66.71±3.55); platelet count ranged from $78X10^3$/mL to 383X 10^3/mL with a mean value (±SE) of 197 (±4.3). 16 (7.9%) patients were scored as F0 (no fibrosis), 54 (26.7%) as F1 (mild fibrosis), 38 (18.8%) as F2 (moderate fibrosis), 40 (19.8%) as F4 (moderate to severe fibrosis), and 54 (26.7%) as F4 (cirrhosis).

Relationship between clinico-laboratory findings and fibrosis

Univariate analysis revealed that serum viral loads, bilirubin, platelet count, AST and ALT levels were significantly different in various fibrosis stages however no statistically significant between age and gender (p=.629, p = 0.825).

As the identification of patients with advanced fibrosis is of clinical importance, the clinical and laboratory features of subjects with no/mild fibrosis (stage F0-F2) were compared with patients with advanced fibrosis (stage F3-F4) and the results are shown in table 2.

There was a significant relationship between fibrosis stages and serum indexes except AST/ALT (AAR) (p >0.05). A gradual increase in the level of AAR, APRI and FIB-4 indexes were observed with advancement of the fibrosis stages. Patients with significant fibrosis or cirrhosis had higher level of ALT, AST and lower platelet count than those without significant fibrosis or cirrhosis in univariate analysis. AST and ALT levels correlated positively (both correlation coefficients r = 0.3, p<0.05) whereas platelet count correlated negatively (r = –0.28, p =0.02) with the stage of fibrosis. In this study, the increasing AAR was associated with advanced fibrosis and a cut-off value of >1 was associated with higher risk of advanced fibrosis.

The AUROCs of the serum non-invasive indexes scores are shown in (Table 3). The FIB-4 score had the best diagnostic accuracy for advanced fibrosis (AUROC 0.68), followed by AST/ALT ratio (AUROC 0.64), and APRI (AUROC 0.62). AUROC of FIB-4 was higher than APRI (p <0.05) for differentiating minimal fibrosis from significant fibrosis (Figures 2,3). By using published cut-off values for each index, we analyzed the sensitivity and specificity of each index for significant fibrosis and cirrhosis. Patients with minimal fibrosis can be identified from advanced/significant or cirrhotic patients using APRI, FIB-4 and AAR with sensitivity of 39.3%, 81% and 23.08%; and specificity of 71.7%, 89.8% and 69.4 respectively. The discrimination of advanced from minimal fibrosis at a cut-off value for APRI >1.3, FIB-4 >2.1 and AAR>1 have 76.4%, 64.9% and 39.1% sensitivity and 88.5%, 89.7% and 76.9% specificity respectively.

As the NPV for FIB-4 score is 89.4% using the lower cut off, this test may have sufficient accuracy to be used clinically to exclude advanced fibrosis. Using this approach, a significant proportion of patients; 133/202 (65.8%) could avoid liver biopsy using this test (Table 4). The NPV for AAR and APRI using the lower cut off values are low (33.3% and 65.7%). As the PPV were modest for all noninvasive tests, ranging from 44.5% to 56.5%, it was felt they were not accurate enough to be used as an alternative to liver biopsy.

Table.1: Baseline characteristic of the studied patients according to metavir system

	F0	F1	F2	F3	F4
Mean age (yrs)±SEM	48.1±2.4	47.1±1.0	46.7±1.4	47.8±6.9	49.0±0.96
Gender (%)	16 (7.9%)	54 (26.7%)	38 (18.8%)	40 (19.8%)	54(26.7%)
ALT (UI/L) Mean ± SEM	58.7±6.0	80.8±7.1	91.8±11.04	113.4±24.4	103.71±10.9
AST (UI/L) Mean ± SEM	41.8±4.4	55.26±5.9	61.84±7.46	67.5±5.9	86.17±8.7
Bilirubin Mean ± SEM	14.0±8.9	12.0±5.6	13.0±7.2	12.0±5.6	16.6±9.8
Platelets count (10^3/mL) Mean ± SEM	217.4±13.2	207.02±7.9	204.39±11.6	199.27±9.6	178.13±8.6

Table 2. Univariate analysis of parameters between patients with and without significant fibrosis, and between patients with and without cirrhosis.

	fibrosis stage (n=202)					
	F0-1 (n=70)	F2-4 (n=132)	*P-value*	F0-3 (148)	F4 (n=54)	*P-value*
ALT (UI/L) Mean ± SEM	75.6±5.8	99.3±9.1	<0.02	89.9±7.7	103.7±10.9	<0.01
AST (UI/L) Mean ± SEM	52.2±4.7	72.49±3.9	<0.01	58.79±3.4	86.2±8.7	<0.001
Bilirubin Mean ± SEM	16.7±7.8	14.3±5.8	<0.05	12.8±6.3	16.6±9.8	<0.05
Platelets count (10^3/mL) Mean ± SEM	209.4±6.7	191.8±3.6	<0.01	205.4±5.0	178.1±8.1	<0.001
APRI Mean ± SEM	0.69±0.48	1.08±0.04	<0.01	0.77±0.56	1.32±0.09	<0.001
FIB-4 Mean ± SEM	1.44±0.78	1.64±0.08	<0.01	1.55±0.91	2.29±0.48	<0.001
AAR Mean ± SEM	0.84±0.07	1.03±0.09	>0.138	0.86±0.09	1.27±0.20	<0.001

The relationship between the fibrosis stages and three indexes: AAR, APRI and FIB-4 are illustrated in Figure 1.

Table 3. Comparison between Cut-off biochemical markers for the prediction of significant fibrosis.

FIB-4	Cut-off	AUC	Sen %	Spec%	PPV	NPV	F0-F1 (n = 70) 34.6%	F2-F4 (n=132) 65.3%
	< 2.1	0.65	64.9	89.7	56.58	92.5	61/47	7/87
	> 1.26	0.68	81.0	89.8	81.5	89.4	88/20	10/84
APRI	Cut-off		Sen %	Spec%	PPV	NPV	F0-F2 (n = 108) 53.4%	F3-F4 (n=94) 46.5
	≤ 0.8	0.62	39.3	71.7)	46.2	65.7	46/24	71/61
	> 1.3	0.76	76.4	88.5	78.5	87.1	15/55	115/17
AAR	Cut-off		Sen%	Spec%	PPV	NPV	F0-F3 (n=148)73.3%	F4(n=54) 26.75%
	<1	0.64	23.08	69.4	44.44	45.95	49/95	30/28
	>1	0.54	39.1	76.9	81.08	33.3	120/28	36/18

Table 4: Percentage of patients avoided liver biopsy using different cut-off value

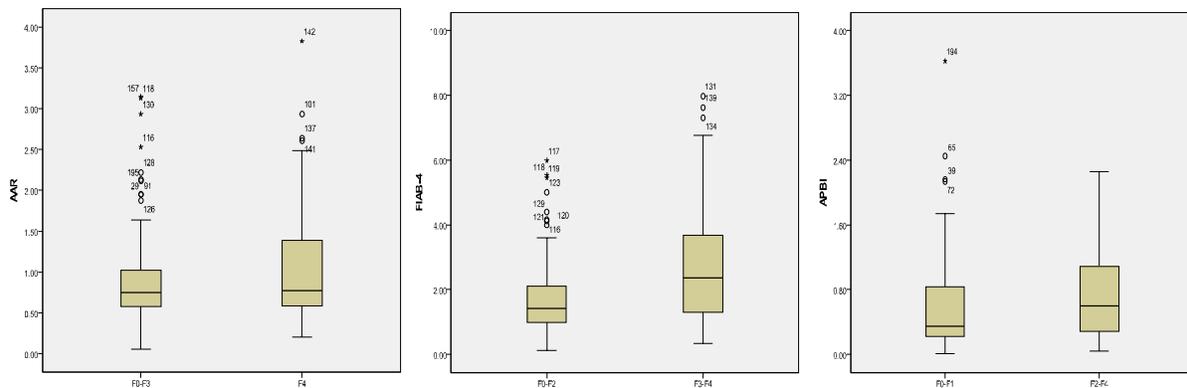|	Cut-off	Patients avoiding liver biopsy*	False negative result
FIB-4	<2.1	133/202 (65.8%)	10 (7.5%)
APRI	≤ 0.8	24/202 (11.9%)	5 (20.8%)
AAR	<1	149/202 (73.8%)	22 (14.8%)

Figure 1: Box plots of the AAR, APRI and FIB-4 for different fibrosis stages.
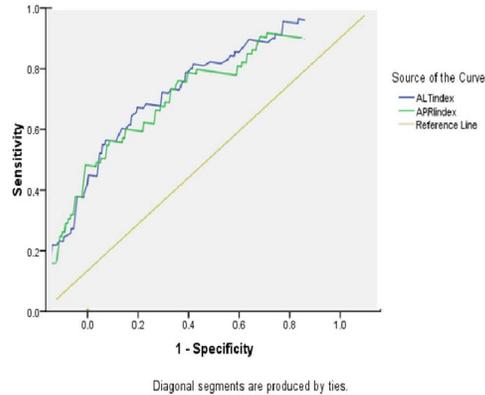
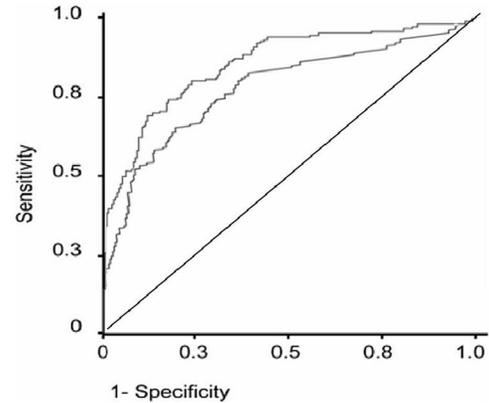**Figure 2: Diagnostic of significant Fibrosis**

Figure 3: Diagnostic of cirrhosis

4. Discussion:

The FIB-4 score was originally developed for HIV-HCV co-infection, but was confirmed also for HCV infection, with performances similar to the Fibro test for the diagnosis of severe fibrosis (F3 and F4), with AUROC 0.85[19,20].

We found that FIB-4 score has the best diagnostic value if we use a cut off value between >1.26 and <2.1. This is in contrary to others who use a cut off value between >1.45 and <3.25[19,20] and in accordance with others [21,22].

Using a cut off value of >1.26, the area under curve (AUC) was 0.68 and it has a sensitivity of 81%, specificity of 89.8%. Our results showed a high NPV (89.4%) for exclusion of advanced fibrosis in patients with chronic HCV genotype 4. This suggests that it could be used clinically to exclude advanced fibrosis in these patients. Using a cut off <2.1 FIB-4 has a PPV of 56.5% with specificity of 89.7% to confirm the presence of advanced fibrosis. Our results are in agreement with Sterling[18] and Vallet-Pichard[19] but using different cut off values. A cut off value of < 1.45 FIB-4 has a NPV for the exclusion of advanced fibrosis of 90%, while a cut off value > 3.25 has a PPV for the diagnosis of advanced fibrosis of 65%.[18] At a cut off value of < 1.45, Vallet-Pichard[19] observed a high NPV of 94.7% with a sensitivity of 74.3% to exclude severe fibrosis. Whereas, for confirming the presence of advanced fibrosis at cutoff value > 3.25, FIB-4 had a PPV of 82.1% with specificity of 98.2%.[18] When we used FIB-4 with cut off value between >1.45 and <3.25, the sensitivity was 47.9% and specificity of 84.6% and NPV of 87% with the low cut off value, while on using the high cut off value, the sensitivity was 15.2%, specificity of 84.84% and PPV of 88.9%. However, our results are in contrary to Ahmad et al[23] who observed a low NPV (70%) for excluding significant fibrosis;

however, they detected a PPV of 83% with specificity of 45% for the presence of advanced fibrosis at cut off value > 3.25.

We are like others who used a new cut off values. Trang et al.,[22] proposed new cut off values of FIB-4 ≤ 1.39 for F0-F1 and ≥2.05 for F2-F4 stage in HCV/HIV co infected patients. At these cut offs, Ahmad et al., [23] observed sensitivity 52%, specificity 76%, PPV 63% and NPV 68% for no/minimal fibrosis and 60%, 63%, 68% and 55% for advanced fibrosis, respectively. Although, they observed low statistical values, their results were in accordance to advance stage prediction. The cut off values proposed by Trang et al., better predict fibrosis stages in co infected patients. When we applied these cut off values, we have the most accurate results and we applied on only HCV infected patients.

FIB-4 can help us to avoid liver biopsy in 133 of 202 patients (65.8%) with low false negatives 10 patients (7.5%). McPherson et al., [24] reported 62% with 5% false negatives.

Strikingly, AUCs for a typical study were shown to fluctuate in a range from 0.67 to 0.98 for the same test and the same type of liver disease depending on the distribution of stages within the cohort[25]. This means that AUCs obtained in different studies should not be compared directly, but a unifying correction for the stage distribution should be performed first[26]. Our studied patients had mainly advanced fibrosis (F2-4 132; 65.3%) with less cases with minimal or no fibrosis (F0-1 70; 34.6%). Therefore, the current utility of non-invasive diagnostic scores remains limited to pre-screening allowing physician to narrow the population of patient before definitive testing of liver fibrosis by biopsy of the liver.

Stibbe et al., in 2011[27] reported that combining different non-invasive tests increases the accuracy of

diagnosis and may reduce the number of liver biopsies. However Park and coauthors specifically addressed this question and concluded that the simultaneous addition of several biomarkers adds only modestly to clinical predictive factors for the risk assessment of individual patients [28].

APRI was less sensitive than FIB-4; 39.3% with specificity of 71.7% and NPV 65.7% at a cut off <0.8. Using a cut off of >1.3, sensitivity was 76.4%, specificity 88.5% and PPV 78.5% for predicting advanced fibrosis. Various studies reported quite different performance scores for the staging of fibrosis in HCV chronic hepatitis: 41-91% sensitivity, 47-95% specificity and 60-82.7% accuracy for predicting significant fibrosis (F ≥ 2 Metavir); 38.4-65.8% sensitivity, 86.7-93% specificity and 60-88% diagnostic accuracy for predicting cirrhosis[16,29,30]. A meta-analysis[31] in 2007 proved that for a cut off value of 0.5, the APRI score had 81% sensitivity and 50% specificity for predicting significant fibrosis (F ≥ 2 Metavir) and that for a cut off value of 1, the sensitivity and specificity for predicting cirrhosis were 76% and 71%. Sirli *et al.,* in 2010[21] reported that, for a cut off value of 0.52, the APRI score had 70% sensitivity and 81% specificity for predicting significant fibrosis (F ≥ 2 Metavir), with 97% PPV and 24.5% NPV. For a cut off value of 1.38, the APRI score had 93.3% sensitivity, and 83% specificity for the diagnosis of cirrhosis. For the cut off value of 0.5 proposed by Shaheen meta-analysis[31], the APRI was slightly more sensitive (73% *vs.* 70%), but not as specific (75% *vs.* 80%) for predicting significant fibrosis (F ≥ 2 Metavir). For cirrhosis prediction, at the cut off value of 1 sensitivity remained at 93.3%, but the specificity decreased significantly to (69% *vs.* 83%). For a cut off value of 0.52, 71% (107/150) of the patients were correctly classified as having or not having significant fibrosis, and for a cut-off value of 1.38, 82% (123/150) of patients were correctly classified as having or not having cirrhosis. For a cut off value of 1, as recommended by Shaheen[31], 70.6% (106/150) were correctly classified. However recently, large meta-analysis suggested that APRI can identify hepatitis C-related fibrosis with only a moderate degree of accuracy[32].

Given the large number of Egyptian patients with chronic HCV genotype 4 who are currently referred for liver clinics for evaluation, use of these non-invasive tests could substantially reduce the number of liver biopsies being performed. This would result in significant benefit to patients by directing liver biopsy to those more likely to have advanced liver disease, as well as lead to cost savings.

On the contrary, the PPVs for the 3 tests were modest and ranging from 45% to 81%. Therefore, these tests do not have sufficient accuracy to be used to diagnose advanced fibrosis. It would therefore seem appropriate to consider liver biopsy in all patients who have a value above the lower cut off for the chosen noninvasive score. Clearly, liver biopsy may also be indicated for individuals in whom the diagnosis in uncertain or where a coexistent disease may be suspected.

More recently, hepatic fibrosis was assessed by liver stiffness measurement using transient elastography. However, transient elastography is not widely available, expensive and the success rate was poor in patients with a BMI>35..

To conclude, FIB-4 index is a new noninvasive test for the assessment of liver fibrosis. FIB-4 is more sensitive and accurate than both AAR and APRI in defining the degree of fibrosis. It can be used efficiently in cases of chronic HCV mono infection. A score of <1.26 and >2.1 enables the correct identification of patients with chronic HCV infection genotype 4 who have significant fibrosis and could avoid liver biopsy examination in 65.8% of cases. Because the FIB-4 index is readily available, inexpensive, and reproducible, it could rapidly replace expensive and/or invasive methods to assess liver fibrosis, especially in developing countries, to detect patients who need antiviral treatment and to monitor liver fibrosis progression or regression.

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References:
1. Sy T, Jamal MM. Epidemiology of hepatitis C virus (HCV) infection. *Int J Med Sci* 2006; 3:41-6.
2. Ray SC, Arthur RR, Carella A, Bukh J, Thomas D. Genetic epidemiology of hepatitis C virus throughout Egypt. *J Infect Dis* 2000; 182:698-707.
3. National Institutes of Health Consensus Development Conference Statement: management of hepatitis C: 2002-June 10-12, 2002. *Hepatology* 2002; 36:S3-20).

4. Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management and treatment of hepatitis C. *Hepatology* 2004; 39: 1147-71.

5. Cadranel JF, Rufat P, Degos F. Practices of liver biopsy in France: results of a prospective nationwide survey. For the Group of Epidemiology of the French Association for the Study of the Liver (AFEF). *Hepatology* 2000; 32:477-481.

6. Castera L, Negre I, Samii K, Buffet C. Pain experienced during percutaneous liver biopsy. *Hepatology* 1999; 1529-1530.

7. Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl *J Med* 2001; 344: 495-500.

8. Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, *et al.* Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002; 97:2614-2618.

9. The French Metavira Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. *Hepatology* 1994; 20:15-20.

10. Colloredo G, Guido M, Sonzogni A, Leandro G. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the smaller the sample, the milder the disease. *J Hepatol* 2003; 39:239-244.

11. Forns X, Ampurdanes S, Llovet JM, Aponte J, Quinto L, Martinez-Bauer E, *et al.* Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; 36:986-992.

12. Poynard T, Munteanu M, Imbert-Bismut F, Charlotte F, Thabut D, Le Calvez S, *et al.* Prospective analysis of discordant results between biochemical markers and biopsy in patients with chronic hepatitis C. *Clin Chem* 2004; 50:1344-1355.

13. McHutchison JG, Blatt LM, de Medina M, Craig JR, Conrad A, Schiff ER, *et al.* Measurement of serum hyaluronic acid in patients with chronic hepatitis C and its relationship to liver histology. Consensus Interferon Study Group. *J Gastroenterol Hepatol* 2000; 15:945-951.

14. Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; 38:1449-1457.

15. Williams AL, Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis. Relationship to cirrhosis. *Gastroenterology* 1988; 95:734-9.

16. 16. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; 38:518-526.

17. Oberti F, Valsesia E, Pilette C, Rousselet MC, Bedossa P, Aube C, *et al.* Non-invasive diagnosis of hepatic fibrosis or cirrhosis. *Gastroenterology* 1997; 113:1609-16.

18. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, Sulkowski SM, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M, APRICOT Clinical Investigators: Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; 43:1317-1325.

19. 19.Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, and Pol S. FIB-4: an Inexpensive and Accurate Marker of Fibrosis in HCV Infection. Comparison with Liver Biopsy and FibroTest. *Hepatology*, 2007; 46,(1):32-36

20. Adler M, Gulbis B, Moreno C, Evrard S, Verset G, Golstein P, Frotscher B, Nagy N, Thiry P. The Predictive Value of FIB-4 versus FibroTest, APRI, FibroIndex and Forns Index to Noninvasively: Estimate Fibrosis in Hepatitis C and Nonhepatitis C Liver Diseases. *Hepatology*, February 2008; 47(2):762-3.

21. Şirli R, Sporea I, Bota S, Popescu A, Cornianu M. A Comparative Study of Non-Invasive Methods for Fibrosis Assessment in Chronic HCV Infection. Hepatitis Monthly 2010; 10(2): 88-94.

22. Trang T, Petersen JR, and Snyder N: Non-invasive markers of hepatic fibrosis in patients co-infected with HCV and HIV: Comparison of the APRI and FIB-4 index. *Clinica Chimica Acta* 2008, 397:51–54.

23. Ahmad W, Ijaz B, Javed FT, Gull S, Kausa H, Sarwar MT, Asad S, Shahid I, Sumrin A, Khaliq S, Jahan S, Pervaiz A and Hassan S. A Comparison of Four Fibrosis Indexes in Chronic HCV. Development of New Fibrosis-cirrhosis Index (FCI). BMC *Gastroenterology*. 2011;11.

24. McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut* 2010;59:1265-1269.

25. Poynard T, Halfon P, Castera L, Munteanu M, Imbert-Bismut F, Ratziu V, Benhamou Y, Bourlière M, de Ledinghen V, FibroPaca Group: Standardization of ROC curve areas for diagnostic evaluation of liver fibrosis markers based on prevalences of fibrosis stages. *Clin Chem* 2007, 53(9):1615-22.

26. 26. Baranova A, Lal P, Birerdinc A and Younossi ZM. Non-Invasive markers for hepatic fibrosis. *BMC Gastroenterology* 2011, 11:91-106.

27. Stibbe KJM, Verveer C, Francke J, Hansen BE, Zondervan PE, Kuipers EJ, de Knegt RJ, van Vuuren A. Comparison of non-invasive assessment to diagnose liver fibrosis in chronic hepatitis B and C patients. *Erasmus Journal of Medicine* 2011, 1(2):7-13.

28. Park SH, Kim CH, Kim DJ, Suk KT, Cheong JY, Cho SW, Hwang SG, Lee YJ, Cho M, Yang JM, Kim YB: Usefulness of multiple biomarkers for the prediction of significant fibrosis in chronic hepatitis B. *J Clin Gastroenterol* 2011, 45(4):361-5.

29. Sebastiani G, Vario A, Guido M*, et al.* Stepwise combination algorithms of non-invasive markers to diagnose significant fibrosis in chronic hepatitis C. J Hepatol. 2006; 44(4):686-93.

30. Sebastiani G, Alberti A. Non invasive fibrosis biomarkers reduce but not substitute the need for liver biopsy. *World J Gastroenterol.* 2006; 12(23):3682-94.

31. Shaheen AA, Myers RP. Diagnostic accuracy of the aspartate aminotransferase-to-platelet ratio index for the prediction of hepatitis C-related fibrosis: a systematic review. *Hepatology.* 2007;46(3):912-21.

32. Lin ZH, Xin YN, Dong QJ, Wang Q, Jiang XJ, Zhan SH, Sun Y, Xuan SY:

33. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. *Hepatology* 2011, 53(3):726-36.

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