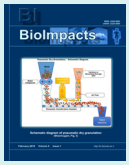
Abdollahi *et al.*, ***BioImpacts,*** 2015, 5(1), 17-23
doi: 10.15171/bi.2015.05
http://bi.tbzmed.ac.ir/

Non-invasive serum fibrosis markers: A study in chronic hepatitis

Mohammadreza Abdollahi[1], Aliasghar Pouri[2*], Morteza Ghojazadeh[2], Rasoul Estakhri[3], Mohammadhossein Somi[2]

[1] Young Researchers and Elite Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[2] Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[3] Department of Pathology, Tabriz University of Medical Sciences, Tabriz, Iran

Article Info



Article Type:
Research Article

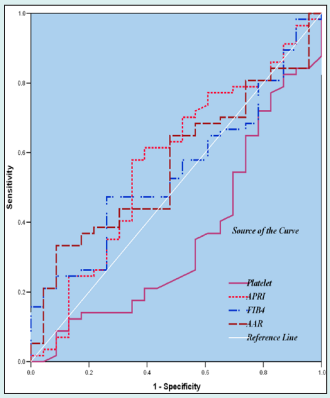
Article History:
Received: 07 July 2014
Revised: 05 Dec. 2014
Accepted: 04 Jan. 2015
ePublished: 22 Feb. 2015

Keywords:
Chronic hepatitis
Fibrosis, Grade
Noninvasive Markers
Stage

Abstract

Introduction: Chronic hepatitis is specified as inflammatory disease of the liver lasting for more than six months. Role of noninvasive fibrosis markers as prognostication factors of the presence or absence of significant fibrosis on liver biopsy of patients with chronic hepatitis is the aim of this study.

Methods: Two hundred twenty-one patients with chronic hepatitis involved in the study between 2011 and 2013. Routine biochemical indices and serum fibrosis markers such as aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio (AAR), AST to platelet ratio index (APRI) and Fibrosis 4 score (FIB-4) were evaluated, and the histological grade and stage of the liver biopsy specimens were scored according to the Ishak scoring system. Diagnostic accuracies of these markers for prediction of significant fibrosis were assessed by Receiver Operating Characteristic (ROC) curve analysis.



Results: Contemporaneous laboratory indices for imputing AAR, APRI, and FIB-4 were identified with liver biopsies. From all, 135 males (61.1%) and 86 females (38.9%), with mean age of 39.6±14.4 were studied. Significant correlation between stages of fibrosis and FIB-4, APRI and AAR were detected, with a correlation coefficient higher than that of other markers in the patients with Hepatitis B (r= 0.46), C (r= 0.58) and autoimmune hepatitis (r=0.28). FIB-4 (AUROC=0.84) and APRI (AUROC=0.78) were superior to AAR at distinguishing severe fibrosis from mild-to-moderate fibrosis and gave the highest diagnostic accuracy.

Conclusion: Application of these markers was good at distinguishing significant fibrosis and decreased the need for staging liver biopsy specimens among patients with chronic hepatitis.

Introduction

Chronic hepatitis, regardless of etiology, is defined as a continuing disease without improvement for at least six months, although in many cases the diagnosis can be made before that time.[1] Of all causes, hepatitis B, hepatitis C and autoimmune hepatitis (AIH) are the most common causes of chronic hepatitis.[2-5] Chronic hepatitis is a common reason for the abnormal liver function tests[6] and forms the background for the cirrhosis progress[7] and hepatocellular carcinoma. Although liver biopsy is expensive, it needs hospitalization for at least 6-18 hours, is invasive and carries a risk of complications with an associated morbidity rate between 0.3% and 0.6%, and with a mortality rate of 0.05%, it remains the deterministic evaluation method for liver histology.[8-10] Moreover, sampling mistakes and inter and intra observer variations may result in under staging of cirrhosis, particularly macro nodular cirrhosis.[11-14] Considering these limitations, recent studies have focused on the progress of non-invasive markers as substitutes for information provided by the percutanous liver biopsy.[15-21] Most concentration has been on the aspartate aminotransferase (AST)–to-platelet ratio index (APRI)[22-24] and the FIB-4 index,[25-27] which is calculated from AST, alanine aminotransferase (ALT), platelet count, and patient age. Thus, based on the histology, this research was performed to explore the association between noninvasive diagnostic parameters and liver biopsy findings.

Materials and methods

We studied 221 patients who had undergone percutaneous

***Corresponding author:** Aliasghar Pouri, Email: aliasghar_pouri@yahoo.com

liver biopsy due to documented hepatitis B or C infections and autoimmune hepatitis at Tabriz University of Medical Sciences clinic, Iran, from 2011 to 2013.

Histologic slides of all qualified patients were reread by one liver pathologist, who had no information about the clinical characteristics of the study patients, to avoid interobserver discrepancy. Biopsies were scored histologically using the criteria described for the Ishak system.[28] Fibrosis was determined as Ishak scores of three or more and cirrhosis as Ishak scores of five or six. None of patients had clinical, histological and biological proofs of chronic liver disease. Beside liver biopsies, a serum sample was taken from each person for further serological examinations. Serum biochemical determinations were done including total bilirubin, indirect bilirubin, ALT, AST, alkaline phosphatase (ALP), albumin, prothrombin time (PT), and platelet count. From these routine laboratory values, AAR (AST: ALT Ratio), APRI (AST: Platelet Ratio Index) and FIB-4 (Fibrosis 4 score) were calculated exactly as originally described.[22,26,29]

AST/ALT [29] = AST: ALT ratio

APRI [26] = AST level (/ULN*) / Platelet count (10^9/L) ×100 (*where ULN = upper limit of normal for that laboratory)

FIB-4 [22] = Age (years) × AST (U/L) / Platelet count (10^9/L) × [ALT (U/L)] $^{1/2}$

The results of quantitative variables are presented as Mean± SD and those of qualitative variables as numbers and percentages. Independent samples *t* test was used to compare quantitative variables, and differences between categorical variables were analyzed by chi-square or Fisher exact test. Defining the effect of different factors on histological findings in liver biopsy specimens was done by logistic regression analysis. Comparisons between the groups were performed using one-way ANOVA or Kruskal-Wallis test for unpaired data or regression analysis with the Spearman correlation coefficient test *(r)*. The difference between the groups was considered to be significant when $P \leq 0.05$.

In addition, the diagnostic value of each index to differentiate significant fibrosis (stage≥3) and mild-to-moderate fibrosis (stage 0-2) was measured by the area under the receiver operating characteristic curve (AUROC) and its corresponding 95% CI according to the procedure suggested by Hanley and McNeil.[30] All calculations were carried out using the SPSS software version 18.0.

Results

Of the 221 patients studied (mean age 39.6±14.4, range 13-83), there were 135 (61.1%) males (mean age 41.6, range 14-83), and 86 (38.9%) females (mean age 36.48, range 13-75). Ninety-five patients (mean age 40.6±13.9, range 13-69; 68 male and 27 female) had hepatitis B, 46 patients (mean age 45.7±11.1, range 23-70; 38 male and 8 female) had hepatitis C and 80 patients (mean age 35±15.3, range 13-83; 29 male and 51 female) had autoimmune hepatitis. Demographic and histological characteristics of subjects with chronic hepatitis are described in Table 1. We compared ALT, AST, ALP, total bilirubin, indirect bilirubin, albumin, PT, platelet count and mentioned serum fibrosis markers. As shown in Table 2, some of the laboratory markers were associated with liver biopsy findings to distinguish whether any other laboratory indices associated with the liver histology. Moreover, noninvasive significant fibrosis identification was done by performing the bivariate Spearman analysis on these three groups of patients considering all functional and biochemical data and looking for an association of parameters that would be able to identify this matter (Table 2). The fibrosis stage repartitions by the resembling scale were 18 (8.14%) F0; 99 (44.8%) F1; 33 (14.93%) F2; 38 (17.2%) F3; and 33 (14.93%) F4. The AAR, APRI and FIB-4 score in patients with different stages of fibrosis are shown in Table 3. Biomarker values were markedly associated with fibrosis stage levels ($P < 0.01$). Significant mean differences among biopsy fibrosis levels were indicated by mutually exclusive mean and its 95% CIs ($P < 0.05$). An increasing APRI and FIB-4 scores were noted with increasing stage of fibrosis in patients with hepatitis C (Table 4).

Noninvasive indexes such as FIB-4, APRI and AAR were associated markedly with the stage of fibrosis, with a correlation coefficient higher than that of other markers in the patients with hepatitis B (r= 0.46), C (r= 0.58) and autoimmune hepatitis (r=0.28). Weak to moderate

Table 1. Gender Specific Demographic and Histological Characteristics of Patients with Chronic Hepatitis

Groups	Gender	No. (%)	Age	Stage	Grade	AAR	APRI	FIB-4
Chronic HBV	Male	68 (71.6)	42.26±13.79	1.99±1.67	4.40±2.83	0.91±0.38	1.24±2.05	1.91±1.88
	Female	27 (28.4)	36.37±13.68	2.04±1.80	4.37±3.04	0.97±0.49	0.81±0.55	1.42±1.18
Chronic HCV	Male	38 (82.6)	45.24±11.99	2.05±1.48	4.47±2.52	0.91±0.33	1.12±0.93	1.93±1.19
	Female	8 (17.4)	48.00±4.03	1.75±1.58	5.75±4.40	1.14±0.54	0.96±1.03	2.42±2.34
Chronic AIH	Male	29 (36.3)	35.38±19.41	3.59±1.47	5.45±2.89	0.90±037	1.41±1.35	1.63±1.24
	Female	51 (63.7)	34.75±12.67	3.20±1.64	6.24±3.10	1.05±0.43	2.44±3.42	2.08±1.45

Abbreviations: HBV, Hepatitis B virus; HCV, Hepatitis C Virus; AIH, Autoimmune Hepatitis.
All Values are mean ± SD; otherwise noted.

Table 2. Correlation of Grade (Modified Hepatic Activity Index) and Stage (Ishak Fibrosis Score) With Standard Laboratory Parameters and Simple Fibrosis Tests

		Bivariate Spearman's Rank Correlation Coefficient					
		HBV		HCV		AIH	
Parameters	Mean ±SD	Grade	Stage	Grade	Stage	Grade	Stage
AST, IU/L	92.2 ±140.3	0.38†	0.27‡	0.52†	0.56†	0.34†	0.002
ALT, IU/L	106.49±145.5	0.27‡	0.15	0.38‡	0.49†	0.19‡	-0.14
Total bilirubin, mg/dL	2.33±4.36	0.15‡	0.22‡	0.04	0.34‡	0.20‡	0.12
Direct bilirubin, mg/dL	0.96±2.84	0.23	0.23‡	-0.001	0.31‡	0.19‡	0.11
ALP, IU/L	369.12±339.93	0.36†	0.28‡	0.01	0.25‡	0.23‡	0.26‡
Platelet count (10^9/L)	194.3±49.5	-0.52†	-0.40†	-0.30‡	-0.35‡	-0.38†	-0.31‡
PT, s	13.83±1.45	0.38†	0.27‡	0.1	0.32‡	0.17	0.20‡
Albumin, g	3.99±0.67	-0.28‡	-0.34†	-0.002	- 0.25‡	-0.09	-0.11
AAR	0.96±0.41	0.13	0.16	0.38‡	0.25	0.25‡	0.28‡
APRI	1.46±2.17	0.54†	0.41†	0.52†	0.58†	0.44†	0.07
FIB-4	1.88±1.55	0.59†	0.46†	0.51†	0.50†	0.38†	0.05

Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, Alkaline Phosphatase; PT, Prothrombin Time; AAR, AST/ALT Ratio; APRI, AST to Platelet Ratio Index; FIB-4, Fibrosis 4 score; HBV, Hepatitis B virus; HCV, Hepatitis C Virus; AIH, Autoimmune Hepatitis.
† $P < 0.001$.
‡ $P < 0.05$.

Table 3. Correlation of Chronic Hepatitis Stage by Invasive (Liver Biopsy Staging) and Noninvasive (ARR, APRI and FIB-4) Scores

Degree of Fibrosis (Stage) (N)	Ishak	Mean AAR (95% CI)	Mean APRI (95% CI)	Mean FIB-4 (95% CI)
No fibrosis (F0) (n = 18)	Stage 0	1.04 (0.83-1.25)	1.08 (0.21-1.95)	1.50 (0.95-2.04)
Fibrous portal expansion (F1) (n = 99)	Stage 1, 2	0.87 (0.80-0.93)	1.09 (0.70-1.47)	1.41 (1.15-1.66)
Few bridges or septa (F2) (n = 33)	Stage 3	0.93 (0.80-1.05)	1.96 (0.69-3.22)	2.07 (1.48-2.65)
Numerous bridges or septa (F3) (n = 38)	Stage 4	0.96 (0.80-1.12)	1.75 (1.36-2.13)	2.23 (1.81-2.66)
Cirrhosis (F4) (n = 33)	Stage 5, 6	1.23 (1.06-1.40)	1.94 (1.24-2.64)	2.89 (2.16-3.61)

Abbreviations: AAR, AST/ALT Ratio; APRI, AST to Platelet Ratio Index; FIB-4, Fibrosis 4 score.

Table 4. Correlation of Chronic Hepatitis Stage by Invasive (Liver Biopsy Staging) and Noninvasive (ARR, APRI and FIB-4) Scores in All Three Groups

Groups	Stage 0	Stage 1, 2	Stage 3	Stage 4	Stage 5, 6
Chronic HBV					
AAR	1.05 (0.75-1.34)	0.85 (0.76-0.94)	0.77 (0.57-0.98)	1.19 (0.74-1.65)	1.05 (0.77-1.33)
APRI	0.70 (0.53-0.87)	0.99 (0.44-1.55)	0.67 (0.42-0.92)	1.26 (0.96-1.55)	2.45 (0.71-4.2)
FIB-4	1.43 (0.66-2.2)	1.31 (0.87-1.76)	1.49 (0.69-2.29)	2.39 (1.75-3.04)	3.95 (2.49- 5.4)
Chronic HCV					
AAR	1.07 (0.41-1.72)	0.86 (0.74-0.98)	0.99 (0.58-1.41)	0.92 (0.61-1.23)	1.63 (0.16-3.1)
APRI	0.4 (-0.002-0.81)	0.77 (0.55-0.99)	1.21 (0.22-2.19)	2 (0.93-3.08)	2.93 (0.36-5.5)
FIB-4	1.26 (0.57-1.95)	1.46 (1.14-1.78)	1.92 (0.81-3.02)	3.52 (2.89-4.14)	5.32 (-0.56-11.2)
Chronic AIH					
AAR	0.93 (-2.04-3.91)	0.92 (0.77-1.07)	0.99 (0.79-1.19)	0.85 (0.67-1.02)	1.27 (1.05-1.5)
APRI	4.77 (-35.26-44.8)	1.73 (0.49-2.98)	2.98 (0.53-5.44)	1.94 (1.3-2.58)	1.49 (0.72-2.26)
FIB-4	2.39 (-10.32-15.1)	1.58 (1.18-1.98)	2.47 (1.43-3.5)	1.79 (1.18-2.4)	1.89 (1.32-2.47)

Abbreviations: HBV: Hepatitis B virus; HCV: Hepatitis C Virus; AIH: Autoimmune Hepatitis.
All Values are Mean (95% CI); otherwise noted.

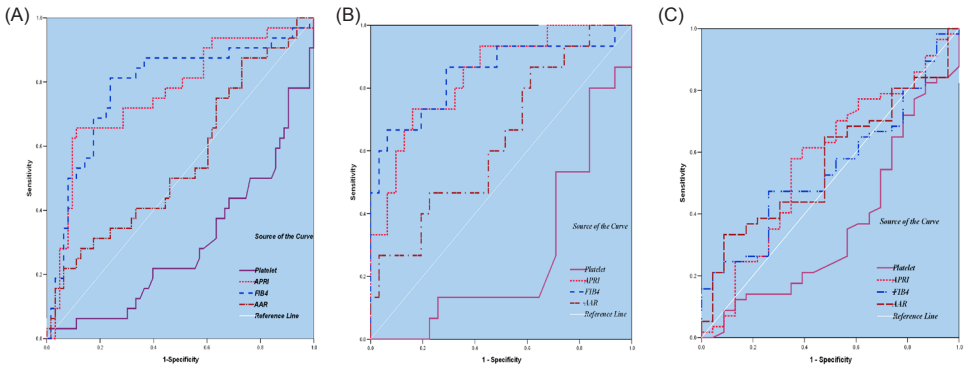


Fig. 1. Receiver operating characteristic (ROC) curves of four simple noninvasive tests for prediction of significant fibrosis (F3-F6) according to the Ishak system in the (A) Hepatitis B (B) Hepatitis C and (C) Autoimmune hepatitis patients. An AUC of 1.0 is characteristic of an ideal test, whereas an AUC of 0.5 or less indicates a test of no diagnostic value. AAR: AST/ALT ratio; APRI: AST to-platelet ratio index; FIB-4: Fibrosis 4 Score.

Table 5. Performance of Simple Fibrosis Tests for Prediction of Significant Fibrosis (F3–F6) in Patients With Chronic Hepatitis

Groups	HBV			HCV			AIH		
Ishak Fibrosis Score vs.	Cutoff	Se/Sp	AUROC (95% CI)	Cutoff	Se/Sp	AUROC (95% CI)	Cutoff	Se/Sp	AUROC (95% CI)
AST	52.5	66/70	0.7(0.59-0.81)	42.5	87/61	0.82(0.69-0.94)	77.5	53/65	0.54(0.4-0.68)
ALT	67.5	47/65	0.6(0.48-0.72)	47	87/61	0.73(0.58-0.88)	84.5	51/61	0.49(0.36-0.63)
PT	13.35	53/75	0.63(0.51-0.76)	13.5	60/77	0.66(0.48-0.83)	13.9	56/61	0.61(0.48-0.73)
Platelet count	221	22/60	0.29(0.18-0.4)	139	80/16	0.27(0.12-0.42)	238.5	12/87	0.36(0.22-0.49)
AAR	1.37	22/94	0.55(0.43-0.68)	0.72	87/39	0.64(0.46-0.81)	1.18	33/91	0.57(0.44-0.7)
APRI	0.96	66/89	0.76(0.65-87)	1.06	73/84	0.84(0.72-0.96)	1.12	58/65	0.57(0.43-0.71)
FIB-4	1.36	81/76	0.78(0.68-0.89)	2.76	67/94	0.84(0.71-0.98)	1.82	47/74	0.55(0.41-0.68)

Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; PT, Prothrombin Time; AAR, AST/ALT Ratio; APRI, AST to Platelet Ratio Index; FIB-4, Fibrosis 4 score; Se, Sensitivity; Sp, Specificity.

correlations were found among Ishak stages of fibrosis versus APRI, FIB-4, AST, Direct bilirubin, Total bilirubin, PT and ALP in both patients with hepatitis B and C infections. In all three groups of patients there were also moderate opposite relationship between platelet count and Ishak stage of fibrosis. Hepatic fibrosis weakly correlated with PT, ALP and AAR in patients with AIH (Table 2).

Prediction of significant fibrosis was done by constructing ROC curves measuring the diagnostic precisions of AAR, APRI, FIB- 4 and platelet count (Fig. 1). Exceptional diagnostic precision of APRI over AAR for prediction of notable fibrosis concluded by comparing AUROCs for continuous variables by the procedure proposed by Hanley and McNeil,[30] especially in patients with hepatitis B and C (Table 5). Optimal cutoff point for AAR to determine considerable fibrosis in hepatitis C was ≥ 0.7, with a sensitivity of 87% and specificity of 39%.

Discussion

At present, pathological examination of liver puncture tissue is the way to diagnose liver fibrosis. Usage of liver biopsy because of its invasive trait and sampling errors is still limited in clinical practice, although it is the gold standard.[31,32] Searching for noninvasive markers to diagnose liver fibrosis has demanded great attention.[33-35] Comparing pathological classification with some non-invasive markers to appraise importance of these markers in expressing pathological differences in three different groups of patients with chronic hepatitis was the main goal of this study.

In a study of long-term outcome of chronic hepatitis B based on histological grade and stage it was concluded that the serum ALT level at the time of liver biopsy was significantly correlated with the grades of lobular and porto-periportal activity. The results proved that histological grade and stage, and biochemical profile during follow-up were important prognostic factors in patients with chronic hepatitis B.[36] In an Italian multi-center study about clinical course and outcome, it was concluded that age, AST, ALT, PT, Albumin level and total bilirubin were prognostic factors,[37] although present study just suggested PT and ALP as prognostic factors in patients with AIH.

The relationship between platelet count and liver fibrosis in patients with chronic hepatitis C has been attractive topic for researchers.[18,22] However, prognostication of liver fibrosis with diagnostic value of platelet count per se have been assessed in only a few studies on these patients.[38-40] In contrast with our findings, diagnostic preciseness of prothrombin time and platelet count in patients with

chronic hepatitis C was assessed by Myers et al[40] and they reported an AUROC of 0.67 for platelet count for prediction of F2-F4 fibrosis (METAVIR system).[40]

Consistent with our findings, in a large observational real-world cohort of chronic hepatitis C patients, FIB-4 and APRI were superior to AAR at distinguishing severe fibrosis from mild-to-moderate fibrosis.[41] Low diagnostic accuracy of AAR in predicting significant fibrosis in patients with hepatitis C was reported by Lackner et al[42] (AUROC 0.57).[42] In this study, we found that the optimal cutoff AAR value for diagnosing significant fibrosis in hepatitis C was ≥ 0.7, with a sensitivity of 87% and specificity of 39%. These results are in contrast with previous findings by Fouad et al;[43] who recommended an AAR value ≥ 1.2 as a cutoff value for diagnosing fibrosis.[43] This study revealed a significant correlation between APRI and both the stage of liver fibrosis and the grade of activity. The optimal cutoff APRI value for the diagnosis of fibrosis in hepatitis C group was ≥ 1.06 that was consistent with findings by Hsieh et al;[44] who reported cutoff values of ≥1 with a sensitivity of 75.5% and specificity of 41.5%. Furthermore our study is consistent with that of Hongbo et al, [45] in which the area under the ROC of APRI was also modest (0.76) in patients with hepatitis B infection.[45] Yilmaz et al[15] reported that the APRI had an acceptable accuracy for the assessment of liver fibrosis in patients with chronic hepatitis C, but not in those with chronic hepatitis B.[15] Parsian et al[46] reported differences between severe and mild liver fibrosis by APRI with 29.0% sensitivity and 22.0% specificity.[46]

In another study Zhang et al[47] assessed the diagnostic value of FIB-4 in 212 patients with chronic hepatitis B by comparing their results with histological features.[47] The AUROC of FIB-4 for significant fibrosis was 0.733. Mahassadi et al[48] had conducted a prospective cohort study to determine the diagnostic accuracy of APRI, AAR, AP and FIB-4 index for the prediction of significant fibrosis or cirrhosis in 117 patients with chronic hepatitis B and APRI and FIB-4 index ruled out significant fibrosis with high specificity of 84.7% and 86.1%, respectively.[48] Distinguishing severe stages (F3–F4) from low or moderate stages (F0–F2) of fibrosis with high FIB-4 scores (e.g. ≥ 2.25) was studied in some researches to date.[49,50] The FIB-4 index may be of value in several respects including simple calculations and no standardization, immediate results during the patient visit and its inexpensiveness with no additional costs.[26]

Conclusion

In conclusion, the current study demonstrated that these low-cost-and-easy-to perform serum fibrosis markers, especially APRI and FIB-4, were simple methods that correlated well with the stages of fibrosis in patients with chronic hepatitis. The combination of these non-invasive markers may replace the requirement for liver biopsy. Therefore further studies with more patients are needed to evaluate these markers.

Research Highlights

What is current knowledge?

√ Hepatitis B, hepatitis C and autoimmune hepatitis (AIH) are the most common causes of chronic hepatitis.

√ Liver biopsy is the deterministic evaluation method for liver histology.

√ Usage of liver biopsy because of its invasive trait and sampling errors is still limited in clinical practice.

What is new here?

√ Low-cost-and-easy-to perform serum fibrosis markers, especially APRI and FIB-4, were simple methods that correlated well with the stages of fibrosis in patients with chronic hepatitis.

√ FIB-4 and APRI were superior to AAR at distinguishing severe fibrosis from mild-to-moderate fibrosis.

√ Significant correlation revealed between APRI and both the stage of liver fibrosis and the grade of activity.

Competing interests

The authors express no opposition of profits.

Ethical issues

There is none to be declared.

References

1. Levy CM, Popper H, Sherlock S. Diseases of the Liver and Biliary Tract: Standardization of Nomenclature, Diagnostic Criteria, and Prognosis. Washington, DC: National Institutes of Health; NIH publication; Fogarty International Center Proceeding 22, **1976**; 76-725
2. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* **2009**; 49(4):1335-74. doi: 10.1002/hep.22759
3. Lok AS, McMahon BJ. Chronic hepatitis B. *Hepatology* **2007**; 45(2): 507-39. doi: 10.1002/hep.21513
4. Czaja AJ. Performance parameters of the conventional serological markers for autoimmune hepatitis. *Digestive diseases and sciences* **2011**; 56(2): 545-54. doi: 10.1007/s10620-010-1501-1
5. Abdollahi MR, Somi MH, Faraji E. Role of international criteria in the diagnosis of autoimmune hepatitis. *World J Gastroenterol* **2013**; 19(23): 3629-33. doi: 10.3748/wjg.v19.i23.3629
6. Berasain C, Betes M, Panizo A, Ruiz J, Herrero J I, Civeira MP, et al. Pathological and virological findings in patients with persistent hypertransaminasaemia of unknown aetiology. *Gut* **2000**; 47:429-435. doi:10.1136/gut.47.3.429
7. Hano H, Takasaki S. Three-dimensional observations on the alterations of lobular architecture in chronic hepatitis with special reference to its angio architecture for a better understanding of the formal pathogenesis of liver cirrhosis. *Virchows Arch* **2003**; 443:655-663. doi: 10.1007/s00428-003-0843-x
8. Degos F, Perez P, Roche B, Mahmoudi A, Asselineau J, Voitot H, et al. Diagnostic accuracy of FibroScan and comparison to liver fibrosis biomarkers in chronic viral hepatitis: a multicenter prospective study (the FIBROSTIC

study). *J Hepatol* **2010**; 53: 1013-1021. doi:10.1016/j.jhep.2010.05.035
9. Poynard T, Imbert-Bismut F, Munteanu M, Messous D, Myers RP, Thabut D, et al. Overview of the diagnostic value of biochemical markers of liver fibrosis (FibroTest, HCV FibroSure) and necrosis (ActiTest) in patients with chronic hepatitis C. *Comparative Hepatology* **2004**; 3(1): 8. doi: 10.1186/1476-5926-3-8
10. Cadranel JF, Rufat P, Degos F. Practices of liver biopsy in France: results of a prospective nationwide survey. *Hepatology* **2000**; 32 (3): 477-481. Doi: 10.1053/jhep.2000.16602
11. Bedossa P. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. *Hepatology* **1994**; 20: 15-20. doi: 10.1002/hep.1840200104
12. Bedossa P, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* **2003**; 38: 1449-1457. doi: 10.1016/j.hep.2003.09.022
13. Maharaj B, Maharaj RJ, Leary WP, Cooppan RM, Naran AD, Pirie D, et al. Sampling variability and its influence on the diagnostic yield of percutaneous needle biopsy of the liver. *Lancet* **1986**; 1: 523-525. doi:10.1016/S0140-6736(86)90883-4
14. 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. doi: 10.1373/clinchem.2004.032227
15. Yilmaz Y, Yonal O, Kurt R, Bayrak M, Aktas B, Ozdogan O. Noninvasive assessment of liver fibrosis with the aspartate transaminase to platelet ratio index (APRI): Usefulness in patients with chronic liver disease. *Hepat Mon* **2011**; 11: 103-106.
16. Leroy V. Other non-invasive markers of liver fibrosis. *Gastroenterol Clin Biol* **2008**; 32: 52-57
17. Pinzani M. Non-invasive evaluation of hepatic fibrosis: don't count your chickens before they're hatched. *Gut* **2006**; 55: 310-312. doi: 10.1136/gut.2005.068585
18. Pohl A, Behling C, Oliver D, Kilani M, Monson P, Hassanein T. Serum aminotransferase levels and platelet counts as predictors of degree of fibrosis in chronic hepatitis C virus infection. *Am J Gastroenterol* **2001**; 96: 3142-3146. doi: 10.1111/j.1572-0241.2001.05268.x
19. Sebastiani G, Alberti A. Noninvasive fibrosis biomarkers reduce but not substitute the need for liver biopsy. *World J Gastroenterol* **2006**; 12: 3682-3694. doi: 10.3748/wjg.v12.i23.3682
20. Leroy V, Halfon P, Bacq Y, Boursier J, Rousselet MC, Bourlière M, et al. Diagnostic accuracy, reproducibility and robustness of fibrosis blood tests in chronic hepatitis C: a meta-analysis with individual data. *Clin Biochem* **2008**; 41: 1368-1376. doi:10.1016/j.clinbiochem.2008.06.020
21. Pinzani M, Vizzutti F, Arena U, Marra F. Technology Insight: noninvasive assessment of liver fibrosis by biochemical scores and elastography. *Nat Clin Pract Gastroenterol Hepatol* **2008**; 5: 95 106. doi:10.1038/ncpgasthep1025
22. Wai CT, Greenson JK, Fontana RJ. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatol*ogy **2003**; 38:518–26. doi: 10.1053/jhep.2003.50346
23. Shaheen AAM, 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**; 46:912–21. doi: 10.1002/hep.21835
24. Lin ZH, Xin YN, Dong QJ, Wang Q, Jiang X J, Zhan SH, et al. Performance of the aspartate aminotransferase- to-platelet ration index for staging of hepatitis C-related fibrosis: an updated meta-analysis. *Hepatology* **2011**; 53:726–36. doi: 10.1002/hep.24105
25. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV co infection. *Hepatology* **2006**; 43:1317–25. doi: 10.1002/hep.21178
26. Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. Comparison with liver biopsy and Fibrotest. *Hepatology* **2007**; 46:32–36. doi: 10.1002/hep.21669
27. Adler M, Gulbis B, Moreno C, Evrard S, Verset G, Golstein P, et al. The predictive value of FIB-4 versus Fibrotest, APRI, Fibroindex and Forn Index to non-invasively estimate fibrosis in hepatitis C and nonhepatitis C liver diseases. *Hepatology* **2008**; 47:762–3. doi: 10.1002/hep.22085
28. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat J. et al. Histological grading and staging of chronic hepatitis. *J Hepatol* **1995**; 22: 696-699. doi:10.1016/0168-8278(95)80226-6
29. Bonacini M, Hadi G, Govindarajan S, Lindsay KL. Utility of a discriminant score for diagnosing advanced fibrosis or cirrhosis in patients with chronic hepatitis C virus infection. *Am J Gastroenterol* **1997**; 92:1302-1304.
30. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology* **1983**; 148: 839-843. doi: 10.1148/radiology.148.3.6878708
31. Lu LG, Zeng MD, Wan MB, Li CZ, Mao YM, Li JQ, et al. Grading and staging of hepatic fibrosis, and its relationship with noninvasive diagnostic parameters. *World J Gastroenterol* **2003**; 9(11): 2574-2578
32. Bravo AA, Sheth SG, Chopra S. Liver biopsy. *N Engl J Med* **2001**; 344(7): 495–500. Doi: 10.1056/NEJM200102153440706
33. Pilette C, RousseletMC, Bedossa P, Chappard D, Oberti F, Rifflet H, et al. Histopathological evaluation of liver fibrosis: quantitative image analysis vs semi-quantitative scores. Comparison with serum markers. *J Hepatol* **1998**; 28: 439-446. doi:10.1016/S0168-8278(98)80318-8
34. Thabut D, Simon M, Myers RP, Messous D, Thibault V, Imbert- Bismut F, et al. Noninvasive prediction of fibrosis in patients with chronic hepatitis C. *Hepatology* **2003**; 37: 1220-1221. doi: 10.1053/jhep.2003.50109
35. Myers RP, Ratziu V, Imbert-Bismut F, Charlotte F, Poynard T. Biochemical markers of liver fibrosis: a comparison with historical features in patientswith chronic hepatitis C. *Am J Gastroenterol* **2002**; 97: 2419-2425. doi:10.1111/j.1572-0241.2002.05997.x
36. Park BK, Park YN, Ahn SH, Lee KS, Chon CY, Moon YM, et al. Long-term outcome of chronic hepatitis B based on histological grade and stage. *J Gastroenterol Hepatol* **2007**; 22(3):383-8. doi: 10.1111/j.1440-1746.2007.04857.x
37. Floreani A, Niro G, Rosa Rizzotto E, Antoniazzi S, Ferrara F, Carderi I, et al. Type I autoimmune hepatitis: clinical course and outcome in an Italian multicentre study. *Aliment Pharmacol Ther* **2006**; 24(7):1051-7. Doi: 10.1111/j.1365-2036.2006.03104.x

38. Giannini E, Risso D, Botta F, Chiarbonello B, Fasoli A, Malfatti F, et al. Validity and clinical utility of the aspartate aminotransferase-alanine aminotransferase ratio in assessing disease severity and prognosis in patients with hepatitis C virus-related chronic liver disease. *Arch Intern Med* **2003**; 163:218-224. doi:10.1001/archinte.163.2.218
39. Murawaki Y, Koda M, Okamoto K, Mimura K, Kawasaki H. Diagnostic value of serum type IV collagen test in comparison with platelet count for predicting the fibrotic stage in patients with chronic hepatitis C. *J Gastroenterol Hepatol* **2001**;16:777-781. doi: 10.1046/j.1440-1746.2001.02515.x
40. Myers RP, De Torres M, Imbert-Bismut F, Ratziu V, Charlotte F, Poynard T. Biochemical markers of fibrosis in patients with chronic hepatitis C: a comparison with prothrombin time, platelet count, and AP index. *Dig Dis Sci* **2003**; 48:146-153. doi: 10.1023/A:1021702902681
41. Holmberg S., Lu M.et al. Noninvasive Serum Fibrosis Markers for Screening and Staging Chronic Hepatitis C Virus Patients in a Large US Cohort. *CID* **2013**:57: 240-246. doi: 10.1093/cid/cit245
42. Lackner C, Struber G, Liegl B, Leibl S, Ofner P, Bankuti C, et al. Comparison and Validation of Simple Noninvasive Tests for Prediction of Fibrosis in Chronic Hepatitis C. *Hepatology* **2005**; 41:1376-1382. doi: 10.1002/hep.20717
43. Fouad SA, Esmat S, Omran D, Rashid L, Kobaisi MH. Noninvasive assessment of hepatic fibrosis in Egyptian patients with chronic hepatitis C virus infection. *World J Gastroenterol* **2012**; 18(23): 2988-2994. doi: 10.3748/wjg.v18.i23.2988
44. Hsieh YY, Tung SY, Lee K, Wu CS, Wei KL, Shen CH, et al. Routine blood tests to predict liver fibrosis in chronic hepatitis C. *World J Gastroenterol* **2012**; 18(8): 746-753. doi: 10.3748/wjg.v18.i8.746
45. Hongbo L, Xiaohui L, Hong K, Wei W, Yon Z. Assessing routine and serum markers of liver fibrosis in chronic hepatitis B patients using parallel and serial interpretation. *Clin Biochem* **2007**; 40: 562-566. doi: 10.1016/j.clinbiochem.2007.01.022
46. Parsian H, Nouri M, Rahimipour A, Somi MH, Qujeq D. *Comparison of Five Liver Fibrosis Indexes with Serum Levels of Laminin and N Terminal Peptide of Procollagen Type III in Chronic Hepatitis Patients*. INTECH;**2011**.
47. Zhang YF, Shi H, Chen LB, Xu QH. Value of FIB-4 for the diagnosis of liver fibrosis in chronic hepatitis B. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* **2010**; 24(3): 215-7.
48. Mahassadi AS, Attia AF, Bathaix FMY, Agbé1 NB, Doffou S, Kissi1 HY, et al. Diagnostic accuracy of biochemical markers of fibrosis in black African patients with chronic hepatitis B. *Health* **2010**; 2: 1413-1420. doi:10.4236/health.2010.212210
49. Poynard T, Ngo Y, Perazzo H, Munteanu M, Lebray P, Moussalli J, et al. Prognostic value of liver fibrosis biomarkers: a meta-analysis. *Gastroenterol Hepatol* **2011**; 7:445–54.
50. Mummadi RR, Petersen JR, Xiao SY, Snyder N. Role of simple biomarkers in predicting fibrosis progression in HCV infection. *World J Gastroenterol* **2010**; 16:5710–5. doi: 10.3748/wjg.v16.i45.5710