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Noninvasive biomarkers FibroTest and ActiTest versus liver biopsy in chronic hepatitis C patients: the Middle East experience

Rafie Yakoob[a], Issam Al Bozom[b], Ragesh Babu Thandassery[a], Mohamed Osman Abdel Rahman[c], Moutaz F. Derbala[a], Muneera J. Al Mohannadi[a], Anil K. John[a], Manik Sharma[a], Hamidulla Wani[a], Saad Al Kaabi[a]

Hamad General Hospital, Doha, Qatar

Abstract

Background The aim of this study was to compare noninvasive biomarkers, FibroTest and ActiTest in predicting fibrosis stage and inflammation grade in chronic hepatitis C (CHC) patients with liver biopsy (LB).

Methods In 107 patients with CHC, levels of six serum biomarkers (alanine aminotransferase, γ-glutamyl transpeptidase, total bilirubin, haptoglobin, apolipoprotein, α-2 macroglobulin) were determined at the time of LB. LB was evaluated by Metavir score for fibrosis and inflammation. Voluntary blood donors (n=106) were taken as controls for the study.

Results Fibrosis estimated by Fibrotest was significantly higher in patients compared to control group. The observed area under the receiver operating characteristic curve (AUROC) for advanced fibrosis (F3, F4) adjusted according to the observed difference between advanced and non-advanced fibrosis prevalence (DANA) was 0.80 (0.69-0.88) and the AUROC for cirrhosis (F4) was 0.94 (0.86-0.98). ActiTest AUROC for moderate to severe activity (A2A3) was 0.72 (0.61-0.81), and for severe activity (A3) was 0.88 (0.78-0.93). The diagnostic values in the group of good quality biopsy (n=41) showed Fibrotest AUROC (DANA-adjusted): for advanced fibrosis 0.90 (0.72-0.99); for cirrhosis 0.93 (0.76-0.98); and ActiTest AUROC: for moderate/severe activity 0.86 (0.67-0.94); and for severe activity 0.90 (0.76-0.93). There was good concordance between FibroTest and LB (with discordance for two or more stages in <20% for advanced fibrosis and <10% for cirrhosis) and between ActiTest and LB. Specificity for FibroTest and ActiTest in the control population were 95% and 100% respectively.

Conclusions Fibrotest and ActiTest had high observed and standardized diagnostic values for predicting fibrosis and activity respectively.

Keywords Liver biopsy, ActiTest, Fibrotest, hepatitis C virus

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Introduction

Liver injury due to chronic hepatitis C (CHC) infection seems to be responsible for induction of fibrogenesis. Assessment of fibrosis is important 1) to estimate the prognosis; 2) for decision on antiviral therapy; and 3) for surveillance of hepatocellular carcinoma [1,2]. Liver biopsy (LB) is widely regarded as the gold standard for assessing hepatitis C virus (HCV)-related fibrosis but the sampling variability (40% for fibrosis staging), a high intra- and inter-pathologist variability, and adverse events limit its utility. In addition, a LB samples only 1/50,000th of the liver parenchyma [3-5].

There are numerous histological scoring systems proposed for grading of inflammation and the staging of fibrosis (Metavir, Ishak, and Scheuer) [6]. Two panels of simple biochemical markers have emerged as an alternative to LB in patients with CHC and other forms of chronic liver disease; FibroTest (FT) and ActiTest (AT) [7,8]. The fibrosis index (FT) consists of an algorithm of five fibrosis markers, α-2 macroglobulin (A2M), apolipoprotein A1, haptoglobin, total bilirubin, and γ-glutamyl transpeptidase (GGT), and the necroinflamatory activity index (AT) combines the same 5 markers, plus alanine

[a]Division of Gastroenterology and Hepatology (Rafie Yakoob, Ragesh Babu Thandassery, Moutaz F. Derbala, Muneera J. Al Mohannadi, Anil K. John, Manik Sharma, Hamidulla Wani, Saad Al Kaabi); [b]Department of Laboratory Medicine and Pathology (Issam Al Bozom); [c]Department of Biochemistry (Mohamed Osman Abdel Rahman), Hamad General Hospital, Doha, Qatar

Conflict of Interest: None

Correspondence to: Ragesh Babu Thandassery, Division of Gastroenterology and Hepatology, Hamad General Hospital, Doha, Qatar, e-mail: doc.ragesh@gmail.com

Guarantor of the article: Rafie Yakoob

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aminotransferase (ALT). These panels demonstrated high predictive values for significant disease in patients with CHC [9]. Recent studies strongly suggest that due to the limitation and risk of LB, as well as, the improvement of the diagnostic accuracy of biochemical markers, LB need not be considered mandatory in all patients with CHC [10]. Since September 2002 these tests (FT and AT) have been used in several countries as an alternative to LB. A recent study compared percutaneous biopsy with laparoscopic biopsy, demonstrating that cirrhosis was missed in almost 30% of cases by percutaneous biopsy. These studies indicate that the potential for error in staging disease can be as high as 40% and that even cirrhosis can be missed in up to 30% of patients. Clearly LB is not the gold standard [5].

The aims of this study were: 1) to estimate the observed diagnostic value of FT by area under the receiver operating characteristic curve (AUROC) and the standardized AUROC for advanced fibrosis and cirrhosis, using LB as reference; 2) to estimate the diagnostic value (AUROC) for AT for moderate to severe and severe necroinflammatory activity using LB as reference; and 3) to evaluate the concordance between biomarkers and histology on LB.

Patients and methods

This is prospective study done in a tertiary care hospital in Doha, Qatar, according to the regulations of the research and ethics committee. A total of 110 patients referred to our center were studied. The inclusion criteria were: reactive to anti HCV antibody test (i.e. HCV-Ab positive); and detectable HCV RNA for the last 6 months. Exclusion criteria included: alcohol consumption regardless its quantity; concomitant liver diseases (presence of HBs antigen, autoimmune hepatitis, hemochromatosis diagnosed by genetic markers, Wilson's disease, and α1 anti-trypsin deficiency); and HIV-Ab positivity.

Signed informed consent was obtained from all patients before their inclusion. LB and biochemical markers, abdominal ultrasound, and coagulation profile were performed on the same day. All LBs were done bedside by a hepatologist using Menghini aspiration needle, 16 G. LB was analyzed by a single histopathologist. A total of 111 voluntary blood donors were prospectively included in the control group. The aim of analyzing seromarkers in this group was to determine the specificity of FT in controls.

Histological analysis

LBs were fixed in 10% buffered formalin, embedded in paraffin; 3-μm thick sections were stained with hematoxylin-eosin, PAS, PAS-diastase, reticulin stain, Perls'-iron staining, and Masson's trichrome stain. Biopsies were interpreted by a single, experienced histopathologist, blinded to patient clinical characteristics and serum biochemical markers. Fibrosis was staged according to Metavir scoring system. Fibrosis was scored on a 5-point scale: stage zero (F0): no fibrosis; stage one (F1): portal fibrosis alone; stage two (F2): portal fibrosis with rare septa; stage three (F3): portal fibrosis with many septa; and stage four (F4): cirrhosis. The presence of stages F2, F3 or F4 was termed "significant fibrosis", whereas the term "advanced fibrosis" was reserved for stages F3 or F4. The biopsies were judged as adequate (or of good quality) if the number of portal tracts was at least 6 and the length of LB at least 15 mm. Necroinflammatory activity, based on assessment of piecemeal and lobular necrosis, was graded on a 4-point scale: A0, no activity; A1, mild; A2, moderate; and A3, severe.

Biochemical markers

The following blood parameters were determined after overnight fasting in the same day as LB in all patients. ALT, total bilirubin, and GGT were measured in fresh serum within 24 h of collection on an automated biochemistry analyzer (Hitachi 917; Roche Diagnostics). A2M, apolipoprotein-A1, and haptoglobin levels were assayed by nephelometry (Image; Backman Coulter). All analyses were performed at our central laboratory of our hospital. The laboratory followed the pre-analytical and analytical recommendations required to obtain the fibrosis marker score FT [11]. The FT provides a quantitative estimate of liver fibrosis ranging from 0.00 to 1.00. The FT cutoffs for presumed fibrosis stages were 0.00-0.21 (F0), 0.22-0.27 (F0-F1), 0.28-0.31 (F1), 0.32-0.48 (F1-F2), 0.49-0.58 (F2), 0.59-0.72 (F3), 0.73-0.74 (F3-F4), and >0.75 (F4)[12].

Statistical analysis

Descriptive statistics included range, mean ± standard error (SE), median, frequencies (number of cases) and percentages when appropriate. Comparisons of numerical variables between the study groups were made using the Mann Whitney *U* test for independent samples. To compare categorical data, chi square (χ^2) test was used. ROC analysis was used to determine the optimum cutoff value for the studied diagnostic markers. Various variables were tested for correlation using the Spearman rank correlation equation for non-normal variables. P values less than 0.05 were considered as statistically significant. All statistical calculations were performed using SPSS (Statistical Package for the Social Sciences; SPSS Inc., Chicago, IL, United States) version 20 for Microsoft Windows. The difference between advanced and non-advanced fibrosis prevalence (DANA) is an estimator of the spectrum bias of the study. DANA is useful to adjust the observed AUROC according to the observed DANA of the study in order to obtain a standardized AUROC. Calculated DANA of the actual study is 1.841, lower than 2.5, that is the DANA of a distribution with equal prevalence for each fibrosis stages [13].

Results

110 patients and 111 controls were included in the study. 3/110 (2.7%) patients were excluded (1 had no LB, 2 were excluded using security algorithms provided with FT calculation for unusual deviation with the value of haptoglobin in FT results). 5/111 (4.5%) controls were excluded using security algorithms provided with FT calculations (1 control for unusual deviation with the median value of haptoglobin, 4 controls for unusual deviation with the median value of A2M). Finally, 107 patients and 106 controls were included.

Mean age for patients was 46.1 years and for controls was 46.7 years, with male ratios being 66% and 48% respectively (P=0.07) (Table 1). The study group had predominantly genotype 4 infection (90%) followed by genotype 1 (10%). The fibrosis stages in LB were: 36% no or minimal fibrosis (F0, F1); 56% bridging fibrosis (F2, F3); and 7.5% liver cirrhosis (F4). However, the fibrosis stages as estimated by FT were: no or minimal fibrosis in 52% patients and in 95% controls; bridging fibrosis in 22% patients and in 4% controls; cirrhosis in 25% patients and in 1% controls (P<0.0001) (Table 2). The activity grades in LB showed that 46% had no or minimal activity (A0, A1) while 54% showed moderate to severe activity (A2, A3). However, AT showed no or minimal activity in 67% patients and in 100% controls (P<0.0001); moderate to severe activity in 33% patients and in 0% controls (P<0.0001). The mean values for biochemical markers, as expected, showed significant differences between patients and controls (Table 3).

Mean FT value was significantly higher in patients than controls [0.49±0.03 vs. 0.18±0.01, P<0.0001]. Prevalence of fibrosis stages according to FT in patients versus controls were, F0-F1 52% vs. 95% (P=0.005), F2-F3 24% versus 4% (P=0.0004), cirrhosis (F4) 25% vs. 1% (P<0.0001), respectively. Mean value of AT was significantly higher in patients vs. controls [0.45±0.02 vs. 0.10±0.01, P<0.0001]. Prevalence of activity grades according to AT in patients versus controls were, A0-A1 67% vs. 100% (P<0.0001) and A2-A3 33% vs 0%, (P<0.0001), respectively. FT-observed AUROC (95%CI) for advanced fibrosis (F3F4) was 0.73 (0.62-0.81)(P<0.0001) (Fig. 1). For advanced fibrosis, the adjusted AUROC (95%CI) according to the observed DANA of the study (=1.841) was 0.80 (0.69-0.88). FT-observed AUROC (95%CI) for cirrhosis (F4) was 0.94 (0.86-0.98) (P<0.0001) (Fig. 1). AT-observed AUROC (95%) for moderate and severe activity (A2A3) was 0.72(0.61-0.81) (P<0.0001) and for severe activity (A3) was 0.88 (0.78-0.93).

Table 1 Comparison of the baseline and study characteristics of the patient and control groups

Characteristics	Patients N=107	Controls N=106	P value
Demographics			
Age, years, mean (standard error)	46.1 (1.2)	46.7 (1.0)	0.65
Male, n (%)	71 (66)	51 (48)	0.07
Fibrosis stage, n (%)			
Biopsy minimal n (%)			
No Minimal fibrosis (F0, F1)	39 (36)	-	
Bridging fibrosis (F2, F3)	60 (56)	-	
Cirrhosis (F4)	8 (7.5)	-	
FibroTest minimal n (%)			
No Minimal fibrosis (F0, F1)	56 (52)	101 (95)	0.005
Bridging fibrosis (F2, F3)	24 (22)	4 (4)	0.0004
Cirrhosis (F4)	27 (25)	1 (1)	<0.0001
Activity grade n (%)			
Biopsy			
No or minimal (A0, A1)	49 (46)	-	
Moderate or severe (A2, A3)	58 (54)	-	
ActiTest			
No or minimal (A0, A1)	72 (67)	106 (100)	<0.0001
Moderate or severe (A2, A3)	35 (33)	0	<0.0001

Concordance between biomarkers and histology

Two-class concordance for advanced fibrosis

For the classification in two classes (advanced versus non-advanced fibrosis) the concordance between FT and LB was 66/107 (62%), kappa=0.24 (P=0.008). 41/107 (38%) patients were misclassified by FT compared to LB. Among the 41 misclassified, the differences between LB and FT classification were: 5/107 (4.7%) of 3 stages, 4/107 (3.7%) of 2.5 stages, 9/107 (8.4%) of 2 stages, 9/107 (8.4%) of 1.5 stages, 6/107 (5.6%) of 1 stage, 8/107 (7.5%) of 0.5 stage of fibrosis. According to the international definition of discordance only 18/107 (17%) were considered true discordant (results with two or more stages of fibrosis difference).

Two-class concordance for cirrhosis

For the classification in two classes (cirrhosis versus non-cirrhosis) the concordance between FT and LB was 88/107 (81%), kappa=0.39 (P<0.0001). 19/107 (19%) patients were misclassified by FT compared to LB for the diagnosis of cirrhosis. Among the 19 misclassified cirrhosis (F4) by FT only 12/107 (11.2%) were classified F3 at biopsy, 3/107 (2.8%) F2, and 4/107 (3.7%) F1. According to the international definition of discordance only 7/107 (6.5%) were considered true discordant results with 2 or more stages of fibrosis difference; the other 12 were classified F3 with FT and F4 with LB.

Two-class concordance for moderate to severe activity

For the classification in two classes (no/minimal versus moderate/severe activity) the concordance between AT and LB was 70/107 (65%), kappa=0.33 (P=0.0002). 37/107 (35%) patients were misclassified by AT compared to LB. Among the 7/107 overscored by AT, the differences between LB and AT classification were: 6/107 (5.6%) of 2 grades, 1/107 (0.9%) of 1 grade. Among the 30/107 (28%) underscored by AT, 5/107 (4.7%) 2 or 3 grades, 7/107 (6.5%) of 1.5 grades, 6/107 (5.6%) of 1 grade, 12/107 (11.2%) a minimal difference only 0.5 grade of activity. According to the international definition of discordance only 11/107 (10.2%) were considered true discordant.

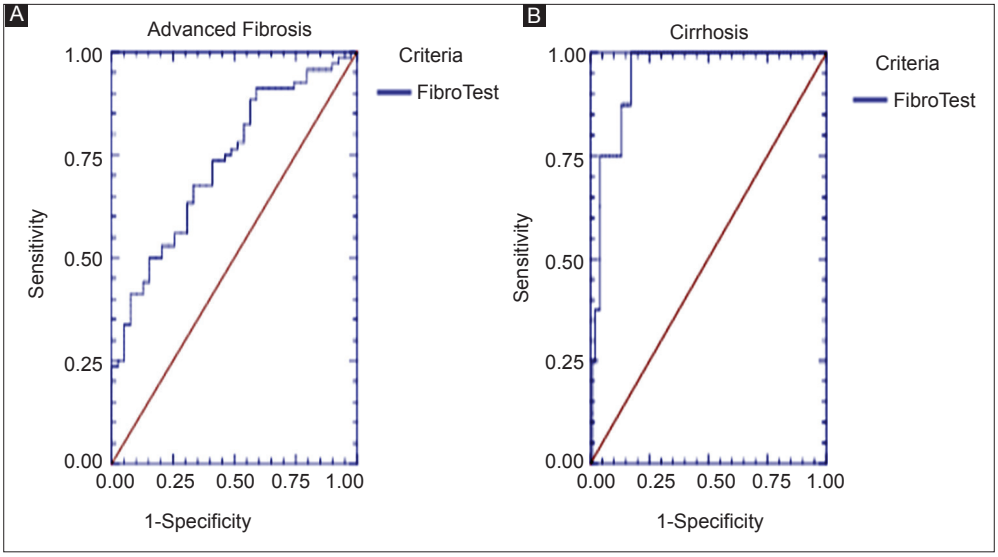

Figure 1 Area under the receiver operating characteristic curve of FibroTest (A) for advanced fibrosis (F3F4 Metavir) = 0.80 (0.69-0.88), and (B) for cirrhosis = 0.94 (0.86-0.98)

Table 2 The distribution of fibrosis and activity scores in the patients' group fibrosis Metavir stage

Fibrosis	**F0**	**F1**	**F2**	**F3**	**F4**	**Total**
Metavir stage Total, n (%)	7 (6.5)	32 (29.9)	31 (29.0)	29 (27.1)	8 (7.5)	107 (100)
Activity	**A0**	**A1**	**A2**	**A3**	**Total**	
Metavir stage Total, n (%)	0 (0)	49 (45.8)	29 (27.1)	29 (27.1)	107 (100)	

Table 3 Serum biochemical markers in the study groups

	Patients	Controls	P value
α-2 Macroglobulin (g/L)	2.56 (0.08)	1.44 (0.05)	<0.0001
Haptoglobin (g/L)	0.92 (0.05)	1.25 (0.06)	<0.0001
Apolipoprotein A1 (g/L)	1.25 (0.02)	1.29 (0.02)	0.25
GGT (IU/L)	80 (7)	29 (2)	<0.0001
Total bilirubin (μmol/L)	13.9 (0.8)	11.6 (0.6)	0.02
ALT (IU/L)	78 (6)	24 (1)	<0.0001
AST (IU/L)	59 (4)	23 (1)	<0.0001
Fasting glucose (mmol/L)	5.8 (0.2)	5.6 (0.2)	0.48
Total cholesterol (mmol/L)	4.1 (0.1)	4.8 (0.1)	<0.0001
Triglycerides (mmol/L)	1.8 (0.1)	1.3 (0.05)	0.03
FibroTest (0.00-1.00)	0.49 (0.03)	0.18 (0.01)	<0.0001
ActiTest (0.00-1.00)	0.45 (0.02)	0.10 (0.01)	<0.0001

GGT, γ-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase

The 7 patients overscored by AT had a significantly higher mean±SE ALT levels 128±13 IU/L compared to the patients not overscored (75±6 IU/L, P=0.002) suggesting a risk of false-negative histology. The 30 patients underscored by AT had a mean±SE ALT level 56±3 IU/L significantly lower than in patients not underscored 87±8 IU/L (p=0.005) suggesting a risk of false-positive histology.

Two-class concordance for severe activity

For the classification in two classes (severe versus not severe activity) the concordance between AT and LB was 80/107 (65%), kappa=0.37 (P=0.0001). 27/107 (25%) patients were misclassified by AT compared to LB. Among the 14 patients overscored by AT, 8/107 (6%) had one-grade difference and 6/107 (5.6%) had two-grade difference between AT and LB. Among 13 patients underscored by AT, 4/107 (3.7%) had one-grade difference, 5/107 (5%) 1.5 grade, 2/107 (2%) 2 grades, 1/107 (1%) 2.5 grades and 1/107 (1%) 3 grades. Overall, 4/107 (3.7%) had a 2 grades or more of difference. According to the international definition of discordance only 10/107 (9.3%) were considered true discordant results with two or more stages of difference.

Concordance analysis in the group of good quality biopsy (n=41)

The concordance for advanced fibrosis was 28/41 (68%). In the subset with good quality LB, 3/41 (7.3%) were misclassified of two or more stages compared to 18/107 (16.8%) in the

overall population (P=0.06). The concordance for cirrhosis was 34/41 (83%), kappa±SE=0.39±0.12, P=0.002. In the subset with good quality LB, 4/41 (9.8%) were misclassified of two or more stages compared to 19/107 (17.8%) overall LB (P<0.0001). The concordance for moderate/severe activity was 27/41 (66%), kappa±SE value 0.34±0.15, P=0.02. In the subset with good quality LB, 2/41 (4.9%) were misclassified of two or more stages compared to 11/107 (10.2%) in overall LB (P=0.0006). The concordance for severe activity was 34/41 (82.9%), kappa±SE 0.55±0.16, P=0.0004. In the subset with good quality LB, 2/41 (4.9%) were misclassified of two or more stages compared to 10/107 (9.3%) in the overall LB (P=0.004).

Diagnostic values in the group of good quality biopsy

FT-observed AUROC (95%Cl) for advanced fibrosis was 0.83 (0.65-0.92) and standardized AUROC (95%Cl) was 0.90 (0.72-0.99). FT-observed AUROC (95%Cl) for cirrhosis was 0.93 (0.76-0.98). AT-observed AUROC (95%Cl) for moderate/severe activity was 0.86 (0.67-0.94). AT-observed AUROC (95%Cl) for severe activity was 0.90 (0.76-0.93).

Discussion

Biomarkers are now recommended as alternative to LB by European [14] and Canadian guidelines [15]. The reported AUROC of FT for predicting ≥F2 fibrosis varies from 0.74 to 0.84 and for predicting F4 fibrosis it varies from 0.71 to 0.87 [16-21]. However there is only limited data showing the efficacy of FT in a cohort with predominantly genotype 4 infection [16]. Halfon *et al* reported high diagnostic accuracy for AT (AUROC of 0.73, CI=0.69-0.77) for predicting A2-A3 necroinflammation [21]. Our study showed that fibrosis estimated by FT was significantly higher in patients compared with controls (mean 0.49 vs. 0.18), P<0.0001. In the patients, the AUROC for advanced fibrosis (F3, F4) was 0.73 (0.62-0.81), but, when adjusted according to the observed DANA, it was 0.80 (0.69-0.88); AUROC for cirrhosis (F4) was 0.94 (0.86-0.98). The AUROC of AT for moderate and severe activity (A2A3) was 0.72 (0.61-0.81); AUROC for severe activity (A3) was 0.88 (0.78-0.93).

The discordance in two classes (advanced versus non-advanced fibrosis) between FT and LB (according to the international definition) was only 18/107 (17%). Likewise, for cirrhosis versus non-cirrhosis the discordance was only 7/107 (6.5%). The activity assessment by AT as compared to LB (no/minimal versus moderate/severe activity) showed a discordance of only 11/107 (10.3%) and for severe versus not severe activity the discordance was 10/107 (9.3%). The concordance rates for FT and AT were much higher for the subgroup of patients with good quality LB. ALT is an intracellular liver enzyme and a good indicator for liver cell necrosis, a common pathway for many hepatotoxic insults; when it is taken as an indicator of activity the discordance analysis suggested that LB could have false-positive and false-negative results for the activity estimation.

Different studies assessing F0-F1 fibrosis vs. F2-F3-F4 in patients with chronic HCV using different seromarkers had comparable results when assessed by AUROC. Imbert-Bismut *et al* showed FT AUROC of 0.8 [19]; Forns *et al* showed FT AUROC of 0.86 [22]; Adams *et al* showed Hepascore (bilirubin, GGT, hyaluronate, A2M, age, sex) AUROC of 0.85 [23]; Patel *et al* showed Fibrospect (TIMP-1, A2M, hyaluronate) AUROC of 0.83 [24]; other studies comparing F0-F1-F2-F3 vs. F4 (cirrhosis) showed FT AUROC of 0.92 [32], and Hepascore AUROC of 0.94 [25], so the results of this study had comparable results to most of other studies.

The combination of non-invasive markers in assessing liver fibrosis showed that performing FT, APRI, and Forns index with LB improves the diagnostic accuracy for liver fibrosis in CHC patients. However, with even in the "best" scenario an AUROC >0.90 can rarely be achieved even for a perfect marker [26]. Newer protocols combining biochemical and imaging (transient elastography) has shown promise in increasing the accuracy [27].

FT is not only good predictive for liver fibrosis, but it can be useful in estimation the degree of portal hypertension. It has significant correlation with hepatic venous pressure gradient (HVPG). In a study by Thabut *et al*, there was a significant correlation between FT and HVPG. In cirrhotic patients, FT was significantly higher when there was severe portal hypertension (0.87±0.15 vs. 0.73±0.14, respectively, P=0.02) [28].

FT is especially useful for evaluating fibrosis progression over time and monitoring the outcomes of therapy, since few patients are eager to undergo frequent repeat biopsies. The

Summary Box

What is already known:

- Liver biopsy is mostly considered as the gold standard to assess hepatitis C virus-related inflammation and fibrosis
- Missing rates of advanced liver fibrosis by percutaneous liver biopsy is up to 40%
- Seromarkers are considered as alternative to liver biopsy, but studies report variable efficacy for these makers

What the new findings are:

- In a cohort of predominantly genotype 4 chronic hepatitis C patients, we report high diagnostic accuracy of FibroTest for predicting advanced fibrosis/cirrhosis
- Likewise, the diagnostic accuracy of ActiTest for predicting moderate and severe necroinflammation is high
- Accuracy of FibroTest in assessing hepatic fibrosis is more impressive in the group of patients with good-quality liver biopsy

limitation of this study was that the number of patients who fulfilled the criteria of good quality LB was limited.

In conclusion, FT-AT had excellent observed and standardized diagnostic values for fibrosis and activity. The concordance between FT and LB was fair, with discordance of two or more stages in less than 20% for advanced fibrosis and less than 10% for cirrhosis. Similar results were obtained for the AT. Sensitivity analysis showed higher diagnostic values for FT and AT when LB samples were longer than 15 mm and not fragmented. Discordant results between LB and biomarkers decreased in the group of good quality biopsy. In our cohort of patients with chronic hepatitis C (predominantly genotype 4) infection, FiboTest and ActiTest appears to be a good non invasive marker for assessment of liver fibrosis and inflammatory activity.

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