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Independent Prospective Multicenter Validation of Biochemical Markers (Fibrotest–Actitest) for the Prediction of Liver Fibrosis and Activity in Patients with Chronic Hepatitis C: The Fibropaca Study

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OBJECTIVES: Fibrotest (FT) and Actitest (AT) are biochemical markers of fibrosis and activity for use as a non-invasive alternative to liver biopsy in patients with chronic hepatitis C virus (HCV). The aim of this study was to perform an external validation of FT and AT and to study the discordances between FT/AT and liver biopsy in patients with chronic hepatitis C.

METHODS: A total of 519 consecutive patients with chronic HCV were prospectively included in five centers, with liver biopsy and biochemical markers taken at the same day. Fifteen patients were excluded because their biopsies could not be interpreted. Diagnostic accuracies were assessed by receiver operating characteristic (ROC) curve analysis.

RESULTS: Median biopsy size was 15 mm (range: 2–58), with 9 portal tracts (1–37) and 1 fragment (1–12). 46% (230/504) were classified F2–F4 in fibrosis and 39% A2–A3 in activity. FT area under ROC curve for diagnosis of activity (A2–A3), significant fibrosis (F2–F4), and severe fibrosis (F3–F4) were 0.73 [0.69–0.77], 0.79 [0.75–0.82], and 0.80 [0.76–0.83], respectively. Among the 92 patients (18%) with 2 fibrosis stages of discordance between FT and biopsy, the discordance was attributable to FT in 5% of cases, to biopsy in 4%, and undetermined in 9%.

CONCLUSIONS: This prospective independent and multicenter study confirms the diagnostic value of FT and AT found in the princeps study and suggests that FT and AT can be an alternative to biopsy in most patients with chronic HCV.

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INTRODUCTION

Chronic infection with hepatitis C virus (HCV) typically induces injury and inflammation of the liver, which appear to be responsible for the associated fibrogenesis. Fibrosis is a physiological mechanism that is at first beneficial but that can subsequently become pathological if viral infection and chronic hepatocellular injury persist (1). The risk of developing cirrhosis involves many factors and is related to the stage (degree of fibrosis) and the grade (degree of inflammation and necrosis) in the initial liver biopsy (2). Necroinflammatory activities and fibrosis are the key factors for treatment indication. Up to now, liver biopsy has been considered the gold standard for assessing HCV-related histological lesions but

its sampling error (33% discordance rate for fibrosis stage) and adverse-event risk limit its utility (3, 4), despite the numerous histological scoring systems proposed to improve the grading of HCV inflammation and the staging of HCV fibrosis (Metavir, Ishak, Scheuer) (5). Non-invasive approaches to assess histology samples include clinical symptoms, routine laboratory tests, and radiology imaging (6–9). Non-invasive approaches for evaluating the serum markers of fibrogenesis include thrombocyte counts (10), prothrombin time (11), the ratio of alaninaminotransferase (ALT) and aspartataminotransferase (AST) levels (6), and the level of gamma-glutamyl transpeptidase (GGT). Another set of markers is based on the direct detection of molecular junctions that activate fibrosis or participate in the generation of the liver extracellular matrix. The most applicable markers are hyaluronic acid (HA) (12), type IV collagen (IV-C), N-terminal propeptide of type III procollagen (PIIIP) (13), metalloproteinases (MMP), inhibitors of metalloproteinases (TIMP), and transforming growth factor beta (TGF-β). At present, however, none of these tests or markers alone is accurate and reliable in predicting histology. To improve diagnostic performance, several scoring systems have been proposed with different thresholds (14–18). Those scoring systems had good correlations, according to the thresholds, with histological scoring systems (19). Among them there is a simple non-invasive routine test named Fibrotest (FT)–Actitest (AT) that uses the biochemical markers of fibrosis and activity. Since September 2002, it has been proposed as a non-invasive alternative to liver biopsy in patients with chronic Hepatitis C (16). French guidelines indicated that non-invasive markers of fibrosis and activity would be of interest for patients with chronic hepatitis if they were validated by an independent multicenter prospective study (20).

The first aim of this study was to validate FT and AT and then to study the discordances between FT/AT and liver biopsy in patients with chronic hepatitis C.

PATIENTS AND METHODS

This was a French, national, multicenter, prospective, cross-sectional study performed according to national regulations in five centers in the southeast region. The centers were hepato-gastroenterology units or internal medicine units known for their expertise in hepatitis C. From November 2002 to December 2003, 519 consecutive patients were studied in the 5 centers (Conception Hospital and Saint-Joseph Hospital (Marseille), Archet Hospital (Nice), Hyères Hospital (Hyères), and Arnault Tzanck Institute (St Laurent du Var).

Patients

All patients had chronic HCV infection without liver complication such as ascites, documented by positivity of HCV RNA in serum. Signed informed consent was obtained from all patients before their inclusion. Liver biopsy and biochemical markers were taken the same day. Liver biopsy was performed and analyzed in each center. Ultrasound examination was performed before liver biopsy in all patients. Information relating to the patient demographic data, risk factors, virological status, clinical examinations, biological data (platelets, prothrombin time ratio, serum albumin level) was prospectively recorded in each center on the day of biopsy. All the data were anonymously recorded in the database.

Liver Biopsies

Liver biopsies were examined by each center and analyzed by the local pathologist for fibrosis stage and activity grade according to the METAVIR scoring system (3). Fibrosis was staged on a scale of 0–4: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis. Activity grading by the METAVIR system (based on the intensity of necroinflammatory activity, mainly on necrosis) was scored as follows: A0 = no histological activity, A1 = mild activity, A2 = moderate activity, and A3 = severe activity.

To assess liver biopsy quality, Regev quality criteria were used (15 mm or more in length, 5 or more portal tracts, and 1 fragment) (3). A biopsy between 10 and 15 mm in length, with less than 5 portal tracts or fragmented is considered as "fair quality biopsy"; a "poor quality biopsy" is less than 10 mm in length.

Biochemical Markers

To do FT and AT, serum samples were taken on the day of biopsy from the patients in fasting state. Six serum biochemical markers were determined: α2-macroglobulin, haptoglobin, GGT, total bilirubin, apolipoprotein A1, and ALT. These markers were analyzed in accredited laboratories following the guidelines recommended for FT and AT assessment by the authors of the initial publication. (16) In the hospital-based cohort, GGT, ALT, and total bilirubin were measured by Hitachi 917 Analyzer and Roche Diagnostics reagents (both Mannheim, Germany). α2-Macroglobulin, apolipoprotein A1, and haptoglobin were measured using a Modular analyzer (BNII, Dade Behring; Marburg, Germany). All coefficients of variation were assessed in a previous study and were lower than 6% (21). Biochemical assays were performed on fresh serum that was decanted and stored 72 h maximum at +2°C/+8°C, protected from light. The assays of the specific proteins (α2-macroglobulin, haptoglobin, and apolipoprotein A1) were carried out on serum stored at +2°C/+8°C for 5 days. All biochemical parameter and FT and AT determinations were done without knowledge of liver biopsy results. In addition, blood samples were collected for further determinations of biochemical and fibrosis markers.

Fibrosis using FT was staged on a scale of 0–4 with respect to Metavir fibrosis staging. For FT score from 0 to 0.21 fibrosis was staged F0, from 0.22 to 0.27 F0–F1, from 0.28 to 0.31 F1, from 0.32 to 0.48 F1–F2, from 0.49 to 0.58 F2, from 0.59 to 0.72 F3, from 0.73 to 0.74 F3–F4, and from 0.75 to 1 F4. Necroinflammatory activity using AT was graded on a

scale of 0–3 with respect to Metavir activity grading. For AT score from 0 to 0.17 activity was graded A0, from 0.18 to 0.29 A0–A1, from 0.30 to 0.36 A1, from 0.37 to 0.52 A1A2, from 0.53 to 0.60 A2, from 0.61 to 0.62 A2–A3, and from 0.63 to 1 A3 (22). FT and AT formulas are available on the USPTO Website (http://www.uspto.gov; Patent No 6,631,330). (22)

Assessment in Discordant Patients

Discordance between FT and biopsy was considered significant if they differed by at least two stages of fibrosis in the METAVIR scoring system. We chose two stages or grades instead of one stage or one grade because the precision of biopsy is not sufficient to discriminate for one stage or one grade difference, 33% and 24% in the same patient, respectively (3).

In patients with an F4 stage on either FT or liver biopsy, all radiological (ultrasound examination (US), computed tomography (CT), and magnetic resonance imaging (MRI)) or endoscopic examinations and biochemical parameter evaluations were done to reveal cirrhosis or portal hypertension. We considered the patients as having cirrhosis if they had at least two criteria of cirrhosis among clinical criteria (abdominal collateral venous circulation and stella angioma), radiological criteria (portal hypertension or liver morphology on US, CT, or RMI), and biological criteria (low blood platelets; below 140.000 g/L (23) or low PT ratio; below 70% (18)).

To attribute the fibrosis discordance either to FT or to liver biopsy, especially in non-cirrhotic patients, biopsy failure was considered as a short biopsy with few portal tracts. FT failure was considered as an isolated abnormal value of one of the five FT components attributable to a clinically identified condition such as hemolysis with haptoglobin <0.30 g/L and/or elevated unconjugated bilirubinemia, inflammation or sepsis with haptoglobin >2 g/L and/or α2-macroglobulin >3 g/L, Gilbert's syndrome, or an extra-hepatic cholestasis with elevated bilirubin and/or GGT.

To summarize, we considered that

1. discordance was highly attributable to biopsy failure if the biopsy was of poor quality (regarding Regev classification: size <10 mm, less than 5 portal tracts) with no associated isolated abnormal marker value (*i.e.*, all values of the FT pattern parameters "evolving" toward either increased or decreased fibrosis)
2. discordance was moderately attributable to biopsy failure if the biopsy was not good (size between 10 and 15 mm) with less than 5 portal tracts or fragmented but with no FT failure.
3. discordance was highly attributable to FT failure if one of the five FT components had an abnormal value attributable to a clinically identified condition.
4. discordance was moderately attributable to FT failure if one of the six FT components had an abnormal value in the absence of a clinically identified condition and was paired with a biopsy of good quality.
5. discordance was "undetermined" if there was no reason for failure or if there was a reason for failure both for FT and biopsy

Statistical Analysis

Statistical analysis was done using Wilcoxon test and Kruskal-Wallis comparison test. The diagnostic values of the FT and AT compared to the METAVIR fibrosis and activity indexes were assessed by logistic regression. Liver biopsy fibrosis stage was the variable to be explained (0 for absence of the studied event and 1 for its presence), and FT numerical result was the explanatory variable. From respective FT cut offs resulting from the logistic regression were deduced respective diagnostic values: area under the receiver operating characteristic (ROC) curves (AUCs), sensitivity, specificity, positive and negative predictive values (PPV and NPV), Spearman rank correlation (SC), kappa score, and Youden index. The most accurate cut off was determined by the highest Youden index in each case. Statistical analysis was performed using SAS V8.2 statistical software (SAS Institute Inc., Cary, NC). The main end points were the AUCs for the diagnosis of significant fibrosis (F2–F4 *vs* F0–F1), severe fibrosis (F3–F4 *vs* F0–F2), and moderate to severe activity (A2–A3 *vs* A0–A1).

RESULTS

Among the 519 patients consecutively included, 15 (2.9%) were excluded because their biopsy could not be interpreted due to either a size below 5 mm or the absence of hepatic parenchyma. The study therefore included 504 patients with simultaneous determinations of fibrosis and activity by FT and AT and liver biopsy. There were more men (54%) and the median [range] age of the population was 45 yr [17–79]. Table 1 gives the biochemical marker results for the whole population.

Liver Biopsy Assessment

We had no major clinical complications of liver biopsy in our 519 patients. For the 504 patients with an interpretable biopsy, Table 1 gives the data on liver biopsy. The median biopsy size was 15 mm [2–58], and the median number of portal tracts was 9 [1–37]. According to Regev quality criteria, 55% of the patients had a biopsy of good quality, 35% of fair quality, and 10% of poor quality. For fibrosis, 12% (58/504) were F0 and 46% (230/504) were F2–F4, 6% (29/504) had cirrhosis, 39% (198/504) were A2–A3 in activity, and 29% (146/504) were simultaneously F2–F4 for fibrosis staging and A2–A3 for activity grading.

Activity Analysis

The box plots of AT according to METAVIR grade are given in Figure 1A. The median (range) AT value was 0.13 (0.03–0.75) for A0 (n = 41), 0.28 (0–0.92) for A1 (n = 265), 0.51 (0.02–0.98) for A2 (n = 183), and 0.78 (0.40–0.96) for A3

Table 1. Characteristics of All Patients, Discordant, and Non-Discordant

	All (N = 504)	Non-Discordant, N = 412 (82%)	Discordant, N = 92 (18%)
Mean age at biopsy (median-range)	45 [17–79]	45 [17–78]	46 [29–79]*
Men (%)	273 (54.2)	218 (53)	55 (59)
Women (%)	231(45.8)	193 (47)	38 (41)
Biopsy size (mm) (median-range)	15 [5–58]	15 [5–58]	14 [5–36]
Portal tract number (median-range)	9 [1–37]	9 [1–37]	8 [2–30]
Number of fragments (median-range)	1 [1–12]	1 [1–5]	1 [1–12]
ALT (UI/L) (median-range)	60.5 [4–549]	60 [4–549]	64 [17–477]
Total bilirubin (μmol/L) (median-range)	10 [1–41]	10 [1–41]	11 [1.5–26.6]
GGT (IU/L) (median-range)	39 [5–983]	37 [5–983]	44 [7–317]
α2-macroglobulin (g/L) (median-range)	2.64 [0.78–10.40]	2.55 [0.78–6.07]	2.90 [0.83–10.40]**
ApoA1 (g/L) (median range)	1.52 [0.59–12]	1.53 [0.59–12]	1.47 [0.86–2.36]
Haptoglobin (g/L) (median-range)	0.93 [0.04–7.71]	0.95 [0.06–7.71]	0.84 [0.04–2.27]

*Significant difference between groups (Wilcoxon test $p < 0.05$).
**Significant difference between groups (Wilcoxon test $p < 0.01$).

(n = 15). Although there was an overlap between the grade of activity for AT and the activity determined by liver biopsy, there was a significant difference in AT levels among liver biopsies (Kruskal-Wallis test; $p < 0.0001$).

The diagnostic values of AT for the grading of necroinflammatory activity are shown in Table 2. The AUCs were 0.73 [0.69–0.77] and the AT threshold giving the highest sensitivity and specificity was 0.32: sensitivity of 78% [71–83], specificity of 60% [54–66], NPV of 81%, PPV of 56%, SC of 0.37, kappa score of 0.35, and Youden index of 0.379. ROC curves with cut-off values are shown in Figure 2A.

Fibrosis Analysis

The box plots of FT according to METAVIR score are given in Figure 1B. The median (range) FT value was 0.12 (0.02–0.79) for F0 (n = 58), 0.21 (0–0.94) for F1 (n = 216), 0.45 (0.03–0.92) for F2 (n = 110), 0.60 (0.10–0.99) for F3 (n = 91), and 0.78 (0.28–0.97) for F4 (n = 29).

Although there was an overlap between the stage of fibrosis for FT and the fibrosis determined by liver biopsy, there was a significant difference in FT levels among liver biopsy (Kruskal-Wallis test $p < 0.0001$).

The diagnostic values of FT for the staging of significant fibrosis are shown in Table 2. The AUCs were 0.79 [0.75–0.82] and the FT threshold giving the highest sensitivity and specificity was 0.36: sensitivity of 73% [66–78], specificity of 72% [67–78], NPV of 76%, PPV of 69%, SC of 0.45, kappa score of 0.45, and Youden index of 0.449. For the diagnosis of severe fibrosis (F0–F2 *vs* F3–F4), AUCs were 0.80 [0.76–0.83] and the FT threshold giving the highest sensitivity and specificity was 0.44: sensitivity of 76% [67–83], specificity

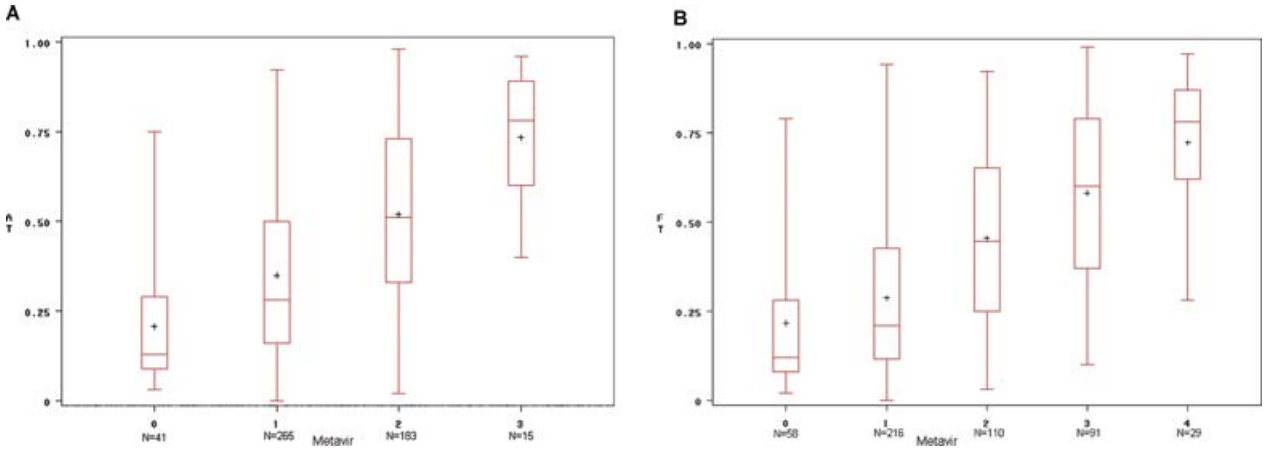

Figure 1. (*A*) Box plots of AT according to METAVIR activity grading. The top and bottom of each box are the 25th and 75th centiles, giving the interquartile range. The line through the box is the median, the bars are the 5th and 95th centiles, and the cross in the box is the mean. The mean AT value was 0.21 (0.17) for A0 (n = 41), 0.35 (0.24) for A1 (n = 265), 0.52 (0.25) for A2 (n = 183), and 0.73 (0.17) for A3 (n = 15). (*B*) Box plots of FT according to METAVIR fibrosis staging. The top and bottom of each box are the 25th and 75th centiles, giving the interquartile range. The line through the box is the median, the bars are the 5th and 95th centiles, and the cross in the box is the mean. The mean FT value was 0.22 (0.21) for F0 (n = 58), 0.29 (0.22) for F1 (n = 216), 0.46 (0.24) for F2 (n = 110), 0.58 (0.25) for F3 (n = 91), and 0.72 (0.18) for F4 (n = 29).

Table 2. Summary of the Diagnostic Values of Fibrotest and Actitest for the Staging of Hepatic Fibrosis and the Grading of Hepatic Activity

Stage/ Grade Studied	AUC [95% CI]	Cut Off	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	NPV (%)	PPV (%)	Spearman Rank Correlation	Kappa Score	Youden Index*
F0–F1 *vs* F2–F4	0.79 [0.75–0.82]	0.10	97 [94–99]	27 [22–33]	91	53	0.33	0.23	0.240
		0.30	77 [71–82]	65 [59–71]	77	65	0.42	0.41	0.420
		0.36†	73 [66–78]	72 [67–78]	76	69	0.45	0.45	0.449
		0.60	44 [38–51]	91 [87–94]	66	81	0.41	0.37	0.355
		0.80	20 [15–26]	98 [96–99]	59	90	0.29	0.18	0.169
F0–F2 *vs* F3–F4	0.80 [0.76–0.83]	0.10	99 [95–100]	21 [17–25]	99	28	0.23	0.11	0.200
		0.30	87 [79–92]	56 [51–61]	93	38	0.37	0.30	0.427
		0.44†	76 [67–83]	70 [65–74]	90	44	0.40	0.37	0.456
		0.60	56 [47–65]	85 [81–88]	86	53	0.40	0.40	0.404
		0.80	29 [21–38]	97 [94–98]	81	73	0.37	0.33	0.258
A0–A1 *vs* A2–A3	0.73 [0.69–0.77]	0.10	98 [94–99]	16 [12–20]	91	43	0.21	0.11	0.132
		0.30	79 [73–85]	56 [50–62]	81	54	0.35	0.33	0.355
		0.32†	78 [71–83]	60 [54–66]	81	56	0.37	0.35	0.379
		0.60	41 [34–48]	85 [80–89]	69	63	0.29	0.27	0.255
		0.80	16 [11–22]	94 [91–96]	63	63	0.16	0.12	0.100

*The Youden index evaluates the diagnostic efficacy of a test. It is expressed as Youden = (S + SP) − 1. If the index is equal to or below 0, the diagnostic efficacy of the test is poor. On the other hand, the closer it is to 1, the higher is its diagnostic value.
†Fibrotest cut off giving the best diagnostic values, corresponding to the highest Youden index value.

of 70% [65–74], NPV of 90%, PPV of 44%, SC of 0.40, kappa score of 0.37, and Youden index of 0.456. ROC curves with cut-off values are shown in Figure 2A and B.

Stage-by-stage fibrosis analysis, assessed by ROC curve analysis between FT and liver biopsy, showed good diagnostic values for differences of two or more fibrosis stages, as reflected by the different AUCs (AUCs from 0.79 to 0.94) (Fig. 3).

Table 3 shows AUCs of FT for fibrosis staging and AT for activity grading on liver biopsy for size <15 mm, size between 15 and 25 mm, and size ≥25 mm. The results were higher in large biopsies but the differences remained not significant (Hanley-McNeil test).

Analysis of Discordant Results: Two Stages or More

In 92 of the 504 patients (18.4%), there was a significant (at least two fibrosis stages) discordance between FT and biopsy results for fibrosis staging.

Fibrosis staging by FT was higher than fibrosis staging by histology in 49 patients (9.7%). The characteristics of patients with or without discordances are given in Table 1. In univariate analysis, patients with discordance were older and had a worse-inflammatory biological profile (α2-macroglobulin significantly higher in discordant patients). In multivariate analysis, assessed by binary multiple logistic regression, only a high α2-macroglobulin level remained significantly associated with discordance ($p = 0.0169$, odds ratio = 1.341).

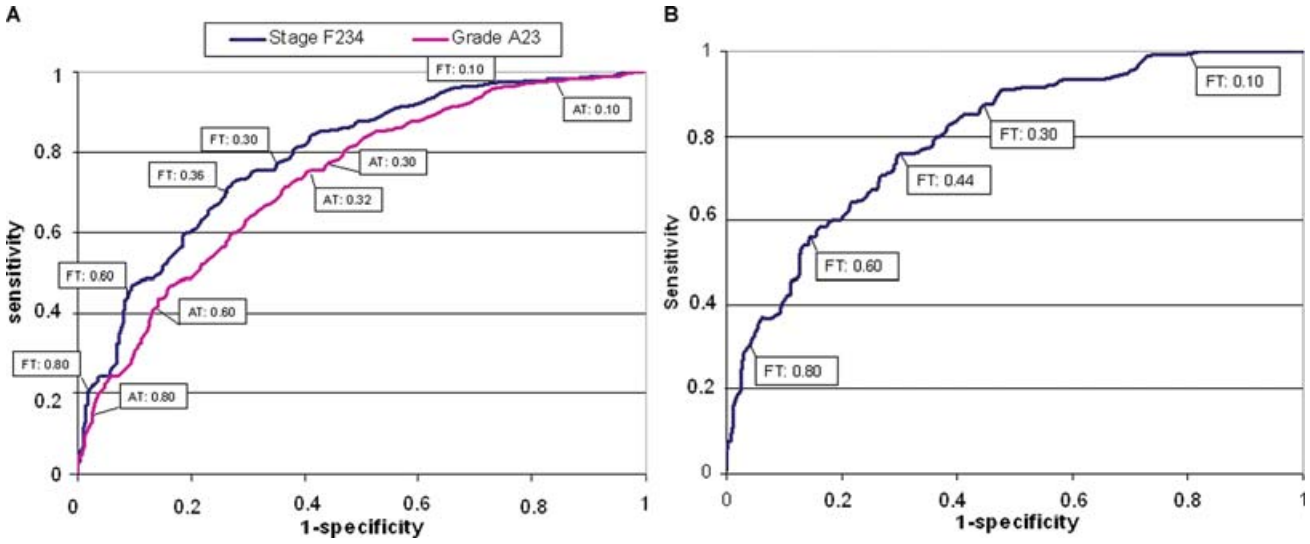

Figure 2. (*A*) ROC curves of significant fibrosis (F2–F4) and significant activity (A2–A3) according to Fibrotest–Actitest (FT–AT). AUC for fibrosis staging, 0.79. AUC for activity grading, 0.72. FT and AT cut-off scores are shown in the figure. (*B*) ROC curves of severe fibrosis (F3–F4) according to FT–AT. AUC, 0.80. FT cut-off scores are shown in the figure.

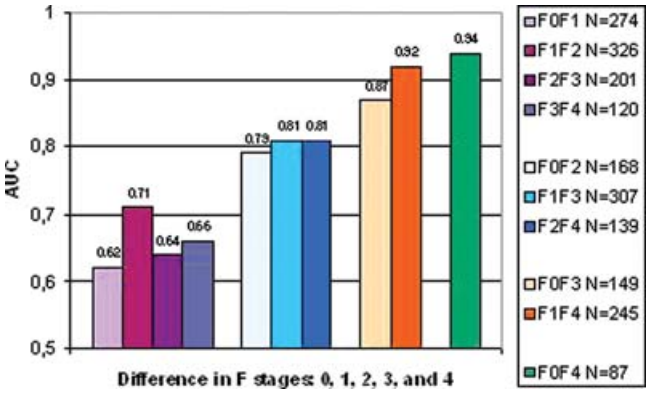

Figure 3. FT stage-by-stage diagnostic value. Respective AUCs were 0.62 [95% CI: 0.56–0.68] for F0 *versus* F1, 0.71 [0.65–0.76] for F1 *versus* F2, 0.64 [0.57–0.71] for F2 *versus* F3, 0.66 [0.57–0.75] for F3 *versus* F4, 0.79 [0.72–0.85] for F0 *versus* F2, 0.81 [0.76–0.85] for F1 *versus* F3, 0.81 [0.73–0.87] for F2 *versus* F4, 0.87 [0.81–0.92] for F0 *versus* F3, 0.82 [0.88–0.95] for F1 *versus* F4, and 0.94 [0.87–0.98] for F0 *versus* F4.

Discordance with Cirrhosis

Among the 92 patients with a discordance of two fibrosis stages between liver biopsy and FT, 33 patients had cirrhosis on one test (liver biopsy or FT). Cirrhosis was diagnosed in 6 patients by liver biopsy and in 27 by FT. The biological, radiological, and endoscopic evaluations suggested cirrhosis in 8 (24%) patients. Among these patients, 5 (15%) were determined F4 by FT and 3 (9%) by liver biopsy. The 25 (76%) other patients had no biological, radiological, and endoscopic signs of cirrhosis. Three (9%) patients were classified F4 by biopsy, all were FT failures. Twenty-two (67%) patients were classified F4 by FT. We therefore classified these patients according to the size of biopsy and evaluation of FT components. Eight (24%) were FT failures, 6 (18%) were liver biopsy failures, and 8 (18%) were unexplained (Table 4).

In patients with cirrhosis on FT or on biopsy, 11 (33%) were biopsy failures, 14 (42%) FT failures, and 8 (24%) had unexplained discordance. Among the 14 FT failures, there were 1 Gilbert's disease, 3 hemolysis, and 5 inflammations; the other 5 FT failures were caused by low bilirubin (two cases) and elevated apolipoprotein A1 (three cases).

Discordance Without Cirrhosis

For the 59 discordant patients without cirrhosis, 21 were overestimated by the FT and 38 by the biopsy. According to the size of biopsy and evaluation of FT components, 8 patients were biopsy failures, 13 patients were FT failures including 6 Gilbert's disease, and 38 patients had unexplained discrepancy.

Discordances were attributable to FT in 27 cases (5.4%) and to biopsy in 19 cases (3.8%); 46 cases (9.1%) had unexplained discrepancy (Table 5).

According to the risk factors, the causes of failure were considered highly attributable to FT in 8 cases (1.6%), moderately attributable to FT in 19 (3.8%), highly attributable to biopsy in 9 (1.8%), and moderately to biopsy in 10 (2%). FT failures were false negative in 10 cases (2%) and false positive in 17 cases (3.4%). Biopsy failures were false negative in 15 cases (3%) and false positive in 4 cases (0.8%).

DISCUSSION

The ultimate utility of any non-invasive model for predicting hepatic fibrosis depends on its practicality and validation by other investigators in a wide range of patients in routine conditions of diagnosis. Among the several systems proposed for assessing severity of inflammation and fibrosis in chronic hepatitis C patients, FT and AT have benefited from assessment in large cohorts of patients (24) but only at a few centers, mainly at the Thierry Poynard Center, where the tests were elaborated. In our prospective independent multicenter study we demonstrate the good diagnostic value both of FT because it distinguishes patients without fibrosis (F0–F1) from patients with fibrosis (F2–F4) (AUC of 0.79) and of AT because it distinguishes patients without activity (A0–A1) from patients with high activity (A2–A3) (AUC of 0.73). Moreover, the advantage of FT/AT over other tests is that it classifies all patients in a linear way for both fibrosis staging and activity grading (14, 15). In the present study, we found a similar AUC when we compared stage by stage the diagnostic values of FT and fibrosis staging by METAVIR on liver biopsy (Fig. 3). Interestingly, when we compared the diagnostic values of FT and fibrosis staging on liver biopsy with more than one stage difference, the AUCs were significantly better (AUCs from 0.79 to 0.94). We believe that this difference is explained by the variability due to the biopsy sample and inter- and intraobserver variability (3). These results are similar to those of Imbert-Bismut *et al*. in terms of AUCs for fibrosis staging and activity grading in their initial and subsequent studies (24). A previous independent study by Rossi *et al*. (25), in fewer patients, reported an AUC for significant fibrosis staging (>F2 on metavir index) of 0.74, which is smaller than the AUC in the princeps study (16) and

Table 3. Distribution of the AUCs According to the Size of Liver Biopsy

	AUC [95% CI]			
Stage/Grade	All (N = 504)	<15 mm (N = 227)	≥15 mm and ≤25 mm (N = 182)	≥25 mm (N = 95)
F2–F4	0.79 [0.75–0.82]	0.78 [0.74–0.82]	0.78 [0.73–0.83]	0.81 [0.71–0.88]
F3–F4	0.80 [0.76–0.83]	0.81 [0.76–0.85]	0.79 [0.74–0.84]	0.81 [0.72–0.88]
A2–A3	0.73 [0.69–0.77]	0.73 [0.69–0.77]	0.74 [0.68–0.79]	0.80 [0.71–0.88]

Table 4. Imputation of Cirrhotic Discordant Patients (N = 33)

Imputation of Discordances		Failure Type	
Biopsy failure	n = 11 (33%)	False-negative	n = 11 (33%)
Fibrotest failure	n = 14 (42%)	False-negative	n = 6 (18%)
		False-positive	n = 8 (25%)
Undetermined	n = 8 (25%)		

Eight patients had radiological, endoscopic, or biochemical cirrhosis, and 17 patients seem to have had cirrhosis with failure determination by either FT or liver biopsy.

in the present study but which is in the range of the monocentric metaanalysis of AUCs. (24). Rossi *et al*. concluded that, because 19% of results were discrepant, FT could not predict the absence or presence of significant liver fibrosis (25). The smaller AUCs may be partially explained by the nonrespect of analytical recommendations for performing FT, the low number of patients, and the lack of information concerning biopsy sample. More important, Rossi *et al*. did not discuss the causes of failures for FT and biopsy. Previous studies and our study highlight the importance of the pre-analytical and analytical steps in the validation of the values of biochemical markers for carrying out FT (26, 27).

In analyzing discordance between liver biopsy and biomarkers, the main difficulty is the absence of a true gold standard of liver injury. In the present study, discordant results with two stages or more of fibrosis between the biopsy and FT were observed in 92 patients (18.4%) and can be explained in half of them. Discordance was attributable to FT marker failure in 27 patients (5.4%), to biopsy failure in 19 patients (3.8%), and was unexplained in 46 patients (9.1%). The most frequent failures attributable to markers were false positives due to Gilbert's disease and inflammation, and false negatives due to inflammation. The most frequent failures attributable to biopsy were false negatives due to small biopsy size. Among the eight cirrhotic patients well defined by radiological, endoscopic, or biochemical criteria with discordant FT and LB, five were not diagnosed by liver biopsy and three were not diagnosed by FT, underlining the absence of a gold standard in determination of liver fibrosis. Among the 22 patients with no radiological or endoscopic criteria for cirrhosis but with F4 on FT, 6 were biopsy failures confirmed by other biochemical markers like hyaluronic acid, and 8 were FT failures.

These results differ from the discordance analysis results by Poynard *et al*. (27) In that study, Poynard *et al.* found that discordance was attributable to marker failure in 13 patients (2.4%) ($p < 0.01$ in comparison with our study), to biopsy failure in 97 patients (18.1%) ($p < 0.001$), and unexplained in 44 patients (8.2%) ($p > 0.05$). Comparing the two studies for fibrosis discordance alone, we found that 1.6% of errors were highly attributable to FT compared with 0.9% for Poynard *et al*. and 3.8% were moderately attributable to FT *versus* 1.5% for Poynard *et al*. The 15 excluded patients with no liver biopsy (2.9% of the cases) must be considered biopsy failures; for these patients, FT and AT permitted liver injury to be assessed. If we consider these patients as discordant, 34 (6.8%) cases were attributable to biopsy, 27 (5.4%) to marker failure, and 46 (9.1%) were unexplained. The difference with Poynard's study might be partly explained by the quality of liver biopsies. Among the 533 patients in the Poynard *et al*. study, 74 (14%) had a liver biopsy of more than 25 mm whereas 95 (19%) had one in the present study ($p < 0.05$). In our FT and AT estimation, the AUCs were higher in patients with a biopsy larger than 25 mm. The number of patients with cirrhosis diagnosis by either liver biopsy or FT was higher in the Poynard *et al.* study (20% *vs* 6.5%) (27), which may also explain their higher rate of failure discordances for liver biopsies.

Liver biopsy remains the gold standard for fibrosis evaluation. However, variability in the distribution of fibrosis within the liver is a potential limitation. In a recent paper, Bedossa *et al*. showed that a length of at least 25 mm is necessary to evaluate fibrosis accurately with a semiquantitative score (METAVIR scoring system) (28). An ideal but impossible study validating biochemical markers would be to perform laparoscopy with two biopsies of 20 mm to reach a total length of 40 mm.

Compared with markers alone, FT was reported superior to traditional markers (prothrombin time, platelet count, and age–platelet index) for predicting significant hepatitis C-related fibrosis (29). Interestingly, it has also been reported that FT has higher predictive values than aspartate platelet ration index for fibrosis diagnosis (30).

The diagnostic accuracy of AT for grading necroinflammatory activity was lower than that of the princeps study (AUCs of 0.73 *vs* 0.75) but not statistically different (Hanley-McNeil test). In our study, as in all the other studies, the AUCs for activity were always lower than AUCs for fibrosis. This may be

Table 5. Imputation of Two-Stage Discordances Between Biopsy and Fibrotest

Imputation of Discordances	Imputation Level		Failure Type	
Biopsy failure, N = 19 (3.8%)	Highly attributable, N = 9 (1.8%)	Moderately attributable, N = 10 (2%)	False-negative, N = 15 (3%)	False-positive, N = 4 (0.8%)
Fibrotest failure, N = 27 (5.4%)	Highly attributable, N = 8 (1.6%)	Moderately attributable, N = 19 (3.8%)	False-negative, N = 10 (2%)	False-positive, N = 17 (3.4%)
Undetermined, N = 46 (9.1%)	Failure of both, N = 19 (3.8%)	Failure unexplained, N = 27 (5.3%)		

partially explained by the rapid fluctuations of ALT in chronic hepatitis C, which is the additional marker used in the AT formula. Another explanation is the much higher variability (either sampling or observer) in the necroinflammatory scoring system than in fibrosis staging (3–5).

In conclusion, the present study validated FT and AT for fibrosis staging and activity grading in an independent cohort. FT and AT bring new insights in the diagnosis of fibrosis in patients with chronic hepatitis C. However, the identification of risk factors of FT and AT failures such as Gilbert's syndrome, inflammation, and hemolysis must be considered before and after the FT-AT interpretation.

The use of non-invasive markers of liver fibrosis and activity simplifies liver injury assessment and should accelerate the management of chronic hepatitis C. New categories of promising markers are elastometry (31) and serum protein glycomics (32). In those two studies, the diagnostic value of FT was also validated *versus* biopsy (31, 32). The challenge for the future is to find a marker or a combination of markers of genomic or proteomic origins with even higher predictive assessment of liver injury that would have an acceptable price.

Part of this study was presented orally in the AASLD 2003 Congress.

STUDY HIGHLIGHTS

What Is Current Knowledge

- Liver biopsy is the gold standard for assessing liver fibrosis.
- Fibrotest is a well characterized non-invasive marker of liver fibrosis although extensive external validation of Fibrotest has not been done.

What Is New Here

- The current prospective study confirmed the diagnostic value of Fibrotest in patients with chronic liver diseases.
- Discordance between Fibrotest and liver biopsy (at least 2 fibrosis stages) occurred in 18% of cases.

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REFERENCES

1. Marcellin P, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. Hepatology 2003;36:S47–56.
2. De Torres M, Poynard T. Risk factors for liver fibrosis progression in patients with chronic hepatitis C. Ann Hepatol 2003;2(1):5–11.
3. Regev A, Berho M, Jeffers LJ, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol 2002;97:2614–8.
4. Colloredo G, Guido M, Sonzogni A, et al. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: The smaller the sample, the milder the disease. J Hepatol 2003;39:239–44.
5. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 1996;24:289–93.
6. Pradat P, Alberti A, Poynard T, et al. Predictive value of ALT levels for histologic findings in chronic hepatitis C: A European collaborative study. Hepatology 2002;36:973–7.
7. Fontana RJ, Lok AS. Noninvasive monitoring of patients with chronic hepatitis C. Hepatology 2002;36(suppl 1):S57–64.
8. Pilette C, Rousselet MC, Bedossa P, et al. Histopathological evaluation of liver fibrosis: Quantitative image analysis vs semi-quantitative scores. Comparison with serum markers. J Hepatol 1998;28:439–46.
9. Aube C, Oberti F, Korali N, et al. Ultrasonographic diagnosis of hepatic fibrosis or cirrhosis. J Hepatol 1999;30:472–8.
10. Renou C, Muller P, Jouve E, et al. Revelance of moderate isolated thrombopenia as a strong predictive marker of cirrhosis in patients with chronic hepatitis C virus. Am J Gastroenterol 2001;96:1657–9.
11. Croquet V, Vuillemin E, Ternisien C, et al. Prothrombin index is an indirect marker of severe liver fibrosis. Eur J Gastroenterol Hepatol 2002;14:1133–41.
12. Patel K, Lajoie A, Heaton S, et al. Clinical use of hyaluronic acid as a predictor of fibrosis change in hepatitis. J Gastroenterol Hepatol 2003;18:253–7.
13. Xie SB, Yao JL, Zheng RQ, et al. Serum hyaluronic acid, procollagen type III and IV in histological diagnosis of liver fibrosis. Hepatobiliary Pancreat Dis Int 2003;2:69–72.
14. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38:518–26.
15. Forns X, Ampurdanes S, Llovet JM, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 2002;36:986–92.
16. Imbert-Bismut F, Ratziu V, Pieroni L, et al. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: A prospective study. Lancet 2001;357:1069–75.
17. Patel K, Muir AJ, McHutchison JG. Validation of a simple predictive model for the identification of mild hepatic fibrosis in chronic hepatitis C patients. Hepatology 2003;37:1222–3.
18. Oberti F, Valsesia E, Pilette C, et al. Noninvasive diagnosis of hepatic fibrosis or cirrhosis. Gastroenterology 1997;113:1609–16.
19. Rozario R, Ramakrishna B. Histopathological study of chronic hepatitis B and C: A comparison of two scoring systems. J Hepatol 2003;38:223–9.
20. Dhumeaux D, Marcellin P, Lerebours E. Treatment of hepatitis C. The 2002 French consensus. Gut 2003;52:1784–7.
21. Imbert-Bismut F, Messous D, Thibaut V, et al. Intralaboratory analytical variability of biochemical markers of fibrosis (Fibrotest) and activity (Actitest) and reference ranges in healthy blood donors. Clin Chem Lab Med 2004;42:323–33.
22. Poynard T. Diagnosis method of inflammatory, fibrotic or cancerous disease using the biochemical markers. United States: Patent No US 6,631,330 B1, 2003.
23. Renou C, Muller P, Jouve E, et al. Revelance of moderate isolated thrombopenia as a strong predictive marker of

cirrhosis in patients with chronic hepatitis C virus. Am J Gastroenterol 2001;96(5):1657–9.
24. Poynard T, Imbert-Bismut F, Munteanu M, 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. Comp Hepatol 2004; 3:8.
25. Rossi E, Adams L, Bulsara M, et al. Validation of the FibroTest biochemical markers score in assessing liver fibrosis in Hepatitis C patients. Clin Chem 2003;49:450–4.
26. Halfon P, Imbert-Bismut F, Messous D, et al. A prospective assessment of the inter-laboratory variability of biochemical markers of fibrosis (FibroTest) and activity (ActiTest) in patients with chronic liver disease. Comp Hepatol 2002;2:3–7.
27. Poynard T, Munteanu M, Imbert-Bismut F, et al. A prospective analysis of discordant results between biochemical markers and biopsy in patients with chronic hepatitis C. Clin Chem 2004;50(8):1344–55.
28. Bedossa P, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology 2003;38;1449–57.
29. Myers RP, de Torres M, Imbert-Bismut F, et al. Biochemical markers of fibrosis in patients with chronic hepatitis C: A comparison with prothrombin time, platelet count and the age-platelet index. Dig Dis Sci 2003;48:146–53.
30. Le Calvez S, Thabut D, Messous D, et al. Fibrotest has higher Predictive values than APRI for fibrosis diagnosis in patients with chronic hepatitis C. (Letter). Hepatology 2004;39:862–3.
31. Castéra L, Vergniol J, Foucher J, et al. Prospective comparison of transient elastography, Fibrotest, APRI and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005;128:343–50.
32. Callewaert N, Van Vlierberghe H, Van Hecke A, et al. Noninvasive diagnosis of liver cirrhosis using DNA sequencer-based total serum protein glycomics. Nature Med 2004;10:1–6.