Clinical Chemistry 50:8
1344–1355 (2004)

Proteomics and
Protein Markers

Prospective Analysis of Discordant Results between Biochemical Markers and Biopsy in Patients with Chronic Hepatitis C

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Background: **The FibroTest and ActiTest are noninvasive biochemical markers of liver injury that are intended for use as alternatives to liver biopsy in patients with chronic hepatitis C. The aims of this study were to assess the quality of biopsy and the prevalence of discordances between biopsy and markers, to identify factors associated with discordances, and to attribute these discordances to either markers or biopsy failure.**

Methods: **Fibrosis stage and activity grade were prospectively assessed on the same day by a liver biopsy and by markers. On the basis of risk factors for failure and independent endpoints, discordance was classified as being attributable to biopsy or to markers.**

Results: **Only 74 of 537 patients (14%) had a biopsy size ≥25 mm. Discordance was observed in 154 of 537 patients (29%), including 16% for fibrosis staging and 17% for activity grading. Steatosis, an inflammatory profile, and biopsy size were associated with discordance. Discordance was attributable to failure of markers in 13 patients (2.4%) and to biopsy failure in 97 (18%; P <0.001 vs Fibrotest and Actitest), and was nonattributable in 44 patients (8.2%). The most frequent failures attributable to markers were false negatives (1.3%) attributable to inflammation. The most frequent failures attributable to biopsy were false negatives of activity grading (10.1%) and of fibrosis staging (4.5%), both associated with smaller biopsy size and steatosis. False positives of fibrosis staging (3.5%) were associated with fragmented biopsies.**

Conclusion: **In this series, the size of liver biopsy is adequate in only a minor proportion (~14%) of patients with chronic hepatitis C. When biopsy and marker results are discordant, a reason can be identified in more than two-thirds of cases and, in those cases, biopsy failure is >7 times more common than diagnostic failure of markers.**

A major clinical problem facing the medical community is how to best evaluate and manage the increasing numbers of patients infected with hepatitis C virus (HCV)[5] *(1)*. According to the latest consensus conferences, liver biopsy is still recommended in most patients *(2, 3)*. Recent studies, however, strongly suggest that because of the limitations *(4–6)* and risks of biopsy *(7)*, as well as improvements in the diagnostic accuracy of biochemical markers *(8)*, liver biopsy should no longer be considered mandatory.

Among the noninvasive alternatives to liver biopsy *(9)*, eight studies have demonstrated the diagnostic accuracy of two combinations of simple serum biochemical markers in patients infected with HCV: FibroTest (FT; Biopredictive) for the assessment of fibrosis, and ActiTest (AT; Biopredictive) for the assessment of necroinflammatory activity *(8, 10–18)*. Similar results have not been obtained with other diagnostic tests *(9–12, 14–16)*. With biopsy as the standard of reference, the diagnostic value of FT for the diagnosis of significant fibrosis (bridging fibrosis), as estimated by the area under the ROC curve, is 0.73–0.86.

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Received February 2, 2004; accepted April 9, 2004.
Previously published online at DOI: 10.1373/clinchem.2004.032227

[5] Nonstandard abbreviations: FT-AT, FibroTest-ActiTest; HCV, hepatitis C virus; GGT, γ-glutamyltransferase; ALT, alanine aminotransferase; CI, confidence interval; and OR, odds ratio.

Identified causes of false positives and false negatives in the combination of FT-AT included Gilbert syndrome, hemolysis, and inflammation *(8)*.

There is no ideal reference for the assessment of liver histology. Liver biopsy has three major limitations: a risk of adverse events *(7)*, sampling error *(4, 5, 19)*, and inter- and intrapathologist variability *(20)*. An overview of published studies summarized the risks of liver biopsy as pain (~30%), severe adverse events (3 in 1000), and death (3 in 10 000) *(7)*. Sampling variation is the major source of variability *(4, 5, 19)*. In a study involving patients with chronic hepatitis C, which included only good-quality biopsies, 30 of 124 patients (24%) had a difference of at least one grade, and 41 of 124 patients (33%) had a difference of at least one stage between the right and left lobes *(4)*. In 18 patients (14.5%), an interpretation of cirrhosis was made in one lobe, whereas stage 3 fibrosis was made in the other *(4)*. Recently, Bedossa et al. *(6)* observed a very high CV (55%) in fibrosis measurements and high discordance rates (35%) for fibrosis staging in biopsies 15 mm in length. The variability significantly improved in biopsies 25 mm in length, but the CV of fibrosis measurement was still high (45%) and the discordance remained high at 25%.

We hypothesize that discordances between biopsy and FT-AT results can be attributable, in many cases, either to FT-AT or to biopsy. It is even possible that biochemical markers such as those described may provide a more diagnostically accurate, as well as quantitative and more reproducible, picture of fibrogenic and necrotic events occurring within the liver than does liver biopsy. The greater diagnostic accuracies of FT-AT when assessed against a reference standard based on biopsy specimens >15 mm in length vs smaller biopsies suggest that some of the discordance between FT-AT and histology is attributable to biopsy specimen sampling error *(8)*. Several case reports have observed false negatives of liver biopsy vs biochemical markers *(8, 10, 13)*. The error was attributable to biopsy because there were overt clinical signs of cirrhosis, such as esophageal varices, low platelet counts, or a dysmorphic liver on ultrasound.

The aims of this study were (*a*) to prospectively assess the quality of biopsy in a large hospital-based cohort; (*b*) to prospectively assess the prevalence and the factors associated with significant discordances between liver biopsy estimates and FT-AT estimates; (*c*) to attribute the cause of discordances to biopsy or to FT-AT; and (*d*) to detect possible causes of failures attributable to FT-AT in a large community-based population by analyzing the distribution of the six components of FT-AT.

Patients and Methods

PATIENTS

Two groups of patients were included. The first, a hospital-based cohort of patients with chronic hepatitis C from our institution, was included prospectively from January 1997 to December 2002 to assess biopsy quality, discordance rate, and the factors associated with discordance and to attempt to attribute the causes of discordances (biopsy or FT-AT). The inclusion criterion was two assessments of liver histology done <24 h apart in our department, one with a liver biopsy and one with a measurement of FT-AT in the serum. Criteria for noninclusion were the absence of a liver biopsy or FT-AT results and a lapse >24-h between the biopsy and collection of serum samples. A total of 537 patients were included, and 2328 were not included (Fig. 1). Included patients were followed every 6 months, and biopsy or FT-AT was repeated as considered necessary by the physician in charge. Procedures followed were in accordance with the current revision of the Helsinki Declaration, and participants gave informed consent.

The second cohort was a community-based population for whom 8524 FT-AT tests have been performed prospectively in 100 private and 10 public French laboratories between September 2002, when the marketing of the FT-AT began, and May 2003. The aim was to identify the risks of failure of FT-AT in the six-component distribution.

LIVER BIOPSIES

Liver biopsies were processed by standard techniques, including Masson's trichrome staining. A single pathologist (F.C.), who was unaware of the results for the biochemical markers, evaluated the stage of fibrosis and grade of activity according to the METAVIR scoring system *(20, 21)*. 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. The grading of activity by the METAVIR system *(20, 21)* (based on the intensity of necroinflammatory activity, mainly on necrosis) was scored as follows: A0, no histologic activity; A1, mild activity; A2, moderate activity; A3, severe activity. Two quality criteria were used. The first, recommended by Regev et al. *(4)*, was a nonfragmented biopsy ≥15 mm in length and with five or more portal tracts (called "a good-quality biopsy"). The second, recommended by Bedossa et al. *(6)*, was a sample length of at least 25 mm.

BIOCHEMICAL MARKERS

We used the previously validated FT-AT *(8, 10–17)*. FT-AT is a noninvasive blood test that combines the quantitative results of six serum biochemical markers—α_2-macroglobulin, haptoglobin, γ-glutamyltranspeptidase (GGT), total bilirubin, apolipoprotein A1, and alanine aminotransferase (ALT)—with patient age and gender in a patented artificial intelligence algorithm (US patent 6,631,330) to generate a measure of fibrosis and necroinflammatory activity in the liver. FT-AT is a continuous linear biochemical assessment of fibrosis stage and necroinflammatory activity grade that provides a numeric quantitative estimate of liver fibrosis ranging from 0.00 to

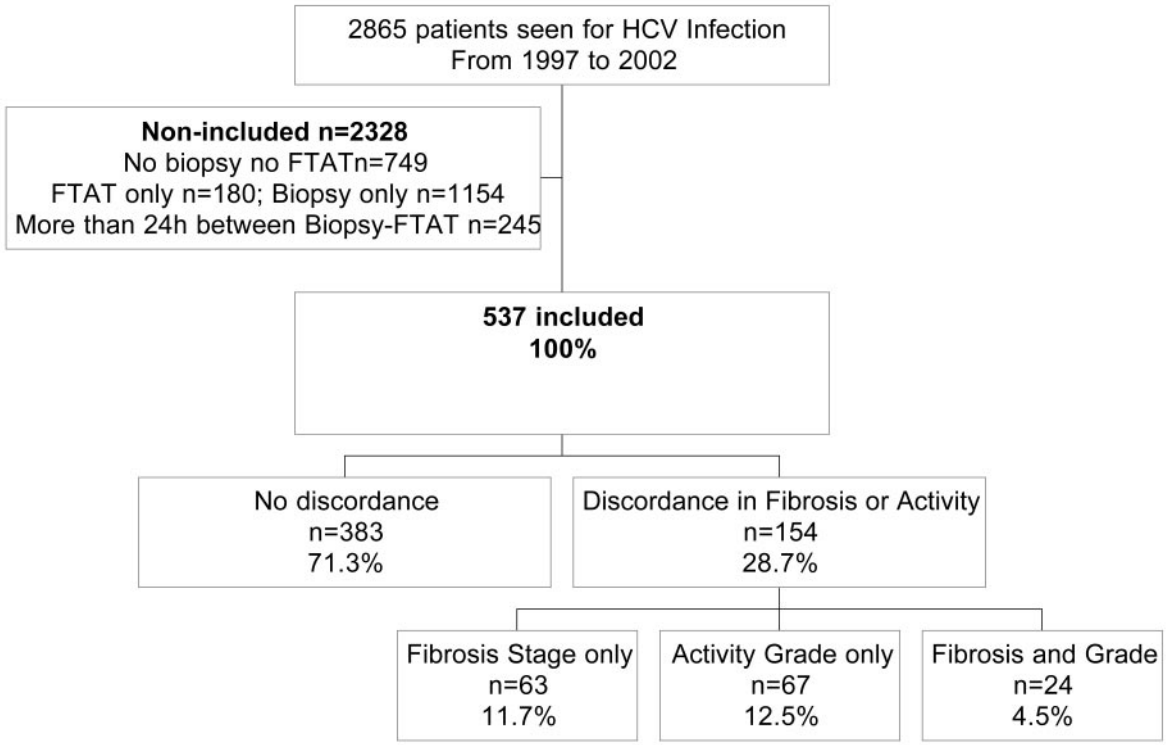

Fig. 1. Study population.

1.00, corresponding to stages F0–F4 and grades A0–A3 of the well-established METAVIR scoring system. Corresponding stages and grades were calculated from median scores and 95% confidence intervals observed in 1270 patients and 300 healthy blood donors *(4, 8, 11)*. Among the 300 controls, the median (SE) FT value was 0.08 (0.004) (95th percentile, 0.23), and the median AT value was 0.07 (0.004) (95th percentile, 0.26). Among the 1270 HCV-infected patients, the FT conversion was 0.0000–0.2100 for F0, 0.2101–0.2700 for F0–F1, 0.2701–0.3100 for F1, 0.3101–0.4800 for F1–F2, 0.4801–0.5800 for F2, 0.5801–0.7200 for F3, 0.7201–0.7400 for F3–F4, and 0.7401–1.0000 for F4. The AT conversion was 0.0000–0.1700 for A0, 0.1701–0.2900 for A0–A1, 0.2901–0.3600 for A1, 0.3601–0.5200 for A1–A2, 0.5201–0.6000 for A2, 0.6001–0.6200 for A2–A3, and 0.6201–1.0000 for A3.

In the hospital-based cohort, GGT, ALT, and total bilirubin were measured on a Hitachi 917 Analyzer with Roche Diagnostics reagents. α_2-Macroglobulin, apolipoprotein A1, and haptoglobin were measured on a Modular analyzer (BNII; Dade Behring). All assay CVs were <6%.

In the community-based population, 110 laboratories assessed the six FT-AT components and applied the preanalytical and analytical recommendations required to obtain the FT-AT results, as detailed elsewhere *(17)*. Abnormal values for the six components, which could increase the risk of false positives or false negatives in the FT-AT, were defined as values outside the 99% percentiles, as determined in the first validation study *(10)*.

ANALYSIS OF DISCORDANCES IN THE HOSPITAL-BASED COHORT

The definition of a significant discordance between FT-AT and biopsy results was a discordance of at least two stages or two grades in the METAVIR scoring system. We chose two stages or grades instead of one stage or grade because the precision of biopsy is not sufficient to differentiate between a one-stage or one-grade difference (33% and 24%, respectively) in the same patient *(4)*. We did perform a sensitivity analysis using one stage or one grade.

Risk factors of failure. For biopsy failure, the risk factors for failure were biopsy size, the number of fragments, and the number of portal tracts. For FT-AT failure, these risk factors were hemolysis, inflammation (confirmed sepsis, a sedimentation rate >20 mm, C-reactive protein >5 mg/L), possible Gilbert syndrome (total bilirubin concentration >17 μmol/L, including >50% nonconjugated bilirubin), acute hepatitis, drugs inducing cholestasis or an increase in transaminases, and extrahepatic cholestasis. We included renal insufficiency (creatinine >120 mmol/L) as a risk factor for failure of FT-AT.

The following factors were also considered but not included in the algorithm for failure definition: diabetes (defined as being treated with antidiabetic drugs or insulin), co-infection with HIV, high alcohol consumption (>50 g/day), and transplantation.

Independent endpoints of FT-AT and biopsy. Independent endpoints used for assessing extensive fibrosis and cir-

rhosis were a platelet count <150 000 cells/mL prothrombin time <70% of control, obvious cirrhosis on ultrasound, a reduction or reversal of portal flow, gradient portal pressure >5 mmHg, and grade 2 or 3 esophageal varices. Serum ferritin was used as an independent marker for necroinflammatory features *(22–25)*.

Repeated assessments. Repeated assessments of liver injury, either by a subsequent biopsy or by a subsequent FT-AT during follow-up, were also considered.

Attribution of discordance to FT-AT failure. Discordance was considered highly attributable to FT-AT failure if (*a*) the FT-AT result was few septa or less, whereas the biopsy result was cirrhosis in the presence of at least two independent signs of cirrhosis; or (*b*) if one of the six FT-AT components had an abnormal value attributable to an identified condition such as hemolysis with haptoglobin <0.30 g/L, inflammation or sepsis with haptoglobin >2 g/L or α_2-macroglobulin >3 g/L, possible Gilbert syndrome, or increased bilirubin or GGT with extrahepatic cholestasis.

Discordance was considered moderately attributable to FT-AT failure if (*a*) the FT-AT result was few septa or less, whereas the biopsy result was cirrhosis in the presence of one independent sign of cirrhosis; (*b*) one of the six FT-AT components had an abnormal value in the absence of an identified condition and was paired with a biopsy of good quality; or (*c*) renal failure was present with a biopsy of good quality.

Attribution of discordance to biopsy failure. Discordance was considered highly attributable to biopsy failure if (*a*) the biopsy result was few septa or less, whereas the FT-AT result was cirrhosis in the presence of at least two independent signs of cirrhosis; or (*b*) the biopsy result was no or minimal activity, and there was an acute flare-up of hepatitis (ALT >200 U/L). A multicenter study observed that at three times the upper limit of the reference interval (ALT >90 U/L), the ALT specificity for the diagnosis of significant liver injury (more than A1F1) was 0.87–1.00 *(26)*.

Discordance was considered moderately attributable to biopsy failure if (*a*) the biopsy result was few septa or less, whereas the FT-AT result was cirrhosis in the presence of one independent sign of cirrhosis; or (*b*) the biopsy was not of good quality, and there was no risk of FT-AT failure.

Undetermined failure. If there was no risk of failure or there was a risk of failure for both FT-AT and biopsy, the discordance imputation was considered undetermined.

RISK OF FT-AT FAILURE IN THE COMMUNITY-BASED POPULATION

Extreme values of the six FT-AT components were defined as values below the 1st percentiles or above the 99th percentiles, as defined in the original validation study *(10)*. A possible risk for false positives was considered attributable to hemolysis when haptoglobin was <0.12 g/L or to Gilbert syndrome when total bilirubin was >30 μmol/L with GGT <50 U/L. A possible risk of failure was considered when one of the two proteins of inflammation had increased values; false positives attributable to inflammation were considered when α_2-macroglobulin was >5.0 g/L without a decrease in haptoglobin, and false negatives attributable to inflammation were considered when haptoglobin was >3.2 g/L.

STATISTICAL ANALYSIS

We performed statistical analyses with the Fisher exact test, χ^2 test, Student *t*-test, and the Mann–Whitney test. We performed variance analyses with the Bonferroni all-pair-wise and Tukey–Kramer multiple-comparison tests to take into account the multiple comparisons and multiple logistic regression for multivariate analysis *(27)*.

Results

A total of 537 of the 2865 patients (19%) seen during this period were included in the cohort (Fig. 1). The included patients did not differ from the nonincluded patients (Tables 1–3). The mean (SE) follow-up of included patients was 31 (1) months; 70 patients (13%) were lost to follow-up.

The diagnostic value of FT for fibrosis staging and of AT for activity grading was similar to those reported previously *(8, 10–18)* with areas under the ROC curves of 0.80 (0.02) for the diagnosis of F2F3F4 and 0.78 (0.02) for the diagnosis of A2A3 (Fig. 2).

QUALITY OF BIOPSY

Only 74 of 537 cohort patients [14%; 95% confidence interval (CI), 11–17%] had a biopsy ≥25 mm in length. A good-quality biopsy *(4)* was observed in 31% of patients. After biopsy, severe pain was observed in 2% of patients, and severe adverse events, without death, were seen in 0.5% of patients.

PREVALENCE OF DISCORDANCES AND RISK FACTORS

In 154 of 537 patients (29%), there was a significant discordance at baseline between FT-AT and biopsy results for fibrosis staging or activity grading. This occurred in 63 patients (12%) for fibrosis staging only, in 67 patients (12%) for activity grading only, and in 24 patients (4.5%) for both.

The characteristics of the patients with or without discordances are given in Tables 1–3. Patients with discordance were older, more frequently were male and diabetic, and had an inflammatory biological profile, renal failure, liver steatosis, more frequent moderate and severe activity, and more extensive fibrosis than patients without discordance (Tables 1–3). Patients with activity discordance had a smaller mean biopsy sample size [14.6 (0.6) mm] than patients without discordance [16.4 (0.3) mm;

Table 1. Characteristics of nonincluded patients and included patients with nondiscordant or discordant results.

		Included		
	Nonincluded	Total	Nondiscordant	Discordant
No. of patients	2328	537	383	154
Mean (SE) age at biopsy, years	47.4 (0.3)	45.7 (0.5)	44.9 (0.6)	47.6 (1.0)[a]
Male, n (%)	1305 (56)	322 (60)	212 (55)	110 (71)[b]
Female, n (%)	1023 (44)	215 (40)	171 (45)	44 (29)
Mean (SE) body mass index, kg/m^2	24.4 (0.3)	24.2 (0.2)	24.2 (0.2)	23.9 (0.3)
Ethnic origin, n (%)				
Caucasian	2031 (87)	454 (85)	321 (84)	133 (87)
African	175 (8)	47 (8)	34 (9)	13 (8)
Asian	122 (5)	36 (7)	28 (7)	8 (5)
Source of infection, n (%)				
Transfusion	626 (27)	113 (21)	74 (19)	39 (25)
Intravenous drug	695 (30)	168 (31)	124 (32)	44 (29)
Other or unknown	1007 (43)	256 (48)	185 (49)	71(46)
Alcohol, n (%)				
0 g/day	1406 (61)	267 (53)	196 (56)	71 (47)
0–50 g/day	633 (28)	169 (33)	111 (31)	58 (38)
>50 g/day	262 (11)	69 (14)	46 (13)	23 (15)
Co-infection by HBV,[d] n (%)	68 (2.9)	18 (3.5)	11 (3.1)	7 (4.5)
Genotype, n (%)				
G1	731 (61)	163 (52)	112 (52)	51 (54)
G2	143 (12)	36 (12)	28 (13)	8 (8)
G3	189 (16)	73 (23)	46 (21)	27 (28)
G4, G5, or G6	133 (11)	39 (13)	29 (14)	10 (10)
Mean (SE) baseline viral load, kIU/mL	1229 (160)	1673 (660)	961.7 (553)	2068 (979)
Risk of failure, n (%)				
Drug	1216 (52)	248 (46)	176 (46)	72 (47)
Immunosuppression	252 (11)	42 (8.3)	27 (7.6)	15 (9.8)
Diabetes, n (%)	102 (4.5)	34 (6.3)	20 (5.2)	14 (9)[a]
Renal failure, n (%)	53 (2.4)	17 (3.2)	7 (1.8)	10 (6.5)[c]
Transplantation, n (%)	74 (3.1)	18 (3.4)	11 (2.9)	7 (4.5)
Co-infection by HIV, n (%)	286 (12)	47 (9.2)	27 (7.6)	20 (13)
Sepsis, n (%)	ND	18/537 (3.4)	6/383 (1.6)	12/154 (9.7)[b]
Severe hemolysis (haptoglobin <0.12 g/L), n (%)	ND	9/537 (1.7)	6/383 (1.6)	3/154 (1.9)

[a–c] Significant difference between groups: [a] $P < 0.05$; [b] $P < 0.001$; [c] $P < 0.01$.
[d] HBV, hepatitis B virus; ND, not determined.

$P = 0.01$]. In multivariate analysis, six factors remained significantly associated with discordance: steatosis, male gender and age (significantly associated with both discordances), an inflammatory profile (fibrosis discordance only), smaller biopsy sample size, and renal failure (activity discordance only; Table 4).

IMPUTATION OF DISCORDANCES

According to the risk factors, the independent endpoints, and the follow-up, the causes of failure among the 154 discordant cases were considered highly attributable to FT-AT in 5 cases, moderately attributable to FT-AT in 8, highly attributable to biopsy in 26, and moderately to biopsy in 71 (Table 5). Discordance was attributable to FT-AT failure in 13 patients (2.4%) and to biopsy failure in 97 (18%; $P < 0.001$ vs FT-AT). Even when we considered all failures of FT-AT (highly and moderately attributable) and only the highly attributable failures of biopsy (worst-case scenario for FT-AT), biochemical markers still had less imputable failures than biopsy (2.4% vs 4.8%; $P = 0.03$).

FACTORS ASSOCIATED WITH ATTRIBUTABLE FAILURES

Among the 13 errors attributable to FT with biochemical markers, 7 were false negatives and 6 were false positives (Table 5). The most frequently identified cause of FT failure was false negatives attributable to inflammation with an isolated increase in haptoglobin (four cases); one false-positive FT result was attributable to hemolysis, and one was attributable to posttransplantation fibrosing cholestasis.

The most frequent biopsy errors were false negatives for activity grading (10.1%), false negatives for fibrosis staging (4.5%), and false positives for fibrosis staging (3.5%; Table 5).

Compared with patients who did not have false-nega-

Table 2. Biopsy results for nonincluded patients and included patients with nondiscordant or discordant results.

		Included		
	Nonincluded	Total	Nondiscordant	Discordant
No. of patients	2328	537	383	154
Patients with A2A3 at biopsy, n (%)	490/1357 (36)	173/537 (32)	141 (37)	32 (21)[a]
Patients with F2F3F4 at biopsy, n (%)	644/1375 (47)	240/537 (45)	156 (41)	84 (55)[b]
Steatosis, n (%)				
0	490/1208 (40)	311/537 (58)	258/383 (67)	53/154 (34)[a]
≤10%	309/1208 (26)	110/537 (20)	65/383 (17)	45/154 (30)
>10% and ≤30%	268/1208 (22)	51/537 (10)	29/383 (8)	22/154 (14)
>30%	141/1208 (12)	65/537 (12)	31/383 (8)	34/154 (22)
Quality of liver biopsy				
Mean (SE) biopsy size, mm	16.8 (0.2)	16.1 (0.3)	16.2 (0.3)	15.8 (0.5)
Mean (SE) portal tracts, n	ND[c]	15.6 (0.4)	15.9 (0.5)	15.0 (0.65)
Mean (SE) number of fragments	ND	2.5 (0.1)	2.4 (0.2)	2.6 (0.2)
Regev criteria				
≥15 mm, n (%)	754/1236 (61)	328/533 (62)	229/380 (60)	99/153 (65)
≥5 portal tracts, n (%)	ND	412/437 (94)	294/309 (95)	118/128 (92)
Not fragmented, n (%)	ND	262/505 (52)	189/353 (54)	73/152 (48)
High-quality biopsy,[d] n (%)	ND	165/537 (31)	118/383 (31)	47/154 (31)
Bedossa criteria (≥25 mm), n (%)	206/1236 (17)	74/533 (14)	56/380 (15)	18/153 (12)
Transjugular biopsy, n (%)	ND	49 (9.1)	27 (7.0)	22 (14)[b]
Warning of pathologist for biopsy, n (%)	ND	22/537 (4.1)	13/383 (3.4)	9/154 (5.8)

[a,b] Significant difference between groups: [a] $P < 0.001$; [b] $P < 0.01$.
[c] ND, not determined.
[d] One fragment ≥15 mm in length with ≥5 portal tracts.

tive activity grading based on biopsy (n = 483), patients who did have false-negative activity grading based on biopsy (n = 54) had a smaller biopsy size [13.5 (0.7) vs 16.4 (0.3) mm; $P = 0.002$], fewer portal tracts [12.9 (0.8) vs 15.9 (0.4); $P = 0.03$], poorer quality biopsies (17% vs 32%; $P = 0.02$), more frequent liver steatosis (64% vs 40%; $P < 0.001$), higher ALT serum activity [203 (17) vs 99 (4) U/L; $P < 0.001$], higher serum ferritin [502 (72) IU/L (n = 31) vs 275 (27) IU/L (n = 135); $P = 0.005$], and were more frequently male (76% vs 58%; $P = 0.01$). In multivariate analysis, three risk factors remained significant: biopsy size [odds ratio (OR) = 1.08, 95% CI, 1.03–1.14; $P = 0.004$], steatosis (OR = 0.44; 95% CI, 0.24–0.80; $P = 0.007$), and male gender (OR = 0.49; 95% CI, 0.25–0.94; $P = 0.03$).

Compared with patients who did not have false-negative fibrosis staging at biopsy (n = 514), patients who did have false-negative fibrosis staging at biopsy (n = 23) had smaller biopsy size [13.0 (0.7) vs 16.3 (0.3) mm; $P = 0.02$], more frequent liver steatosis (74% vs 41%; $P = 0.002$), higher serum ALT activity [159 (18) vs 96 (4) U/L; $P < 0.001$], and were older [57.3 (2.8) vs 45.2 (0.5) years; $P < 0.001$]. In multivariate analysis, two risk factors remained significant: biopsy size (OR = 1.08; 95% CI, 1.01–1.17; $P = 0.04$) and steatosis (OR = 0.27; 95% CI, 0.10–0.70; $P = 0.007$).

Compared with patients who were not false positive for fibrosis staging at biopsy (n = 518), patients who were false positive for fibrosis staging at biopsy (n = 19) had more frequent fragmented biopsies (84% vs 51%; $P = 0.004$) and poorer quality biopsies (0% vs 32%; $P < 0.001$). In multivariate analysis, fragmented biopsy remained significant (OR = 0.18; 95% CI, 0.05–0.63; $P = 0.007$).

A total of 36 patients (6.7%) were discordant for the diagnosis of cirrhosis; 18 (3.4%) were false negative based on incorrect biopsy, 3 (0.6%) were false positive based on incorrect biopsy, 3 (0.6%) were false positive based on incorrect FT results (one with Gilbert syndrome, one with hemolysis, and one with fibrosing cholestasis), 2 (0.4%) were false negative based on incorrect FT results, 7 (1.3%) were failures for both, and 3 were unclassified.

ABNORMAL VALUES OF BIOCHEMICAL COMPONENTS OF FT-AT IN THE COMMUNITY-BASED POPULATION

A total of 8524 consecutive tests were computed, and the distribution of the six FT-AT components is shown in Table 6. The most frequent abnormal value observed in 402 patients (4.7%) was haptoglobin <0.12 g/L. For 227 of these patients (2.7%), extensive fibrosis was still predicted by FT if low haptoglobin was replaced with a value within the reference interval (1 g/L). The remaining 2% were at risk of having false positives for FT-AT attributable to significant hemolysis. Gilbert syndrome was suspected in 120 patients (1.4%). A possible false positive attributable to acute inflammation with highly increased α_2-macroglobulin values and without a concomitant increase in haptoglobin was observed in only six patients (0.07%).

Table 3. Biochemistry results for nonincluded patients and included patients with nondiscordant or discordant results.

		Included		
	Nonincluded	Total	Nondiscordant	Discordant
No. of patients	2328	537	383	154
Mean (SE) blood glucose, mmol/L	5.4 (0.3)	5.2 (0.1)	5.06 (0.2)	5.2 (0.2)
Mean (SE) ALT, U/L	89 (3.9)	109 (4.1)	94.6 (4.4)	146 (8.7)[a]
Mean (SE) total bilirubin, μmol/L	14.8 (1.4)	11.7 (0.3)	11.6 (0.4)	12.1 (0.7)
Mean (SE) GGT, U/L	95 (7)	96 (7)	85 (6)	125 (16)[a]
Mean (SE) α_2-macroglobulin, g/L	2.79 (0.05)	2.52 (0.04)	2.46 (0.05)	2.69 (0.08)[b]
Mean (SE) ApoA1,[c] g/L	1.44 (0.02)	1.46 (0.01)	1.47 (0.02)	1.44 (0.03)
Mean (SE) haptoglobin, g/L	0.9 (0.03)	0.93 (0.02)	0.95 (0.03)	0.87 (0.04)
Mean (SE) conjugated bilirubin, μmol/L	11.4 (4.1)	4.3 (0.26)	4.1 (0.26)	4.7 (0.65)
Gilbert syndrome possible,[d] n (%)	ND	48/496 (9.7)	35/347 (10)	13/149 (8.7)
Mean (SE) ferritin, μg/L	ND	318 (26)	258 (40)	367 (65)[b]
Mean (SE) fibrosis index (range, 0.00–1.00)	0.51 (0.01)	0.41 (0.01)	0.38 (0.01)	0.48 (0.02)[a]
Mean (SE) activity index (range, 0.00–1.00)	0.47 (0.01)	0.52 (0.01)	0.46 (0.01)	0.65 (0.02)[a]
Follow-up				
Lost to follow-up, n (%)	ND	70 (13)	55 (10)	15 (3)
Mean (SE) duration of follow-up, months	ND	31 (1)	30 (1)	33 (2)

[a,b] Significant difference between groups: [a] $P < 0.001$; [b] $P < 0.01$.

[c] ApoA1, apolipoprotein A-1; ND, not determined.

[d] Gilbert syndrome possible if unconjugated bilirubin is greater than conjugated bilirubin with a total bilirubin >17 μmol/L.

SENSITIVITY ANALYSIS USING ONE-STAGE OR ONE-GRADE DISCORDANCE

In 209 of 537 patients (39%), there was a significant discordance at baseline between FT-AT and biopsy results for fibrosis staging or activity grading. This occurred in 78 patients (14%) for fibrosis staging only, in 91 patients (17%) for activity grading only, and in 24 patients (7.4%) for both.

The same risk factors were observed for the discordance of one stage/grade or more compared with the discordance of two or more stages/grades. Patients with discordance were older, were more frequently male and diabetic, and had an inflammatory biological profile, renal failure, liver steatosis, more frequent moderate and severe activity, and more extensive fibrosis than patients without discordance. Patients with activity discordance had a smaller mean biopsy sample size [14.5 (0.5) mm] than patients without discordance [16.6 (0.3) mm; $P = 0.001$]. In multivariate analysis, four factors remained significantly associated with discordance: steatosis ($P < 0.001$; significantly associated with both discordances), an inflammatory profile (fibrosis discordance only; $P < 0.001$), smaller biopsy sample size ($P = 0.04$), and renal failure ($P = 0.004$; activity discordance only).

According to the risk factors, the independent endpoints, and the follow-up, the causes of failure among the 54 cases discordant by only 1 or 1.5 stages or grades were considered highly attributable to FT-AT in 1 case (hemolysis), moderately attributable to FT-AT in 1 (isolated low α_2-macroglobulin), highly attributable to biopsy in 2 (false negative for fibrosis staging), and moderately attributable to biopsy in 14 (mostly falsely negative for activity grading). In 36 cases it was not possible to attribute the failure. Among the 54 discordant cases, only 27 patients had a

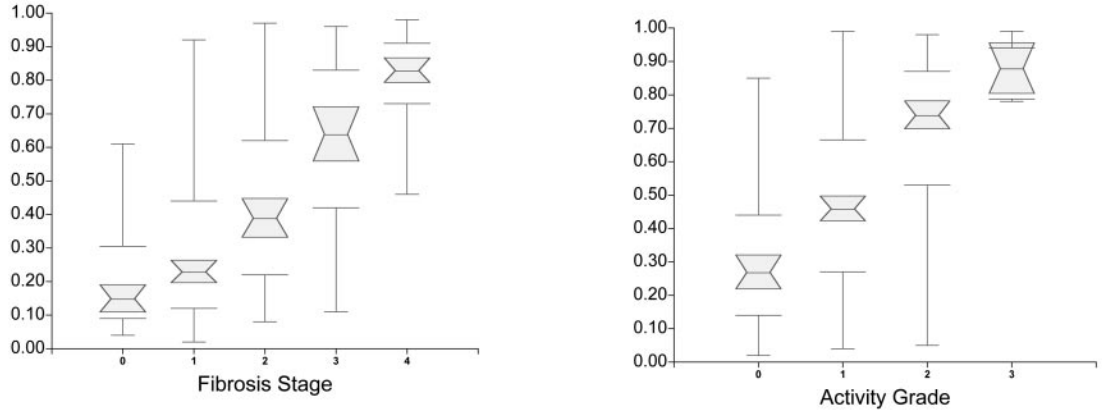

Fig. 2. Diagnostic value of FT for the diagnosis of fibrosis (*left*) and AT for the diagnosis of necrotico-inflammatory activity (*right*).

Table 4. Multivariate analysis of factors associated with discordance.[a]

Factor	OR exp, *B*	Lower 95% confidence limit	Upper 95% confidence limit	*P*
Inflammatory profile				
All discordance	0.25	0.08	0.74	0.01
Discordance in fibrosis	0.26	0.09	0.72	0.01
Discordance in activity	0.96	0.29	3.18	0.94
Renal failure				
All discordance	0.22	0.08	0.65	0.006
Discordance in fibrosis	0.38	0.12	1.19	0.10
Discordance in activity	0.20	0.07	0.60	0.004
Steatosis				
All discordance	0.56	0.47	0.67	<0.0001
Discordance in fibrosis	0.64	0.52	0.79	<0.0001
Discordance in activity	0.58	0.47	0.72	<0.0001
Size of biopsy				
All discordance	1.01	0.98	1.04	0.62
Discordance in fibrosis	0.98	0.94	1.01	0.20
Discordance in activity	1.05	1.01	1.09	0.02
Male gender				
All discordance	0.45	0.29	0.70	0.0004
Discordance in fibrosis	0.53	0.31	0.90	0.02
Discordance in activity	0.45	0.26	0.77	0.004
Age				
All discordance	0.98	0.96	0.99	0.02
Discordance in fibrosis	0.98	0.96	0.99	0.03
Discordance in activity	0.98	0.96	0.99	0.03
Intercept				
All discordance	6.93	2.32	20.67	0.001
Discordance in fibrosis	11.1	2.91	42.80	0.0004
Discordance in activity	4.30	1.18	15.65	0.03

[a] A total of 533 patients had all of the data for the six factors, and 153 had discordance of more than one stage or more than one grade. The other factors significantly associated with discordance in univariate analysis (Tables 1–3) had no independent significant values in multivariate analysis.

biopsy ≥15 mm length, and among them only 17 had a nonfragmented biopsy.

Discussion

This study is the first to investigate the prevalence and the causes of discordance observed between the degree of liver injury estimated by liver biopsy, the classic "gold standard", and that estimated by a panel of biochemical markers. The main difficulty in this discordance analysis was the absence of a true reference standard for liver injury.

Biopsy and FT-AT are two surrogate markers of liver injury, each with its own risks of failure. The two main causes of failure for biopsy are sampling error *(4–6)* and observer error *(20)*. For FT-AT, the two main causes of failure are individual errors for each of the six components *(17, 28–30)* and, indirectly, the failure of biopsy because FT-AT was initially validated against liver biopsy *(10)*. It is therefore paramount to consider separately the quality of liver biopsy and the factors associated with discordant results, both of which are objective criteria, and the less objective criterion of imputability.

An ideal, but impossible to perform, study for the validation of biochemical markers would involve laparoscopy with two biopsies of 20 mm to reach an estimate based on a total liver sample length of 40 mm. The dispersion of fibrosis estimates has been found to be acceptable with biopsy size >40 mm *(6)*. This threshold, which could define a true standard, was obtained in only 10 patients of 1773 (0.2%) in the present study. The usual recommendations are far beyond this 40-mm length and are associated with extreme variability *(4–6)*. Even these usual recommendations were difficult to obtain in our experienced center because the mean size was 16 mm, only 14% of samples were ≥25 mm, and only 31% met the quality criteria recommended by Regev et al. *(4)*.

This analysis uncovered factors already known to be risks of failure, such as short sample length for biopsy failure and an inflammatory profile for FT-AT failure.

Steatosis was highly associated with discordance. This point is important in chronic hepatitis C because steatosis is frequent, with a mean of 50%, reaching 90% in patients infected by genotype 3 hepatitis C *(31)*. Detailed analysis of cases showed that steatosis was associated with possible false-negative biopsy results for both staging and grading. We have no definitive explanation for this. One hypothesis is that in the presence of steatosis, the pathologist underscores the fibrosis staging and necrosis grading. Another hypothesis is that steatosis increases the false-positive rate of FT-AT through an increase in GGT independent of fibrosis and necrosis. This second hypothesis seems very unlikely because we recently demonstrated that FT has excellent predictive values for fibrosis staging in both alcoholic liver disease *(32)* and fatty liver disease *(33)*, in which GGT and steatosis are often highly increased.

Renal failure was associated with discordance in activity. One explanation for this could be the low activity of transaminases often observed in hemodialysis patients *(34)*. The significant but moderate associations between discordance and age and gender could be seen as a technical artifacts because these factors are included in the calculations of FT-AT *(10)*.

Because of the limitations of biopsy, biopsy quality as a factor for biopsy failure was taken into account, although only for the "moderately attributable" grade.

Small sample size increases the risk of false-negative biopsy results *(5, 6)*. Colloredo et al. *(5)* demonstrated that the risk of false negatives was increased by 27% for grading and 20% for staging when biopsy specimens 10 mm in length were compared with specimens 30 mm in length. In the present study, false negatives were associated mainly with smaller biopsies and steatosis.

Our definition of false-negative biopsy results can be challenged because we used ALT >200 U/L to confirm biopsy failure. However, among these patients, serum ferritin, which is a surrogate marker of necrosis *(22–25)*

Table 5. Imputation of discordances observed between biopsy and biochemical markers.

Imputation of discordance[a]	Degree of imputation	Type of failure
Biochemistry failure,[b] n = 13 (2.4%)	Highly attributable, n = 5 (0.9%) Moderately attributable, n = 8 (1.5%)	False-negative biochemistry,[c] n = 7 (1.3%) Fibrosis, n = 7 (1.3%) Activity, n = 0 False-positive biochemistry, n = 6 (1.1%) Fibrosis, n = 6 (1.1%) Activity, n = 0
Biopsy failure, n = 97 (18%)	Highly attributable, n = 26 (4.8%) Moderately attributable, n = 71 (13%)	False-negative biopsy, n = 77 (14%) Fibrosis, n = 23 (4.3%) Activity, n = 54 (10%) False-positive biopsy, n = 22 (4.1%) Fibrosis, n = 19 (3.5%) Activity, n = 3 (0.6%)
Unclassified, n = 44 (8.2%) Failure for both, n = 16 (3.0%) No evidence of failure, n = 28 (5.2%)		Unclassified,[d] n = 42 (7.8%)

[a] A total of 154 discordant cases were observed from a total of 537 included cases (29%)

[b] Significantly lower than biopsy failure: $P < 0.001$.

[c] Percentage calculated from a per-patient analysis. When there was discordance for both fibrosis and activity, the misclassified case was attributed to the certain case and to fibrosis if there was the same degree of association (highly or moderately attributable for both).

[d] Two patients had failure for both but were attributed to biopsy failure, which was more likely than for biochemistry (highly vs moderately attributable).

and which is not included in AT, was also highly increased in 81% of patients (25 of 31), with a mean of 501 (72) μg/L. It is also possible that centrolobular necrosis was underscored by the METAVIR scoring system. However, the risk of false negatives attributable to small sample size has also been observed with the Ishak scoring system, which scores centrolobular necrosis differently *(5)*.

In the present study, fragmented biopsies were associated with false-positive fibrosis, suggesting that pathologists in this setting could overestimate bridging fibrosis.

Liver biopsy can be useful in patients with chronic hepatitis C for purposes other than staging and grading, such as identifying other causes of liver injury. In the present study, the only case in which the biopsy was useful was in the diagnosis of graft rejection in liver transplantation patients. We never made a management decision based on biopsy results for the following other causes: alcohol abuse, steatohepatitis, drug-induced liver disease, hemochromatosis, or co-infection with hepatitis B virus or HCV.

Despite the fact that noninvasive markers have been validated by biopsy *(10)*, biochemical markers such as those described here probably provide a more accurate (quantitative and reproducible) picture of fibrogenic and necrotic events occurring within the liver than does liver biopsy. Supporting evidence for FT-AT are (*a*) the decrease in false positives and false negatives according to the quality of biopsy *(8)*; (*b*) an increase in the linearity of correlations between histologic scores and FT-AT scores when the number of patients studied increases *(35)*; (*c*) the correction of discordance by use of repeat biopsies *(8)*; and (*d*) confirmation of biopsy error by use of independent endpoints, as demonstrated in the present study, as well as by portal pressure *(36)* or endoscopy *(37)*.

The variability of each of the six biochemical components *(17, 28–30)* is a possible source of variability for FT-AT and can be related to preanalytical and inter- and intralaboratory variability *(17–38)*. The same reference laboratory measured all components for the hospital-based cohort. In the large community-based population, all 110 laboratories agreed with the analytical recommendations, and it was reassuring that the percentages of abnormal values were very similar to those observed in the original study (Table 5).

Table 6. Prevalence of values outside the 1st and 99th percentiles as defined by the reference laboratory.[a]

	Below 1st percentile		Above 99th percentile		
	Cutoff concentration	n (%)	Cutoff concentration	n (%)	Within the 1st to the 99th percentiles, n (%)
α_2-Macroglobulin, g/L	1.1	170 (2.0)	5.0	105 (1.2)	8249 (96.8)
Haptoglobin, g/L	0.12	402 (4.7)	3.2	17 (0.2)	8105 (95.0)
Apolipoprotein A1, g/L	0.73	152 (1.8)	2.5	40 (0.5)	8332 (97.7)
Total bilirubin μmol/L	3	270 (3.2)	50	96 (1.1)	8158 (95.6)
GGT, U/L	8	75 (0.9)	1140	16 (0.2)	8433 (98.9)
ALT, U/L	14	239 (2.8)	622	21 (0.3)	8264 (96.9)

[a] Study of 8524 consecutive FT-AT results.

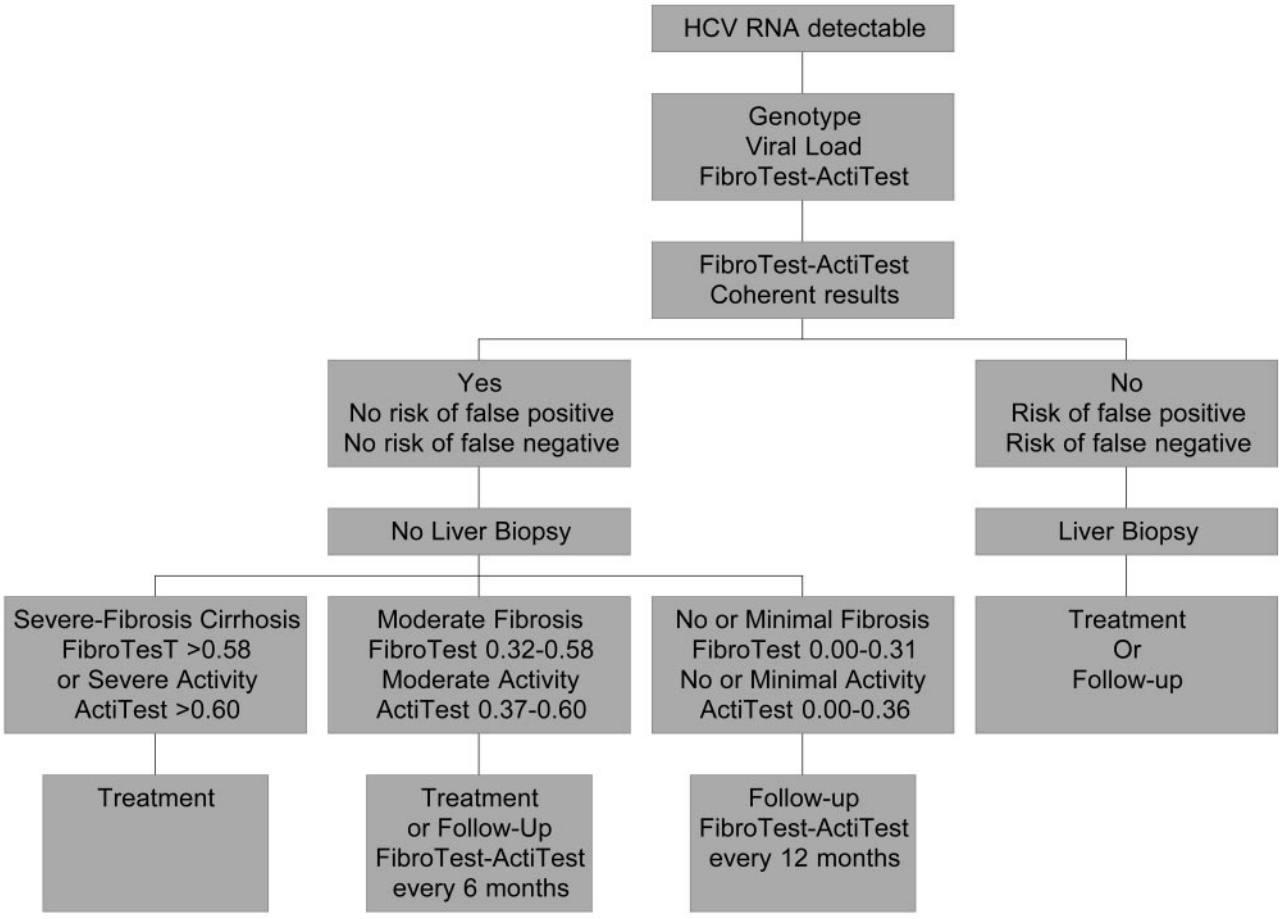

Fig. 3. Suggested algorithm for management of patients according to FT and AT results.

False negatives for fibrosis were mainly associated with an isolated increase in haptoglobin, which can be observed in acute inflammation. This low prevalence is possibly attributable to the inverse relationship in fibrosis of the two proteins of inflammation, haptoglobin and α_2-macroglobulin *(10)*. In the community-based population, the prevalence of haptoglobin above the 99% percentile was rare (0.2%).

False-positive results for fibrosis based on FT-AT scores occurred in only 1.1% of patients in the hospital-based cohort and were attributable to various causes. There was one patient each with Gilbert syndrome (an increase in unconjugated bilirubin), hemolysis (an increase in unconjugated bilirubin and a decrease in haptoglobin), and acute inflammation (an isolated increase in α_2-macroglobulin). One transplanted patient with fibrosing cholestatic hepatitis (a large increase in total bilirubin and GGT) was classified as false positive for the FT test but died 2 years later with decompensated cirrhosis.

In the community-based population, the prevalence of haptoglobin below the 99% percentile was not rare (4.7%). When the other components do not suggest fibrosis (2% of cases in this study), causes of hemolysis should be ruled out, such as drug-induced hemolysis (e.g., ribavirin or azathioprine), cardiac prosthesis, and genetic hemoglobin disease *(29)*.

Highly increased α_2-macroglobulin values without a concomitant increase in haptoglobin were very rare, which should limit the false-positive risk attributable to inflammation *(28)*.

Gilbert syndrome is a frequent cause of increases in unconjugated bilirubin *(30)*. Despite the fact that genetic screening was not used, Gilbert syndrome was suspected in 14% of the hospital cohort when they were assessed for unconjugated bilirubin. Nevertheless, the consequences were minimal for false-positive FT-AT results because only one case with Gilbert syndrome (0.2%) was associated with a false-positive FT-AT result and was suspected in 1.4% of patients in the community-based population.

False-positive FT-AT results attributable to undiagnosed extrahepatic cholestasis were not observed. This can probably be explained by the routine ultrasound usually performed.

The FT-AT scores are derived from six components, which are available in most countries. Since September 2002, the FT-AT has been on the market in France, Morocco, Mexico, Switzerland, Portugal, and Germany, and >25 000 tests have been performed up to February

2004. In the US, the FT-AT has been on the market under the name FibroSure™ (LabCorp.) since March 2004.

When compared with routine laboratory tests, which are predictive of activity or fibrosis *(9)*, the FT-AT has better diagnostic value. This is true of ALT, aspartate aminotransferase, GGT, bilirubin (alone or in combination) *(10)*, hyaluronic acid *(11)*, the age-platelets index, and prothrombin time *(14)*; other published scores combine GGT, cholesterol, platelets, and age *(14)*; AST and platelets *(16)*; and historical features *(12)*. The FT is not genotype dependent, whereas the index developed by Forns et al. *(14)* includes serum cholesterol, which varies with HCV genotype. The FT has the same diagnostic value for fibrosis stages in the subpopulation of HCV patients with transaminase values within the appropriate reference intervals as in patients with increased transaminases *(8–10)*. Another advantage of the FT-AT compared with the other noninvasive markers is that this combination provides fibrosis staging and activity grading, as well as a continuous estimate of fibrosis and necrosis. We suggest that the FT-AT be used as a first-line estimate of liver injury (Fig. 3).

The identification of risk factors for FT-AT failure is important for issuing precautions for use of the FT-AT. In the presence of acute inflammation, i.e., sepsis, or of acute hemolysis, FT-AT analysis must be postponed. Regarding precautions for use of the FT-AT, inflammation should be ruled out in case of an isolated increase in haptoglobin or in case of an isolated increase in α_2-macroglobulin. Hemolysis should be ruled out in case of a very low haptoglobin concentration, particularly if the five other components of the FT-AT are within reference values. Patients should be questioned for a previous history of Gilbert syndrome. When unknown, Gilbert syndrome must be suspected in the presence of an isolated increased total bilirubin, and the unconjugated bilirubin should be measured. If Gilbert syndrome is confirmed, the FT-AT score can be calculated with conjugated bilirubin instead of total bilirubin. Drugs that can induce hemolysis, such as ribavirin or azathioprine, and those that can increase unconjugated bilirubin, such as indinavir and atazanavir, can cause false-positive FT-AT scores.

In conclusion, even in an experienced medical center, liver biopsy does not meet the minimum standard of quality for accurately assessing the severity of liver injury. One concern is the occurrence of false negatives because treatment would not be initiated in these patients despite the real presence of significant fibrosis or necroinflammatory activity. Of similar concern are false positives in patients; in those patients, costly treatment with side effects may be initiated without the actual presence of significant liver damage. The results strongly suggest that liver biopsy was responsible for most of the significantly discordant results, mainly as a result of sampling error. Because of its associated limitations and risks, liver biopsy should no longer be considered mandatory in the first-line management of patients with chronic hepatitis C (Fig. 3). Worldwide, there is an increase in mortality related to HCV infection, a lack of screening, and undertreatment despite the efficacy of antiviral agents. A simplification of liver injury assessment could accelerate the management of chronic hepatitis C.

This work was supported by research grants from the Association pour la Recherche sur le Cancer (ARECA) and from the Association pour la Recherche sur les Maladies Hépatiques Virales. T.P. is a consultant and has a capital interest in Biopredictive, the company marketing FT-AT.

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