

Biochemical Surrogate Markers of Liver Fibrosis and Activity in a Randomized Trial of Peginterferon Alfa-2b and Ribavirin

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Liver fibrosis and activity indexes were validated in patients infected by hepatitis C virus (HCV) nontreated and treated by interferon. The aim was to validate their usefulness as surrogate markers of histologic features using the data of a randomized trial of combination peginterferon alfa-2b and ribavirin. Three hundred fifty-two patients who had had 2 interpretable liver biopsies and stored serum sample before and after treatment were selected. Two hundred eight patients received peginterferon alfa-2b 1.5 mcg per kg and ribavirin and 144 patients interferon alfa-2b 3 MU three times a week and ribavirin for 48 weeks. A fibrosis and an activity index combining 5 and 6 biochemical markers were assessed at baseline and at end of follow-up (24 weeks after treatment). The biochemical markers have significant predictive values both for the diagnosis of fibrosis and for activity. For the diagnosis of bridging fibrosis and/or moderate necroinflammatory activity, the area under the receiver operating characteristics curve of the activity index was 0.76 ± 0.03 at baseline and 0.82 ± 0.02 at end of follow-up. A cutoff of activity index at 0.30 (range, 0.00-1.00) had 90% sensitivity and 88% positive predictive value for the diagnosis of bridging fibrosis or moderate necroinflammatory activity. Sensitivity analyses with biopsy specimens of size greater than 15 mm suggest that a part of discordances between biochemical markers and histology were due to biopsy specimen sampling error. In conclusion, these biochemical markers of fibrosis and activity could be used as surrogate markers for liver biopsy in patients with chronic hepatitis C, both for the initial evaluation and for follow-up. (HEPATOLOGY 2003;38:481-492.)

In patients infected with hepatitis C virus (HCV), recent studies have demonstrated the predictive value of combinations of simple serum biochemical markers: Fibrotest (FT) for the diagnosis of significant fibrosis

(ranging from few septa to cirrhosis) and Actitest (AT) for the assessment of necroinflammatory activity fibrosis and activity.¹⁻⁶ Such results were not obtained by other diagnostic tests.^{2,7-10}

The usual indication for liver biopsy in patients with chronic hepatitis C is to aid in the discussion of treatment options with the patient and for the long-term follow-up of patients to determine whether their disease is stable or whether it has progressed.⁷⁻¹² Prior data suggest that FT-AT, if accurate, could act as a surrogate and lead to a significant reduction in the number of liver biopsies performed.¹⁻⁶ The aim of this study was to validate the usefulness of FT-AT as surrogate markers of histologic features using the data generated from a recent randomized trial of peginterferon alfa-2b and ribavirin.¹³

Patients and Methods

Specific Aims. The primary aim was to assess the diagnostic value of FT-AT in patients at baseline and at end of follow-up. In contrast to previous reports, we used both the METAVIR and Knodell scoring systems, including

Abbreviations: HCV, hepatitis C virus; FT, Fibrotest; AT, Actitest; ALT, alanine aminotransferase; SVR, sustained virologic response, GGT, γ -glutamyl transpeptidase; ROC, receiver operating characteristic; AUROC, area under the ROC curves.

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the components of the histologic activity index. The secondary aims were to assess the variation of FT-AT according to virologic response; to assess the concordance between FT-AT and histologic variations; to compare a decision algorithm without liver biopsy, using FT-AT, versus the standard strategy with liver biopsy; and, finally, to compare FT-AT and biopsy as trial end points for evaluating histologic impact.

Patients. A total of 1,530 patients from a randomized trial comparing 3 interferon plus ribavirin combination regimens¹³ were considered for this retrospective study. Patients were previously untreated for their hepatitis C, had HCV RNA detectable in the serum, and had elevated serum alanine aminotransferase (ALT) level. Patients with stored serum sample and biopsy specimens interpretable at baseline and at 24 weeks follow-up and treated by 1 of 2 48-week regimens were retrospectively selected. The old standard was the combination of interferon 3 MU three times a week and ribavirin (1,000 mg if weight <75 kg, 1,200 mg \geq 75 kg) and the new combination of 1.5 μ g/kg peginterferon and ribavirin (800 mg).

Serum Samples and Biochemical Markers. Serum samples were collected in each center and centrally stored at -80°C . Sustained virologic response (SVR) was defined as undetectable HCV RNA in serum at the end of follow-up (quantitative PCR assay with a sensitivity of 100 copies, or 50 IU per mL [National Genetics Institute, Los Angeles, CA]).

We used the previously validated FT-AT.¹⁻⁶ FT combines the following 5 markers: α_2 -macroglobulin, haptoglobin, γ -glutamyl transpeptidase (GGT), total bilirubin, and apolipoprotein A1. AT combines the same 5 markers plus ALT with a high predictive value for the diagnosis of significant fibrosis and activity features.¹

Hitachi 747 or 911 automates assessed GGT, ALT, and total bilirubin using Roche Diagnostics reagents (Roche Diagnostics, Indianapolis, IN). Apolipoprotein A1, α_2 -macroglobulin, and haptoglobin were determined in serum samples by using an automatic nephelometer (Beckman Instrument). All CV assays were lower than 3%.

Liver Biopsies. Liver biopsies were performed before treatment and 72 weeks after randomization (24 weeks follow-up). Liver biopsy specimens were processed using standard techniques and evaluated for stage of fibrosis and grade of activity according to the METAVIR and Knodell scoring systems.¹⁴⁻¹⁶ Fibrosis was staged using the METAVIR system on a scale of 0 to 4: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis. Reproducibility of results between pathologists using this method has been established.¹⁵ The grading of activity by

the METAVIR system, previously described^{14,15} (the intensity of necroinflammatory activity mostly based on necrosis), was scored as follows: A0 = no histologic activity, A1 = mild activity, A2 = moderate activity, A3 = severe activity. Liver biopsy specimens were analyzed by a single pathologist (Z.D.G.), who was unaware of the patient's identity, treatment regimen, response, or timing of the biopsy relative to treatment. According to consensus recommendations, the presence of septal (bridging) fibrosis (F2F3F4) or moderate or severe inflammatory activity (A2A3) have been considered as significant features indicative of patients that should be considered for antiviral therapy.¹¹

Statistical Analysis. Statistical analysis used the Student's *t* test, Mann-Whitney test, and variance analysis using the Bonferroni all-pairwise multiple comparison test receiver-operating characteristic (ROC) curves.¹⁷ The diagnostic values of FT-AT were assessed by sensitivity, specificity, positive and negative predictive values, and ROC curves. The respective overall diagnostic values were compared using the area under the ROC curves (AUROC). The variations of FT-AT according to the treatment were analyzed by generalized variance analysis and Tukey-Kramer multiple-comparison test to take into account the repeated measures and the virologic response.

Results

A total of 352 out of the 1,530 randomized patients were included because serum was available, which is 34% of the 1,025 randomized patients in the 2 regimens. One hundred forty-four patients received the standard interferon and ribavirin regimen and 208 the peginterferon plus ribavirin regimen. The 352 retrospectively included patients did not differ from the overall population included in the prospective trial (Table 1).

Diagnosis of Fibrosis and Activity Using the METAVIR Scoring System

Using the METAVIR scoring system, the box plots of FT-AT according to the fibrosis stage and according to the activity grade at baseline and end of follow-up are shown in Fig. 1. FT-AT increases parallel the increase in fibrosis stage and activity grade. FT varied, at baseline, from 0.24 for F0 to 0.69 in F4 and, at the end of follow-up, from 0.13 to 0.62. AT varied, at baseline, from 0.32 for A0 to 0.68 for A3 and from 0.05 to 0.61 at end of follow-up.

The AUROC of FT for the diagnosis of fibrosis (stages F2-F3-F4 or stages F3-F4) varied from 0.73 to 0.77 (Table 2). The AUROC of AT were even higher, for the diagnosis of activity (grades A2-A3 or grade A3), particularly at the end of follow-up (Table 2).

Table 1. Characteristics of Included and Not Included Patients

Characteristics	Peginterferon 1.5-Ribavirin	Interferon-Ribavirin	All Randomized Patients
Number of patients	208	144	1,530
Age at biopsy (y) mean (SD)	45 (8)	45 (7)	43 (6)
Duration between biopsies (mean) (mo)	21 (4)	21 (4)	21 (4)
Male (%)	129 (62)	95 (66)	1,003 (66)
Female (%)	79 (38)	49 (34)	527 (34)
Transfusion (%)	52 (25)	24 (17)	320 (21)
Genotype 2 or 3 (%)	55 (26)	40 (28)	445 (29)
Baseline viral load (millions copies mL)	5.9 (7.7)	5.6 (6.3)	5.9 (7.7)
Metavir fibrosis stage (%)			
No fibrosis (F0)	11 (5)	0 (0)	15 (1)
Portal fibrosis or (F1)	129 (62)	73 (51)	994 (70)
Few septa (F2)	34 (16)	42 (29)	238 (17)
Many septa (F3)	12 (6)	17 (12)	92 (6)
Cirrhosis (F4)	18 (9)	12 (8)	89 (6)
Metavir activity grade (%)			
None (A0)	4 (2)	1 (1)	21 (1)
Mild (A1)	37 (18)	18 (13)	225 (16)
Moderate (A2)	73 (35)	44 (31)	539 (55)
Severe (A3)	94 (45)	81 (56)	643 (45)
Patients with F2F3F4 or A2A3 (%)	168 (81)	129 (90)	1,190 (84)
Biopsy specimen size, mm (SD)	16 (10)	18 (25)	17 (20)
Markers (SD)			
ALT U/L (upper normal value)	2.8 (1.9)	3.6 (3.2)	3.0 (2.2)
Total bilirubin μ mol/L (2-17)	11 (4)	11 (5)	ND
GGT U/L (7-32 female) (11-49 male)	70 (85)	76 (73)	ND
α_2 -Macroglobulin g/L (female 1.6-4.0 male 1.4-3.3)	3.20 (1.23)	3.08 (1.3)	ND
ApoA1 g/L (1.2-1.7)	1.27 (0.27)	1.24 (0.25)	ND
Haptoglobin g/L (0.35-2.00)	0.76 (0.46)	0.72 (0.43)	ND
Fibrosis index (0.00-1.00)	0.46 (0.26)	0.50 (0.26)	ND
Virologic response (%)			
Sustained responder	113 (55)	71 (49)	753 (49)
Relapser	28 (13)	14 (10)	142 (9)
Nonresponder	67 (32)	59 (41)	635 (42)

NOTE. There was no significant difference between groups.

The AUROC were significantly higher for AT for the diagnosis of A2A3 F2F3F4 according to the quality of biopsy specimen, biopsy specimen size greater than 15 mm when the number of portal tracts was greater than or equal to 6 (Table 2).

Diagnosis of Fibrosis and Activity Using the Knodell Scoring System

When the Knodell scoring system was used instead of the METAVIR scoring system, results were similar both for fibrosis (Fig. 2A and B) and for activity (Fig. 2C and D). There was a significant association between AT and the 3 components of the Knodell scoring system: periportal bridging necrosis (Fig. 3A), parenchymal injury (Fig. 3B), and portal inflammation (Fig. 3C).

Variation of Fibrosis and Activity Indexes According to Virologic Response

There was a significant decrease of FT among the 184 sustained virologic responders, from 0.39 ± 0.02 (mean \pm SE), median 0.34 at baseline, to 0.28 ± 0.02 ,

median 0.23 at 72 weeks, in comparison with 126 nonresponders (from 0.59 ± 0.03 , median 0.64 at baseline, to 0.55 ± 0.02 , median 0.56 at 72 weeks; $P < .001$) and in comparison with 42 relapsers (from 0.49 ± 0.04 , median 0.49 at baseline, to 0.45 ± 0.02 , median 0.46 at 72 weeks; $P < .001$).

There was also a significant decrease of AT among the 184 sustained virologic responders, from 0.55 ± 0.02 (mean \pm SE), median 0.53 at baseline, to 0.08 ± 0.02 , median 0.05 at 72 weeks, in comparison with 126 nonresponders (from 0.58 ± 0.02 , median 0.60 at baseline, to 0.50 ± 0.03 , median 0.51 at 72 weeks; $P < .001$) and in comparison with 42 relapsers (from 0.58 ± 0.03 , median 0.58 at baseline, to 0.43 ± 0.03 , median 0.41 at 72 weeks; $P < .001$).

There was a significant concordance between FT and fibrosis stage variations (Table 3) as well as between AT and activity grade variations (Table 4). At baseline, 32 patients had cirrhosis. For 15 patients, stage remains F4, and FT did not significantly change (0.67 ± 0.05 vs.

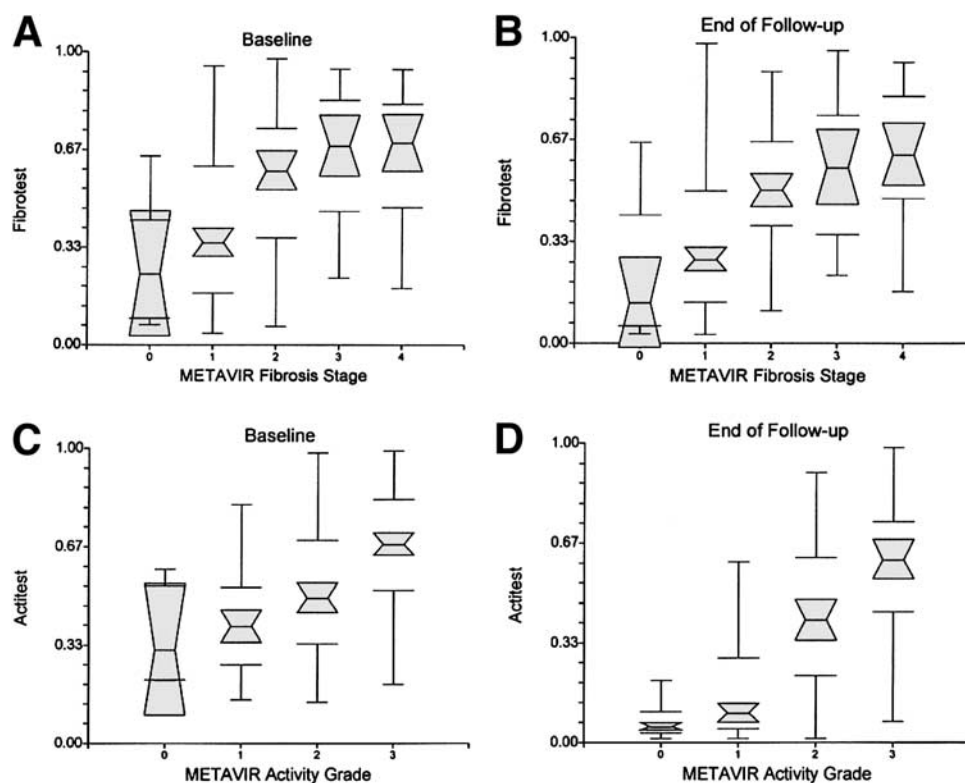


Fig. 1. Fibrosis score according to fibrosis stage. (A) Baseline fibrosis index: F0, $n = 6$, median = 0.24; F1, $n = 206$, median = 0.34; F2, $n = 76$, median = 0.60; F3, $n = 32$, median = 0.68; F4, $n = 32$, median = 0.69. A line is drawn through the middle of the box at the median (the 50th percentile). The notched box represents the 95% confidence interval of the median. If the notches of 2 boxes do not overlap, the medians are significantly different. The adjacent values are displayed as lines. The first adjacent value at top and bottom of the box are median plus or minus the 25th and 75th percentiles. The length between the 2 first adjacent values is thus the interquartile range. The upper adjacent value is the largest observation that is less than or equal to the 75th percentile plus 1.5 times interquartile range. The lower adjacent value is the smallest observation that is greater than or equal to the 25th percentile minus 1.5 times interquartile range. Analysis of variance shows significant differences between stages 0 or 1 versus stages 2, 3, or 4 (Bonferroni all-pairwise multiple comparison test; $P < .001$). (B) End of follow-up fibrosis index: F0, $n = 15$, median = 0.13; F1, $n = 222$, median = 0.28; F2, $n = 64$, median = 0.50; F3, $n = 25$, median = 0.58; F4, $n = 26$, median = 0.62. Analysis of variance shows significant differences between stages 0 or 1 versus stages 2, 3, or 4 ($P < .001$). (C) Baseline activity index: A0, $n = 5$, median = 0.32; A1, $n = 55$, median = 0.40; A2, $n = 117$, median = 0.50; A3, $n = 175$, median = 0.68. Analysis of variance shows significant differences between stages 0 or 1 versus grades 2 and 3 and between grades 2 and 3 ($P < .001$). (D) End of follow-up activity index: A0, $n = 83$, median = 0.05; A1, $n = 136$, median = 0.10; A2, $n = 82$, median = 0.41; A3, $n = 51$, median = 0.61. Analysis of variance shows significant differences between stages 0 or 1 versus grades 2 and 3 and between grades 2 and 3 ($P < .001$).

0.64 ± 0.05). For 17 patients, there was at least 1 fibrosis stage improvement. These patients had a very significant FT improvement ($P < .0001$) from 0.68 to 0.44 for 3 stages improvement, from 0.60 to 0.47 for 2 stages improvement, and from 0.61 to 0.56 for 1 stage improvement. For 11 patients, the biopsy found cirrhosis at the end of follow-up, which was not observed at baseline. The respective mean FT was 0.64 ± 0.06 versus 0.75 ± 0.05 ($P = .06$).

Simulation of Decision Algorithm Without Liver Biopsy

A possible algorithm for making treatment decisions without a liver biopsy using FT-AT is described in Fig. 4. Two scenarios have been constructed, 1 using a specific cutoff for AT at 0.40 (Fig. 4A) and 1 sensitive cutoff at 0.30 (Fig. 4B).

AT greater than 0.40 has a positive predictive value of 90% for the presence of septal (bridging) fibrosis (F2F3F4) or moderate or severe activity (A2A3) (Fig 4A), with 80% sensitivity. Among the 60 false negatives, 43 had minimal fibrosis, and only 17 had extensive fibrosis: 16 patients with few septa (AT range, 0.20 to 0.37) and one with many septa (AT = 0.38); none had cirrhosis. Fifteen patients were considered as false positive patients for the FT-AT. At the follow-up biopsy, 6 of these patients' fibrosis stage worsened despite treatment, 5 progressed to F2 and one to F4 (Fig. 4A).

With the more sensitive cutoff at 0.30, the positive predictive value was 88% with 90% sensitivity (Fig. 4B). When the activity cutoff at 0.30 was used in patients with biopsy specimens over 15 mm, the positive predictive value was 88% with 91% sensitivity (Fig. 4C), and the

Table 2. Areas Under ROC Curve for the Fibrosis and Activity Indexes

Markers	Baseline n = 352	End of Follow-Up n = 352
Fibrosis index		
F2-F3-F4 vs. F0-F1	0.733 ± 0.03	0.766 ± 0.03
F3-F4 vs. F0-F1-F2	0.730 ± 0.04	0.762 ± 0.04
Activity index		
A2-A3 vs. A0-A1	0.754 ± 0.03	0.862 ± 0.02
A3 vs. A0-A1-A2	0.720 ± 0.03	0.867 ± 0.03
A2A3-F2F3F4 vs. A0A1-FOF1	0.762 ± 0.03	0.822 ± 0.022
Biopsy size ≥15 mm	0.879 ± 0.03*	0.853 ± 0.03
Biopsy size <15 mm	0.705 ± 0.04	0.831 ± 0.03
Portal tracts ≥6	0.803 ± 0.03†	0.850 ± 0.03‡
Portal tract <6	0.615 ± 0.09	0.789 ± 0.11

NOTE. All the areas under the ROC curves were significantly higher than the 0.500 nonpredictive value ($P < .001$ for all comparisons).

*Significantly higher in 152 patients with biopsy specimen size greater or equal to 15 mm than in 200 patients with smaller biopsy specimens ($P < .001$).

†Significantly higher in patients with biopsy specimen including 6 or more portal tracts in comparison with patients with less portal tracts ($P < .001$).

‡Significantly higher in 313 patients with biopsy specimen including 6 or more portal tracts in comparison with 39 patients with less portal tracts ($P = .04$).

specificity increased in comparison with smaller biopsy specimens (50% vs. 36%).

Comparison of Analyses of Treatment Impact on Histology Using Either Biopsy or Biochemical Markers

Finally, analyses using FT-AT gave similar results to liver histology with a higher power for biochemical markers. FT-AT permitted us to demonstrate more significant differences between groups in comparison with liver biopsies: fibrosis improvement in sustained responders, activity improvement in relapsers, and fibrosis improvement in patients treated with peginterferon and ribavirin or standard interferon and ribavirin.

Treatment Impact on Histology According to Virologic Response.

Analysis using FT showed 8 significant differences between sustained responders at baseline (SR1) and at end of follow-up (SR2) versus both relapsers at baseline (RE1) and at end of follow-up (RE2) and versus nonresponders at baseline (NR1) and at end of

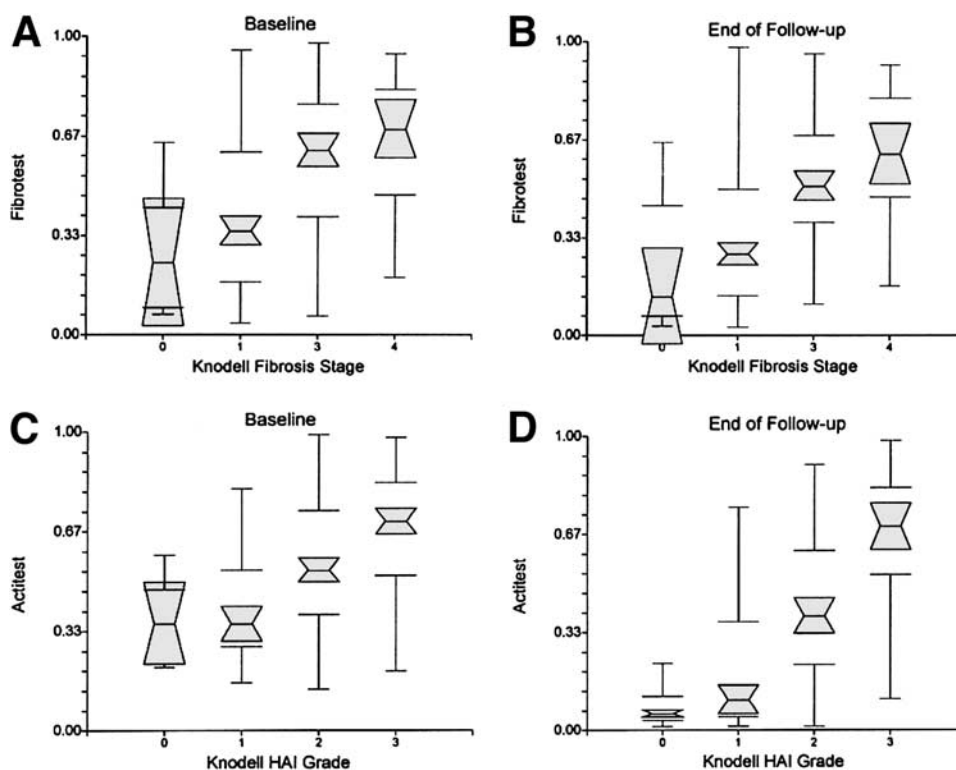


Fig. 2. Fibrosis and activity indexes according to Knodell fibrosis stage and Knodell HAI activity grade. (A) Fibrosis index at baseline. Baseline fibrosis index: F0, n = 6, median = 0.24; F1, n = 205, median = 0.35; F3, n = 108, median = 0.52; F4, n = 32, median = 0.69. Analysis of variance shows significant differences between stages 0 or 1 versus stages 3 or 4 ($P < .001$). (B) Fibrosis index at the end of follow-up. End of follow-up fibrosis index: F0, n = 13, median = 0.13; F1, n = 224, median = 0.28; F3, n = 88, median = 0.51; F4, n = 26, median = 0.62. Analysis of variance shows significant differences between stages 0 or 1 versus stages 3 or 4 ($P < .001$). (C) Activity index at baseline. The HAI activity scoring system has been divided in 4 classes: 0 = 0-2 HAI; 1 = 3-6 HAI; 2 = 7-9 HAI; 3 = 10-12 HAI. HAI 0-2, n = 8, median = 0.36; HAI 3-6, n = 46, median = 0.36; HAI 7-9, n = 174, median = 0.54; HAI 10-12, n = 123, median = 0.70. Analysis of variance shows significant differences between stages 0 or 1 versus grades 2 and 3 and between grades 2 and 3 ($P < .001$). (D) Activity index at the end of follow-up. HAI 0-2, n = 106, median = 0.06; HAI 3-6, n = 111, median = 0.11; HAI 7-9, n = 100, median = 0.39; HAI 10-12, n = 34, median = 0.69. Analysis of variance shows significant differences between all comparisons among the 4 stages ($P < .001$).

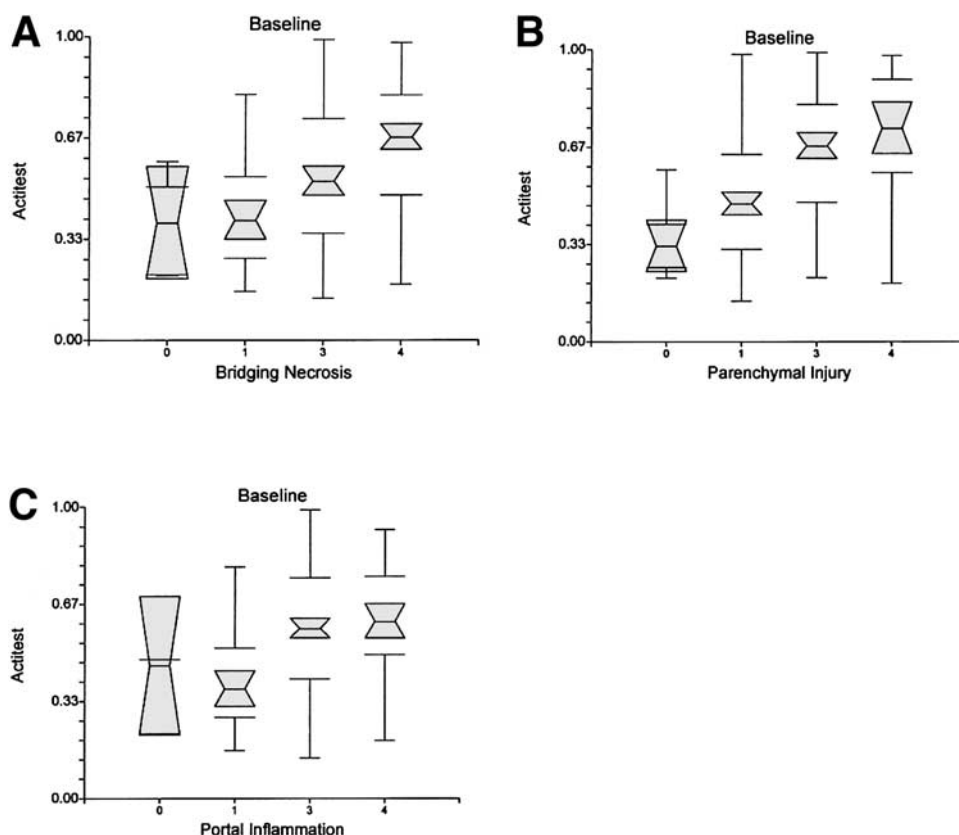


Fig. 3. Activity index according to the components of the Knodell activity scoring system: periportal bridging necrosis (A), parenchymal injury (B), and portal inflammation (C). All differences were significant ($P < .001$).

follow-up (NR2) (repeated variance analysis with Bonferroni all pairwise multiple comparison test, F-ratio = 78, $P < .0001$). SR2 FT was significantly lower than SR1, RE2, RE1, NR2, and NR1. SR1 FT value was significantly lower than NR2 and NR1. RE2 FT was significantly lower than NR1.

Analysis using the fibrosis stages showed 4 significant differences (F-ratio = 16, $P < .0001$). SR2 FT value was significantly lower than RE2, RE1, NR2, and NR1.

Analysis using AT showed 9 significant differences (F-ratio = 118, $P < .0001$). SR2 AT was significantly lower than SR1, RE2, RE1, NR2, and NR1. RE2 AT was significantly lower than RE1, SR1, and NR1. NR2 AT was significantly lower than NR1.

Analysis using the activity grades showed 9 significant differences (F-ratio = 51, $P < .0001$). SR2 AT was significantly lower than SR1, RE2, RE1, NR2, and NR1. RE2 AT was significantly lower than SR1 and NR1. NR2 AT was significantly lower than NR1 and SR1.

Treatment Impact on Histology According to Treatment Regimen. Manns et al.¹³ have shown that response is optimized for doses of ribavirin greater than 10.6 mg per kg per day, a dose corresponding to 800 mg in a 75-kg individual. FT as well as AT at the end of follow-up was significantly lower among patients receiving the combination regimen (peginterferon 1.5 μg per kg and more than

10.6 mg of ribavirin per kg, $n = 90$): 0.34 ± 0.03 and 0.20 ± 0.02 versus 0.43 ± 0.02 and 0.30 ± 0.02 in patients receiving less than 10.6 mg ribavirin ($n = 157$) versus 0.43 ± 0.02 and 0.32 ± 0.02 in patients receiving interferon-ribavirin ($n = 171$) ($P = .02$ for both FT comparisons and $P = .003$ for both AT comparisons).

Analysis using FT-AT showed 3 significant differences between FT values before and after treatment, both for peginterferon 1.5 $\mu\text{g}/\text{kg}$ ribavirin (pegR1 vs. pegR2; $P < .001$) and for interferon-ribavirin (IR1 vs. IR2; $P < .001$). There was no difference between treatments (F-ratio = 2.8, $P = .10$). PegR2 FT was significantly lower than pegR1 and IR1. IR2 FT was significantly lower than IR1. Analysis using liver biopsy showed no significant difference for fibrosis stage.

For activity comparison, the same significant differences were observed (3 significant differences between treatments: PegR2 AT was significantly lower than pegR1 and IR1; IR2 AT was significantly lower than IR1), but the level of significance was higher for AT (F-ratio = 8, $P = .005$) versus activity grade (F-ratio = 3.8, $P = .05$).

Discussion

This study is the seventh demonstrating that a combination of 5 (FT) or 6 biochemical markers (AT) can have

Table 3. Concordance Between Fibrosis Biochemical Markers and Fibrosis Stage Variations

Difference in stages	Time	Number	Fibrosis Index		Significance	
			Mean	Standard Error		
All patients						
-3	Baseline	5	0.72	0.10	<i>P</i> < .001	
	End of follow-up	5	0.47	0.10		
-2	Baseline	18	0.59	0.06		
	End of follow-up	18	0.44	0.06		
-1	Baseline	75	0.50	0.02		
	End of follow-up	75	0.41	0.02		
0	Baseline	193	0.42	0.01		
	End of follow-up	193	0.35	0.01		
1	Baseline	52	0.56	0.03		
	End of follow-up	52	0.50	0.03		
2	Baseline	6	0.46	0.10		
	End of follow-up	6	0.48	0.10		
3	Baseline	3	0.71	0.14		
	End of follow-up	3	0.70	0.14		
Sustained responders						
-3	Baseline	4	0.68	0.10		<i>P</i> < .001
	End of follow-up	4	0.39	0.10		
-2	Baseline	11	0.54	0.06		
	End of follow-up	11	0.35	0.06		
-1	Baseline	43	0.44	0.06		
	End of follow-up	43	0.31	0.03		
0	Baseline	106	0.34	0.03		
	End of follow-up	106	0.25	0.03		
1	Baseline	17	0.44	0.05		
	End of follow-up	17	0.29	0.05		
2	Baseline	2	0.46	0.15		
	End of follow-up	2	0.41	0.15		
3	Baseline	1	0.70	0.21		
	End of follow-up	1	0.69	0.21		
Nonresponders						
-3	Baseline	1	0.88	0.23	<i>P</i> = .003	
	End of follow-up	1	0.80	0.23		
-2	Baseline	7	0.66	0.08		
	End of follow-up	7	0.59	0.08		
-1	Baseline	32	0.59	0.04		
	End of follow-up	32	0.53	0.04		
0	Baseline	87	0.52	0.04		
	End of follow-up	87	0.47	0.04		
1	Baseline	35	0.62	0.04		
	End of follow-up	35	0.60	0.04		
2	Baseline	4	0.47	0.11		
	End of follow-up	4	0.51	0.11		
3	Baseline	2	0.71	0.16		
	End of follow-up	2	0.70	0.16		

NOTE. For all comparisons, there was a significant association between stages and fibrosis index variations (repeated variance analysis).

high positive or negative predictive values for diagnosing significant fibrosis and significant activity in patients with chronic hepatitis C.¹⁻⁶ Although retrospective, the analyses of this study were made with an independent assessment of FT-AT, of fibrosis stages, and of activity grades. During the randomized trial, all sera were prospectively stored, and there were no differences between the patients with stored serum in comparison with those patients without serum (Table 1). The population of the present study was different than that of the first study,¹ with lower fibrosis grade and higher activity grade.

These scores are derived from tests that are not yet routine in many countries. However, all the 6 components are available in most countries. In France, these tests (Fibrotest and Actitest; Biopredictive, Houilles France) have been on the market since September 2002, and more than 6,000 tests have been performed up to April 2003 (U.S. Patent Application Serial No. 09/687,459). When compared with routine laboratory tests found to be predictive of activity or fibrosis,^{11,18-20} we always found better diagnostic values for our scores: to ALT, AST, GGT, bilirubin, (alone or in combination)¹; to hyaluronic acid²;

Table 4. Concordance Between Activity Biochemical Markers and Activity Grade Variations

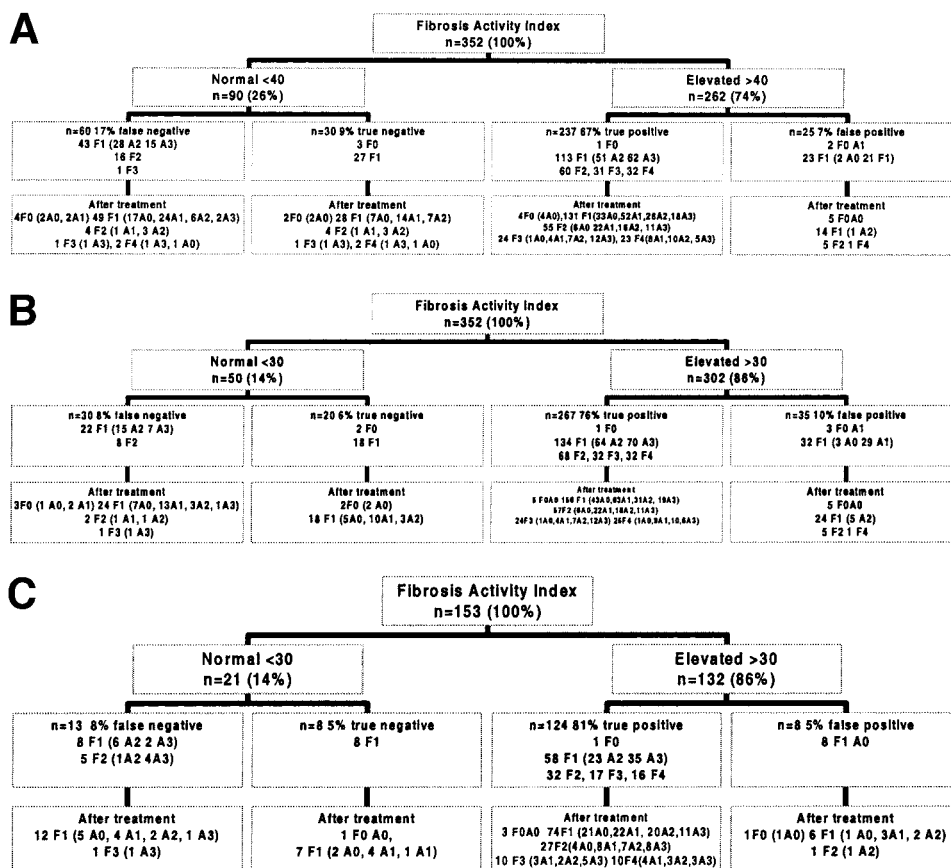
Difference in Grades	Time	Number	Activity Index		Significance	
			Mean	Standard Errors		
All patients						
-3	Baseline	32	0.64	0.04	<i>P</i> < .001	
	End of follow-up	32	0.11	0.04		
-2	Baseline	102	0.57	0.02		
	End of follow-up	102	0.15	0.02		
-1	Baseline	96	0.55	0.02		
	End of follow-up	96	0.25	0.02		
0	Baseline	89	0.56	0.02		
	End of follow-up	89	0.43	0.02		
1	Baseline	31	0.52	0.04		
	End of follow-up	31	0.49	0.04		
2	Baseline	2	0.43	0.15		
	End of follow-up	2	0.44	0.15		
Sustained responders						
-3	Baseline	30	0.62	0.03	<i>P</i> = .82	
	End of follow-up	30	0.08	0.03		
-2	Baseline	85	0.57	0.02		
	End of follow-up	85	0.08	0.02		
-1	Baseline	50	0.50	0.02		
	End of follow-up	50	0.07	0.02		
0	Baseline	15	0.47	0.05		
	End of follow-up	15	0.09	0.05		
1	Baseline	4	0.58	0.09		
	End of follow-up	4	0.10	0.09		
Nonresponders						
-3	Baseline	2	0.85	0.15		<i>P</i> = .08
	End of follow-up	2	0.42	0.15		
-2	Baseline	17	0.63	0.05		
	End of follow-up	17	0.49	0.05		
-1	Baseline	46	0.62	0.03		
	End of follow-up	46	0.45	0.03		
0	Baseline	74	0.58	0.02		
	End of follow-up	74	0.50	0.02		
1	Baseline	27	0.51	0.04		
	End of follow-up	27	0.55	0.04		
2	Baseline	2	0.43	0.14		
	End of follow-up	2	0.44	0.14		

NOTE. For comparisons including all patients, there was a significant association between grades and activity index variations (repeated variance analysis).

to age-platelets index and to prothrombin time⁵; to a recently published score combining GGT, cholesterol, platelets, and age^{6,21}; and to historical features.³ In addition to superior diagnostic power, FT is not genotype dependent, whereas the Forns et al. index includes serum cholesterol, which varies with HCV genotype.²¹ FT in the present study can distinguish fairly well between stages F0-F2 and F3-F4, which is a progress in comparison with hyaluronic acid² and the Forns et al. index.²¹ Studies including more stage F2F3F4 are needed to assess the diagnostic value of FT between F2 and F3 and between F3 and F4. Furthermore, we previously observed that FT has the same diagnostic value for fibrosis stages in the subpopulation of HCV patients with normal ALT than in patients with elevated ALT.¹ We did not include at purpose ALT in FT to detect fibrosis in patients with normal ALT. This study confirms also the diagnostic value of AT

(which includes ALT, the only variable adding independent predictive value to the 6 components used for fibrosis alone) for moderate and severe activity as observed in previous studies.¹ In the present population, activity grades and fibrosis stages were significantly associated: Spearman correlation coefficient was 0.34 at baseline and 0.46 at the end of follow-up biopsy (*P* < .0001 for both). The associations between FT and fibrosis are biologically plausible. Age and gender are independent predictors of fibrosis.²² Hyperbilirubinemia is related to early fibrosis stages without any liver dysfunction.¹ GGT has been associated with epidermal growth factor and hepatocyte growth factor, which rise as fibrosis progresses,¹ and has been implicated in fibrogenesis.²⁴ Haptoglobin has a strong negative association with fibrosis,¹ possibly because of its association with transforming growth factor- β 1.²⁵ Conversely, α ₂-macroglobulin increases as fibrosis

Fig. 4. Treatment algorithm with-
 out liver biopsy. The prevalence of
 the screened disease (A2A3 or
 F2F3F4) is 84% (297 out of 352).
 (A) Specific cutoff. For the specific
 cutoff (0.40), sensitivity of activity
 index is 80% (237 out of 297 pa-
 tients), specificity is 55% (30 out of
 55), positive predictive value is 90%
 (237 out of 262), and negative pre-
 dictive value is 33% (30 out of 90).
 (B) Sensitive cutoff. For the sensitive
 cutoff (0.30), sensitivity of activity
 index is 90% (267 out of 297 pa-
 tients), specificity is 36% (20 out of
 55), positive predictive value is 88%
 (267 out of 302), and negative pre-
 dictive value is 40% (20 out of 50).
 (C) Algorithm without biopsy evalu-
 ated in patients with liver biopsy
 specimen greater than 15 mm. The
 prevalence of the screened disease
 (A2A3 or F2F3F4) is 90% (137 out
 of 153). For the sensitive cutoff
 (0.30), sensitivity of activity index is
 91% (124 out of 137 patients),
 specificity is 50% (8 out of 16),
 positive predictive value is 88%
 (124 out of 132), and negative pre-
 dictive value is 38% (8 out of 21).



progresses¹; α_2 -macroglobulin is produced by hepatocytes and expressed by activated hepatic stellate cells,²⁶ which are integral in liver fibrosis.²⁷ Furthermore, α_2 -macroglobulin is a protease inhibitor²⁸; increased synthesis may enhance fibrosis by inhibiting the catabolism of other extra cellular matrix proteins. Serum levels of apoA1 are reduced in hepatitis C as well as in other liver fibrotic diseases.²⁹ *In vitro* studies and biopsies showing deposition on fibrous septa suggest that this is due to binding to extracellular matrix proteins.³⁰ ApoA1 is trapped and down-regulated by extra cellular matrix,³¹ and serum concentration starts to decrease before any liver dysfunction.³²

Because of their predictive values and their reproducibility in different populations, FT-AT could be used as surrogate markers of liver biopsy both for the initial liver biopsy and for follow-up of treatment in chronic hepatitis C patients. To date, liver biopsy has been considered mandatory for the management of HCV-infected patients, particularly for the staging of fibrosis.^{9,10} For some patients and general practitioners, liver biopsy may be considered an aggressive procedure.³³⁻³⁵ Reviews of morbidity and mortality of intercostal liver biopsy have shown an observed mean occurrence of pain in 30% of patients, 3 out of 1,000 severe adverse events, and 3 out of 10,000

deaths.³⁵ Furthermore, because treatment is now so effective in patients with genotype 2 or 3 infection, the utility of biopsy in this setting could be challenged.¹⁰⁻¹²

Initial Estimate of Liver Damage. There is no ideal gold standard for the assessment of liver histology. Even liver biopsy is dependent on inter- and intraobserver (pathologist) differences. There are also potential problems with liver biopsy sampling variation. In a study with 3 consecutive samples through a single entry site, only 50% of patients with cirrhosis were scored as cirrhosis on the 3 samples.³⁶ In a recent study using laparoscopy,³⁷ 30 of 124 patients (24.2%) had a difference of at least 1 grade, and 41 of 124 patients (33.1%) had a difference of at least 1 stage between the right and left lobes. In 18 patients (14.5%), interpretation of cirrhosis was given in 1 lobe, whereas stage 3 fibrosis was given in the other. A difference of 2 stages or 2 grades was found in 3 (2.4%) and 2 (1.6%) patients, respectively. Therefore, it is possible that biochemical markers such as those described may provide a more accurate (quantitative and reproducible) picture of fibrogenic events occurring within the liver.

One concern with FT-AT is the false negative rate because, in these patients, treatment would not be initiated despite the presence of significant fibrosis and disease. However, it was reassuring that, among the 60 false

negatives, only 17 had extensive fibrosis and none had cirrhosis. Another concern is the false positive patients in whom a costly treatment with side effects may be initiated without the presence of significant liver damage. The follow-up of these patients after treatment strongly suggests that at least 6 could be false negative of the first liver biopsy as 5 progressed to F2 and one to F4, despite the treatment. Hemolysis, Gilbert disease, acute sepsis and extrahepatic cholestasis were not observed but are possible causes of FT-AT false positive.

The variability of all 6 biochemical components^{38,39} are a possible source of variability for FT-AT. We analyzed the preanalytical and interlaboratory variability, showing that the variability of FT-AT was acceptable if standardized methods and assay calibration were used.⁴⁰ The intraindividual variations have also been studied in 55 patients with 2 samples in a week (Imbert-Bismut personal communication). The strength of agreement between the 2 FT was almost perfect for fibrosis staging (κ statistic = 0.83) and moderate between the 2 AT for activity grading because of attempted transaminases variation in these patients (κ statistic = 0.44).

Biochemical Markers Predictive Value Increases With Biopsy Specimen Size. The diagnostic value of FT-AT improved significantly when the biopsy specimen size was greater than 15 mm or when biopsy specimens with less than 6 portal tracts were excluded. Only 8 out of 132 patients were false positive by AT (0.30 cutoff) when biopsy specimens were greater than 15 mm versus 35 out of 302 patients with smaller biopsy specimens. These observations also argue for the utilization of FT-AT in practice. An ideal but impossible study to validate FT-AT would be to have a larger specimen (such as a wedge biopsy) or the entire liver.

Follow-Up of Liver Damage. The results show that FT-AT can also be used as surrogate markers of the histologic impact of treatment. Both indexes were associated with the virologic responses and with the histologic variations. In chronic hepatitis C, the impact of treatment on fibrosis progression and activity is related to the virologic response and, for virologic nonresponders, to the baseline stage of fibrosis and to the duration of treatment.⁴¹⁻⁴⁵ Therefore, FT-AT could be used as surrogate markers in trials evaluating the risk-benefit of maintenance therapy, without increasing the risk and the cost because of repeated liver biopsies. FT significantly decreases during interferon monotherapy in virologic responders from 0.35 ± 0.03 to 0.23 ± 0.04 at 24 weeks versus 0.41 ± 0.02 to 0.42 ± 0.03 in nonresponders ($P < .05$).² In the present study, we have not measured the parameters during combination treatment. Because ribavirin can induce hemolysis, and therefore a decrease of haptoglobin and an

increase of unconjugated bilirubin, there is a risk of false positive FT-AT during combination treatment.

Organization of Future Randomized Trials Without Liver Biopsies. From previous results and those presented here, it would be possible to perform randomized clinical trials without liver biopsy. We retrospectively compared what would have been the interpretation of a simulated trial, using FT-AT only with a classical trial using liver biopsies. At inclusion, 14% of the patients would have not been included without significantly changing the main characteristics of patients randomized. Similar conclusions concerning the impact of virologic responses and treatment effect would have been obtained but with more powerful statistical differences with FT-AT. Four important end points would have been significant using FT-AT and not significant using liver biopsies: the fibrosis improvement in sustained responders, the activity improvement in relapsers, and the fibrosis improvement in patients treated by peginterferon ribavirin or by interferon-ribavirin. Furthermore, FT-AT permitted us to observe a significant histologic impact of the optimized combination regimen in comparison with the other regimens. However, the seemingly greater histologic improvement using FT-AT versus actual histology may indeed be due to lag in histologic improvement among virologic responders or an effect of treatment that is unrelated to histologic improvement.

The disadvantage of FT-AT would have been the possible inclusion of patients with nonidentified liver disease because of other causes such as alcohol, NASH, or hemochromatosis. However, this risk could be reduced by using different biochemical markers in obese patients⁴⁶ and HFE gene for hemochromatosis (or transferrin saturation if too expensive) exclusion and by excluding morbid obesity, heavy drinkers, and patients with uncontrolled diabetes. Advantages of FT-AT include simplification of the screening phase and inclusion, inclusion of patients with biopsy contraindications or those who refuse biopsy, and an increase in available histologic evaluation. In this particular study, a total of 466 patients (32% of the total randomized) had no paired biopsy specimens available. Intention to treat analysis was usually impossible because of the high percentage of patients without paired biopsies. Another advantage of FT-AT is the ability to provide a long-term follow-up of liver damage without the constraints of repeating an invasive procedure. For patients, there is an obvious advantage in the absence of adverse events related to biopsy.

Recommendation of Future Management of Chronic Hepatitis C Without Liver Biopsies. From the previous results and those presented here, a simplification of the management of chronic hepatitis C is possi-

ble, particularly using a cutoff of 0.30 for AT. Because this analysis is retrospective, a randomized trial of 2 strategies comparing a strategy without and with biopsy is certainly the best scientific comparison of the respective utilities. However, this type of trial would require a very large number of patients to estimate the severe adverse events.

Because of the improvement of biochemical markers¹⁻⁷ and the limits and the risk of biopsy,³³⁻³⁷ liver biopsy should not be mandatory anymore. It is perhaps time to leave the decision regarding liver biopsy to the physician and to the patient. There is, worldwide, a lack of screening and an under prescription of treatment despite its efficacy. A simplification of liver damage assessment should accelerate the management of chronic hepatitis C.

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