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Protein Markers

A Prospective Analysis of the Prognostic Value of Biomarkers (FibroTest) in Patients with Chronic Hepatitis C

Yen Ngo,[1] Mona Munteanu,[2] Djamila Messous,[3] Frederic Charlotte,[4] Françoise Imbert-Bismut,[3] Dominique Thabut,[1] Pascal Lebray,[1] Vincent Thibault,[5] Yves Benhamou,[1] Joseph Moussalli,[1] Vlad Ratziu,[1] and Thierry Poynard[1*]

Background: **FibroTest, a noninvasive method of measuring biomarkers of liver fibrosis, is an alternative to liver biopsy for determining the severity of chronic hepatitis C virus (HCV) infection. We compared the 5-year prognostic value of the FibroTest with biopsy staging for predicting cirrhosis decompensation and survival for patients with chronic HCV infection.**

Methods: **Fibrosis stage was assessed on the same day by FibroTest and biopsy in a prospective cohort of 537 patients. Disease classification at baseline was 157 patients with severe fibrosis (FibroTest >0.58), 137 with moderate fibrosis (FibroTest 0.32–0.58), and 243 with no or minimal fibrosis (FibroTest <0.32).**

Results: **In 64 untreated patients with severe fibrosis, survival without HCV complications was 73% [95% confidence interval (CI), 59%–086%; 13 complications], and survival without HCV-related death was 85% (95% CI, 73%–96%; 7 HCV deaths). Survival rates were higher in patients with moderate fibrosis, [99% (95% CI, 97%–100%; 1 complication; *P* <0.001) and 100% (no HCV death; *P* <0.001) for with and without HCV-related complications, respectively], and in patients with minimal fibrosis [100% (no complication; *P* <0.001 vs severe) and 100% (no HCV death; *P* <0.001 vs severe), respectively]. FibroTest was a better predictor than biopsy staging for HCV complications, with area under the ROC curves (AUROC) = 0.96 (95% CI, 0.93%–0.97%) vs 0.91 (95% CI, 0.85%–0.94%; *P* = 0.01), respectively; it was also a better predictor for HCV deaths: AUROC = 0.96 (95% CI, 0.93%–0.98%) vs 0.87 (95% CI, 0.70%–0.94%; *P* = 0.046), respectively. The prognostic value of FibroTest was still significant (*P* <0.001) in multivariate analyses after taking into account histology, treatment, alcohol consumption, and HIV coinfection.**

Conclusion: **The FibroTest measurement of HCV biomarkers has a 5-year prognostic value similar to that of liver biopsy.**

Finding the best method to evaluate and manage patients infected with the hepatitis C virus (HCV)[6] continues to be a challenge *(1, 2)*. Liver biopsy for determining disease grade and stage has limitations *(3–5)* and risks *(6)*; noninvasive alternatives to liver biopsy in patients infected with HCV *(7)* include 2 combinations of simple serum biochemical markers: FibroTest (FT) (Biopredictive) for the assessment of fibrosis, and ActiTest (AT) (Biopredictive) for the assessment of necroinflammatory activity *(8–16)*. With biopsy as the standard of reference, the diagnostic value of FT for a diagnosis of significant fibrosis (bridging fibrosis), as estimated by the area under the ROC curve (AUROC), is 0.73–0.86 *(8)*. AUROC values inside the range of other studies have been reported, but the usefulness of FT compared with biopsy was not

[1] Service d'Hépato-Gastroentérologie, Groupe Hospitalier Pitié-Salpêtrière, Université Paris VI, CNRS, Paris, France.
[2] Biopredictive, Paris, France.
[3] Laboratoire de Biochimie, Groupe Hospitalier Pitié-Salpêtrière, Paris, France.
[4] Service d'Anatomie Pathologique Groupe Hospitalier Pitié-Salpêtrière, Paris, France.
[5] Laboratoire de Virologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, France.
* Address correspondence to this author at: Service d'Hépato-Gastroentérologie, Groupe Hospitalier Pitié-Salpêtrière, 47 Boulevard de l'Hôpital 75651 Paris Cedex 13, France. Fax (33-1)-42-16-14-27; e-mail tpoynard@teaser.fr.
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[6] Nonstandard abbreviations: HCV, hepatitis C virus; FT, FibroTest; AT, ActiTest; AUROC, area under the ROC curve; F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis; F4, cirrhosis; HCC, hepatocellular carcinoma; CI, confidence interval.

established *(17)*. Discordance between FT and biopsy may be related to biopsy failure due mainly to sampling errors and to false-positive and false-negative FT results related to Gilbert syndrome, hemolysis, or acute inflammation *(8, 18)*. Biomarkers have shown error rates similar to *(15, 16)* or lower than *(18)* those of small liver biopsies.

Despite its limitations, liver biopsy is the only validated prognostic marker for chronic HCV infection *(19–21)*. To be useful as alternatives to liver biopsy, noninvasive biomarkers must demonstrate prognostic value based on hard clinical endpoints: liver disease–related mortality and severe hepatic complications.

We prospectively assessed the prognostic value of FT for 5-year HCV mortality and morbidity compared with prognostic values of histologic features of biopsy specimens, the current standard of reference, and other indexes.

Patients and Methods

PATIENTS

Study patients belonged to a prospective hospital-based cohort of 2865 patients with chronic HCV, seen at our institution from January 1997 to December 2002 (Fig. 1). Survival analysis included a core cohort of 537 patients who had been previously studied at baseline to assess the discordance rate between biopsy and FT *(18)*. Inclusion criteria were 2 assessments of liver histology done <24 h apart, 1 of a liver biopsy specimen and the other with FT measured in serum. Patients had been referred by general practitioners, private specialists, or public general hospitals for the staging and treatment of HCV infection *(18)*. Most patients had no severe complications, and the disease had been discovered by the detection of HCV antibodies. Liver biopsy was indicated for all patients without a previous biopsy but was not performed in patients with contraindications or who refused. All patients received FT unless it was not available, the patient refused, or the hospital laboratory was unable to perform the FT the day of the biopsy.

Follow-up of core group patients was performed every 6 months, and biopsy or FT was repeated as considered necessary by the physician in charge. A group of 395 patients (validation group) who underwent a baseline FT either with no liver biopsy or with a >24-h lapse between liver biopsy and FT were analyzed separately to check the prognostic value of FT observed in the core group.

All procedures were followed in accordance with the current revision of the Helsinki declaration, and all participants gave informed consent. Biopsy was performed for routine management of chronic HCV infection and was not related to the study protocol. Consenting patients underwent FT testing if biochemistry unit personnel were available to perform the test and were blinded to the clinical data. The result of the FT was known after the results of the biopsy, and the clinical management at baseline was decided according to biopsy results before 2002.

LIVER BIOPSIES

Liver biopsies were processed with standard techniques. A single pathologist (F.C.), who was unaware of the biochemical markers, evaluated the stage of fibrosis and

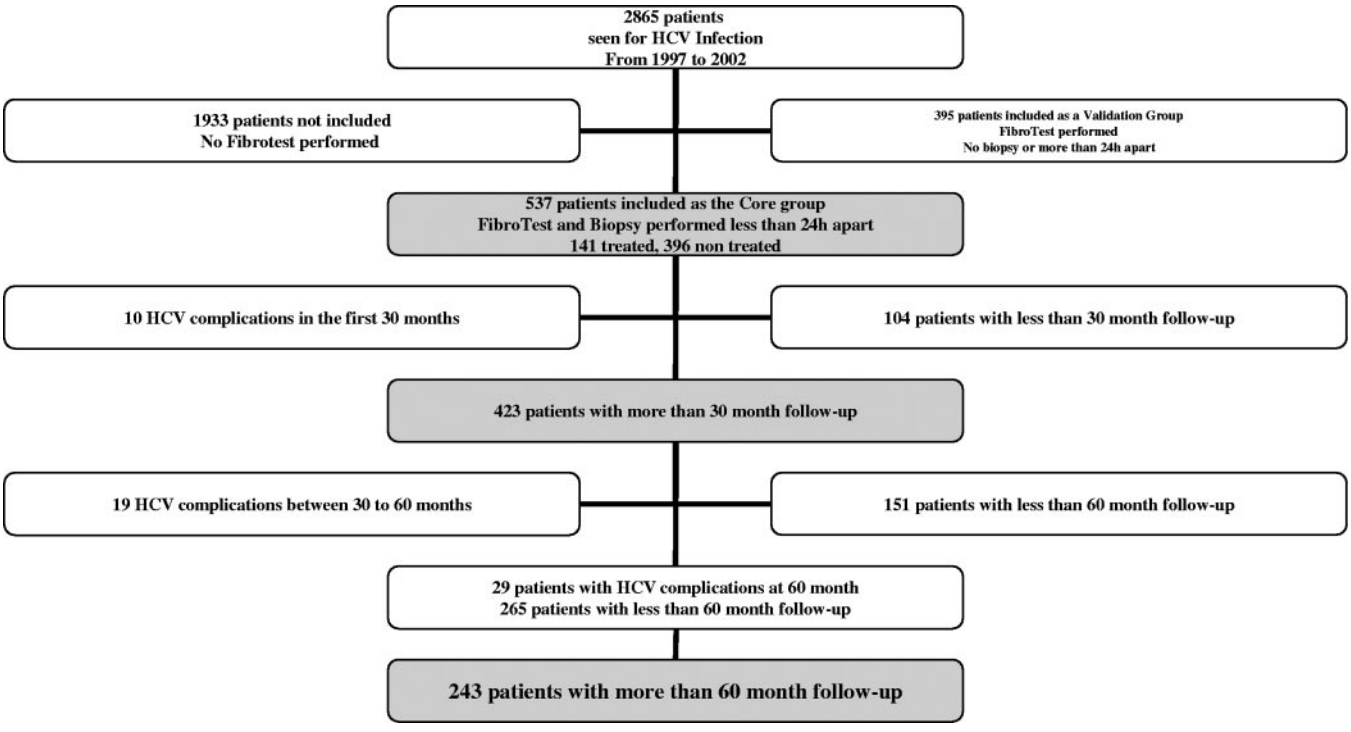

Fig. 1. Study design and patients included.

grade of activity according to the METAVIR scoring system *(22, 23)*. 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.

BIOCHEMICAL MARKERS

FT is a noninvasive blood test that combines the quantitative results of 6 serum biochemical markers, [α2-macroglobulin, haptoglobin, γ-glutamyl transpeptidase, total bilirubin, apolipoprotein A1, and alanine aminotransferase (ALT)] with patient age and sex data in a patented artificial intelligence algorithm *(24)* to generate a measure of fibrosis and necroinflammatory activity in the liver *(8–16)*. This method provides a numerical quantitative estimate of liver fibrosis ranging from 0.00 to 1.00, corresponding to the METAVIR scoring system, which grades fibrosis from F0 (no fibrosis) to F4 (cirrhosis). An algorithm has been suggested that would classify patients into 3 groups: no or minimal fibrosis (FT 0–0.31), moderate fibrosis (FT 0.31–0.58), and severe fibrosis (FT 0.58–1.00) *(8, 18)*.

We measured γ-glutamyl transpeptidase, ALT, AST, and total bilirubin with a Hitachi 917 analyzer and Roche Diagnostics reagents, and we measured α2-macroglobulin, apolipoprotein A1, and haptoglobin with a Dade Behring Nephelometer II. All tests were performed by personnel blinded to all patient characteristics, including biopsy results. All analytical studies, including intra- and interobserver and reproducibility studies, were performed independently of the present study, with CVs $<$10%, and have been reported previously *(25–30)*.

SURVIVAL ANALYSIS

The 5-year survival without HCV-related cirrhosis or complications related to liver disease [defined as: liver-related death, liver transplantation, or one of the following: decompensation, variceal bleeding, or hepatocellular carcinoma (HCC)] was the a priori main end-point used to compare the prognostic value of FT with histologic staging of biopsy samples. Decompensation was determined by the presence of ascites or hepatic encephalopathy or jaundice (total bilirubin, $>$51 μmol/L). Ascites was deemed to be present when ascitic fluid was confirmed by paracentesis and/or abdominal imaging. HCC was diagnosed by histologic examination of liver tissue obtained by liver biopsy, or at autopsy, or if 1 or more hepatic space–occupying lesions observed at ultrasonography or computed tomography were shown to have vascular patterns typical of HCC by angiography, dual-phase spiral tomography, or magnetic resonance imaging. Variceal bleeding was diagnosed on the basis of endoscopic findings in patients presenting with upper gastrointestinal hemorrhage.

To validate that the classification of HCV patients into 3 classes according to FT values, as previously recommended for disease management *(8, 18)*, was actually associated with mortality and morbidity, we used as 2 secondary endpoints survival without death related to HCV and the overall survival whatever the cause of death. We retrospectively compared FT with other indexes: the classical Child–Pugh score *(31)*, the APRI index *(32)*, and the Forns index *(33)*.

The survival time was calculated from the date of FT to the endpoint date. This interval was censored at the time of last follow-up. For decompensated patients at baseline, only complications occurring during follow-up were taken into account. When several complications occurred, the first one was taken into account. Each year, for patients who had not been seen at our hospital in the previous 12 months, we found out whether they were living and, if not, the date and the cause of death. For patients who were still alive, we either interviewed the patients or obtained information through their physicians. For deceased patients who died outside our hospital, we obtained information about the date and cause of death from their physicians or family. If we could not obtain information on the patient, we sent a letter to the city of their birth to find out whether they were still living and, if not, the date of death.

STATISTICAL ANALYSIS

We used the χ^2 test for qualitative comparisons, the Mann–Whitney test for quantitative comparisons *(34)*, time-dependent Kaplan–Meier analysis for survival curves, and the log-rank test and the Cox proportional hazard model for multivariate analysis *(35)*. We checked the assumption of proportional hazards by plotting the scaled Schoenfeld residuals *(36)*.

We compared patient survival with the survival expected in the French population, matched for age, sex, and follow-up period. The survival curve of the French population was calculated on the basis of age, sex, and follow-up period and conditional probabilities of death from official published census tables *(35)*. For each patient, beginning from the date of FT assessment, we used the Ederer II method to calculate a yearly predicted cumulative survival rate from a person of the same age and sex having a similar period of follow-up, and we used the Z test for comparisons between actual and predicted survivals *(36)* to compare the prognostic values of FT and fibrosis staging. FT data, as well as other score values, were entered as continuous variables. We calculated AUROCs with an empirical nonparametric method according to DeLong et al. *(37)* or the binormal method if the sample size of the endpoint was $<$30 *(38)*, and we compared results with the method of Zhou et al. *(39)*. We used 2-sided statistical tests for all analyses; a P value of $\leq$0.05 was considered substantial. We used Number Cruncher Statistical Systems 2003 software for all analyses *(34)*.

Data from patients who had advanced cirrhosis at baseline was excluded from sensitivity analysis, which including only data from Pugh class A patients.

Results

PATIENTS

Of the 2865 patients seen during the study period, 537 (19%) were included in the core group and 395 in the validation group (Table 1). Core group patients were younger, with lower body-mass index, more infections attributable to intravenous drug use, higher transaminases and ALT, more genotype 3, less fibrosis, and less

Table 1. Characteristics of included patients.

	Core group with concomitant FT and biopsy, mean (SD)	Group with FT but no concomitant biopsy, mean (SD)	Significance, *P* value
Number of patients	537	395	
Characteristics			
Age at biopsy, years	45.5 (12.4)	52.1 (13.4)	<0.0001
Male, %	322 (60)	235 (60)	0.90
Female, %	215 (40)	160 (40)	
Body mass index, kg/m^2	24.2 (4.1)	24.9 (4.3)	0.01
Ethnic origin, %			
Caucasian	454 (85)	342 (87)	0.64
African	4 (8)	32 (8)	
Asian	36 (7)	21 (5)	
Source of infection, %			0.01
Transfusion	113 (21)	114 (29)	
IV drug	168 (31)	90 (23)	
Other or unknown	256 (48)	191 (48)	
Alcohol consumption, %			0.59
0 g/day	267(53)	210 (56)	
0–50 g/day	169 (33)	114 (30)	
Over 50 g/day	69 (14)	52 (14)	
Coinfection HBV, %	18 (3.5)	6 (1.6)	0.08
Genotype, number performed, %	310	237	0.009
G1	163 (52)	147 (62)	
G2	36 (12)	33 (14)	
G3	73 (23)	30 (13)	
G4, G5, G6	39 (13)	27 (11)	
Baseline viral load, KUI/mL	1673 (3200)	1301 (2867)	0.11
Risk factors			
Diabetes, %	34 (6.3)	23 (5.8)	0.74
Renal failure, %	17 (3.2)	5 (1.3)	0.06
Coinfection HIV, %	47 (9.2)	23 (6.1)	0.09
Biopsy			
Number performed	537	223	
Patients with A2 or A3 at biopsy, %[a]	173/537 (32)	79 (36)	0.34
Patients with F2, F3, or F4 at biopsy, %	240/537 (45)	120 (54)	0.02
Steatosis, %			<0.0001
No	311/537 (58)	52/155 (33)	
Yes	226/537 (42)	103/155 (67)	
Quality of liver biopsy			
Biopsy size, mm	16.1 (6.5)	17.2 (7.6) n = 183	0.17
25 mm or more, %	74/533 (14)	29/183 (16)	0.50
Biomarkers			
Number performed	537	395	
ALT UI/L	109 (96)	90 (83)	0.001
Total bilirubin, μmol/L	11.7 (8.2)	15.1 (28.9)	0.16
γ-Glutamyl transpeptidase, IU/L	96 (152)	92 (133)	0.35
α2-Macroglobulin, g/L	2.52 (0.93)	2.81 (1.02)	<0.0001
Apolipoprotein A1, g/L	1.46 (0.34)	1.44 (0.41)	0.45
Haptoglobin, g/L	0.93 (0.55)	0.89 (0.53)	0.41
FT (0.00–1.00)	0.41 (0.28)	0.51 (0.29)	<0.0001
AT (0.00–1.00)	0.52 (0.27)	0.47 (0.28)	0.01

[a] A0, no histological activity; A1, mild activity; A2, moderate activity; A3, severe activity.

steatosis than the validation group. Comparisons between the patients of core group and the 2328 nonincluded patients were previously published *(18)*.

CORE GROUP

A total of 396 patients (74%) had not been treated, and 141 (26%) had received treatment, mostly with a combination of polyethylene glycol interferon and ribavirin; patients with severe fibrosis had been treated more frequently (59%; 93 of 157) than those without severe fibrosis (17%; 47 of 280); and 50% of treated patients (70 of 140) achieved a sustained virologic response. A total of 59 patients (11%) had a histologic diagnosis of cirrhosis, with only 1 patient with Pugh class C decompensated cirrhosis at baseline and 9 with Pugh class B. At baseline in the severe group, 12 patients had signs of portal hypertension: 3 with esophageal varices, 2 with gastropathy, and 8 with signs of portal hypertension by ultrasound. During follow-up, 8 patients were treated with beta-blockers for portal hypertension. The median follow-up of the cohort was 57 months (range, 1–93 months). Details of the number of patients at risk at each interval are given in Fig. 1.

COMPLICATIONS AND SURVIVAL

A total of 29 complications, including 2 liver transplantations, were observed during the 5-year follow-up, with a total of 20 deaths, including 9 deaths attributable to HCV (Table 2A). One patient treated with beta-blockers bled from esophageal varices but survived.

The survival outcomes of patients classified according to previously defined FT cutoffs are presented in Table 2B. At 5 years, the survival rate without HCV complications was 93% [95% confidence interval (CI), 91%–96%; 29 complications], and the survival without HCV related death was 98% (95% CI, 96%–99%; 9 deaths). The overall survival was 95% (95% CI, 93%–97%; 20 deaths), which was lower, although not substantially, than that for paired controls (97%; 95% CI, 97%–98%; $P = 0.06$) (Table 2B).

No HCV-related complications or deaths occurred in the minimal severity group; 1% complications (1 nontreated patient) and no death occurred in the moderate group; and 22% (n = 28) complications and 7% (n = 9) HCV-related deaths occurred in the severe group (Fig. 2). In the severe group, 13 (28%) complications (7 deaths) occurred among the 64 untreated patients, 12 (26%) complications (2 deaths) in 54 virologic nonresponders, and 3 (8%) complications (no death) among the 39 sustained virologic responders. Overall survival of the untreated severe group was substantially lower than that of paired controls (Table 2B).

COMPARISON WITH BIOPSY AND OTHER INDEXES

The ROC curves comparing the sensitivity and specificity of FT vs fibrosis at biopsy for complications, HCV-related death, and overall death are shown in Fig. 3. FT had a substantially higher prognostic value than biopsy for HCV complications and HCV-related deaths compared (Table 3).

Table 2. Death and survival analysis.

A. Causes of death and complications during the 5-year follow-up.

Death related to HCV, n = 9	Complications without death, n = 20	Death unrelated to HCV, n = 11
Decompensation, n = 4	Decompensation, n = 4	Suicide, overdose, n = 3
HCC and hemorrhage, n = 3	Hemorrhage, n = 8 (1 transplanted)	Plane accident, n = 1
HCC, n = 1	HCC, n = 8 (1 transplanted)	Car accident, n = 1
Hemorrhage, n = 1		Regurgitation, n = 1
		Nonliver lymphoma, n = 1
		Cholangiocarcinoma, n = 1
		Other, 3

B. Survival according to baseline FT value.

Baseline FT value	n	HCV complications	Survival without HCV complications	HCV Related Death	Survival without HCV Death	Death	Overall survival	Survival in paired controls
0.00–0.31	243	0	100	0	100	2	98.9 (97.3–1.00)[a]	98.7 (98.6–98.9)
No or minimal fibrosis								
0.32–0.58	137	1	98.8 (96.6–1.00)	0	100	6	93.4 (88.1–98.7)[b]	97.4 (96.9–97.9)
Moderate fibrosis								
0.59–1.00	157	28	78.5 (71.2–85.9)[c]	9	92.7 (88.0–97.3)[c]	12	90.5 (85.3–95.7)[c]	95.1 (92.3–96.0)[c]
Severe fibrosis								
Not treated	64	13	72.5 (58.7–86.2)	7	84.5 (73.2–95.7)	10	78.5 (66.2–90.9)[d]	94.2 (92.5–96.1)
Nonresponders	54	12	74.0 (61.0–87.1)	2	95.1 (88.45–1.00)	2	95.1 (88.5–1.00)	95.8 (94.8–96.7)
Sustained responders	39	3	92.3 (83.9–1.00)	0	100	0	100	95.6 (94.6–96.7)
All	537	29	93.2 (90.7–95.7)	9	97.8 (96.3–99.3)	20	95.0 (92.8–97.2)	97.3 (97.1–97.6)

[a] There was no significant difference for survival curves between minimal or moderate groups, with the exception indicated between overall survival ($P = 0.02$).
[b] Survival of the severe fibrosis group was not significantly lower than that of the moderately severe group (NS).
[c] Survivals of the severe fibrosis group were significantly lower than the two other groups ($P < 0.001$).
[d] Overall survival of the nontreated severe fibrosis group was significantly lower than that of paired controls ($P = 0.01$).

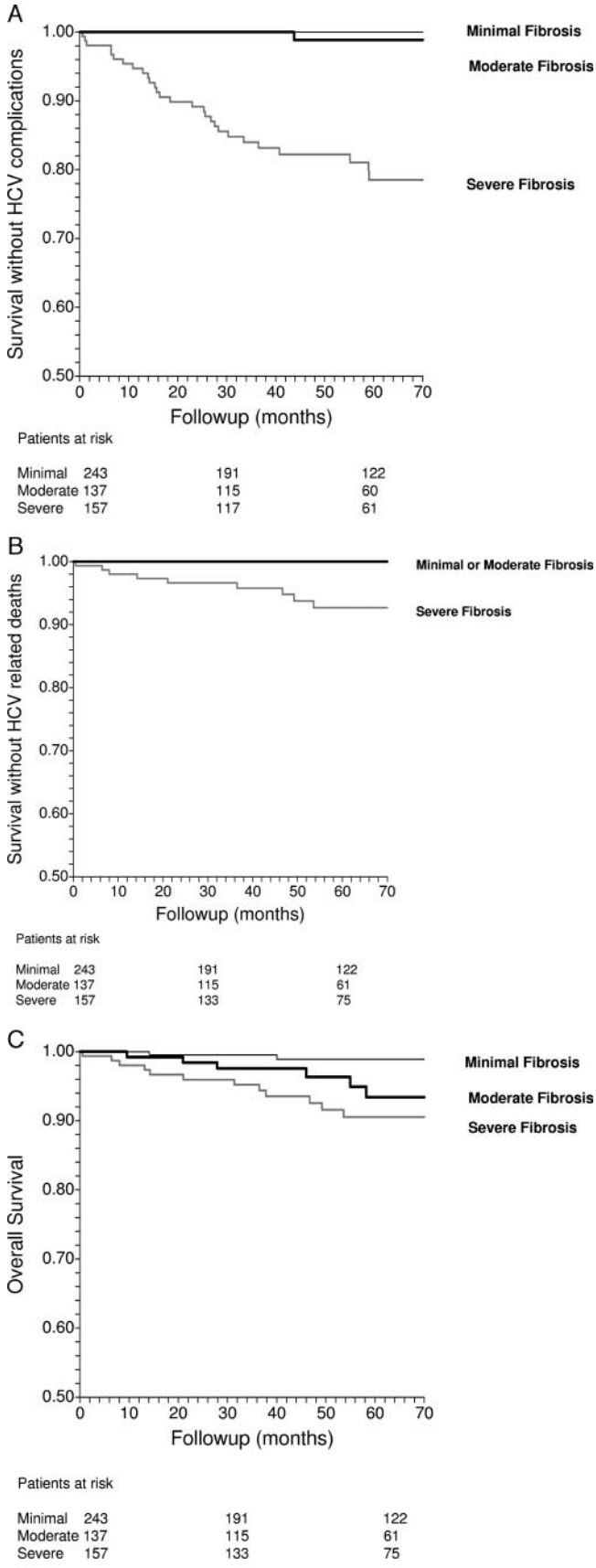

Fig. 2. Five-year survival curves according to FibroTest severity groups at baseline.

(*A*), group 1, no or minimal fibrosis; patients with baseline FibroTest of 0.00–0.31. (*B*), group 2, moderate fibrosis: patients with baseline FibroTest of 0.32–0.58. (*C*), group 3, severe fibrosis: patients with baseline FibroTest of 0.58–1.00. Survival without HCV complications (*A*), survival without HCV death (*B*), and overall survival (*C*) were higher in patients with minimal or moderate FibroTest values in comparison with severe values (all log-rank tests, $P < 0.001$), except between moderate and severe groups for overall survival. We found no substantial difference for survival curves between minimal or moderate groups except for overall survival ($P = 0.02$).

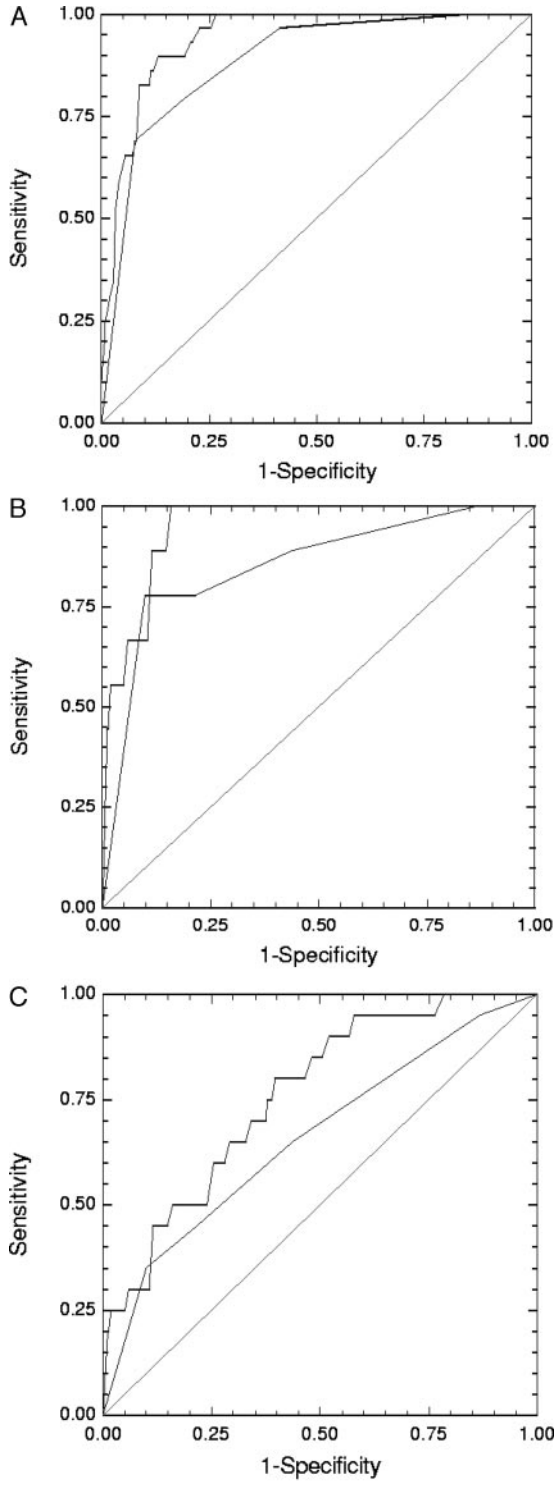

Fig. 3. ROC of FibroTest and biopsy.

(*A*), prognostic value (ROC curves) (AUROC) of FibroTest vs biopsy fibrosis staging for survival without HCV-related complications. FibroTest AUROC (0.96; 95% CI, 0.93%–0.97%) was greater than fibrosis staging AUROC (0.91; 95% CI, 0.85%–0.94%; $P = 0.01$). (*B*), prognostic value (AUROC) of FibroTest vs biopsy fibrosis staging for survival without HCV-related death. FibroTest AUROC (0.96; 95% CI, 0.93%–0.98%) was greater than fibrosis staging AUROC (0.87; 95% CI, 0.70%–0.94%; $P = 0.046$). (*C*), prognostic value (AUROC) of FibroTest vs biopsy fibrosis staging for overall survival. FibroTest AUROC (0.76; 95% CI, 0.63%–0.84%) was not substantially different from fibrosis staging AUROC (0.66; 95% CI, 0.52%–0.78%).

The FT AUROC for survival without complications (0.96; 95% CI, 0.93%–0.97%) was also greater (all $P < 0.01$) than the AUROCs of the other indexes (Pugh, APRI, and Forns; Table 3), and each of the 6 components of FT analyzed separately (platelets, prothrombin time, and albumin; see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol52/issue10). FT had also better prognostic value than the ActiTest or histologic activity grade (data not shown).

variables associated with complications and survival (see table 2 in the online data supplement)

FT was the only variable significantly associated with both survival without HCV-related complications and overall survival. Fibrosis staging at biopsy was not associated with overall survival. Heavy alcohol consumption (>50 g/day) and absence of HCV treatment were associated with overall death.

test values on ft and biopsy for persons with complications and death

Nine patients died from HCV-related causes: all of them had been classified by FT at baseline as having cirrhosis; 7 of these patients had the same classification made by biopsy, but 2 patients had been classified as noncirrhotic by biopsy, one as stage F1 and the other F2.

Among the 20 patients who had nonlethal complications, 12 had been classified by both biopsy and FT as having cirrhosis at baseline, 2 were classified as stage F2 by biopsy and FT, 5 were classified as cirrhotic by FT but not by biopsy (2 F2 and 3 F3), and 1 was classified as F3 by FT and cirrhotic by biopsy.

repeated ft

For 72 patients with baseline severe fibrosis, a second FT was performed after a mean (SE) of 5.0 (0.3) years. Notable improvement of the FT was observed in 23 sustained virologic responders, including reversal of cirrhosis in 9 patients, [mean (SE) baseline FT, 0.76 (0.02); last FT, 0.60 (0.03); $P < 0.0001$], but improvement was not observed in 36 nonresponders to virologic treatment [mean (SE) baseline FT, 0.76 (0.02); last FT, 0.73 (0.03); $P = 0.25$] or in 13 nontreated patients [mean (SE) baseline FT, 0.80 (0.03); last FT, 0.77 (0.04); $P = 0.42$].

sensitivity analyses

When the 10 patients with baseline severe cirrhosis were excluded, (5 of them died from HCV and 8 had complications), all the main results were the same (see Table 3 in the online Data Supplement).

validation group

No HCV-related complications or HCV-related death occurred in the minimal severity group (n = 129); 1% complications (1 complication) and 1% death (1 death)

Table 3. Comparison of AUROC for survival endpoints determined from biomarkers and biopsy specimen histology

Marker	Number of patients	Survival without HCV complications		Survival without HCV death		Overall survival	
		AUROC	95% CI	AUROC	95% CI	AUROC	95% CI
FT	537	0.96[a]	0.93–0.97	0.96[b]	0.93–0.98	0.76	0.63–0.84
Nontreated	396	0.97	0.94–0.99	0.99	0.97–0.99	0.81	0.68–0.89
Treated	141	0.89	0.83–0.93	0.85	0.68–0.94	0.85	0.68–0.94
Fibrosis staging at biopsy	537	0.91	0.85–0.94	0.87	0.70–0.94	0.66	0.52–0.78
Nontreated	396	0.91	0.79–0.96	0.88	0.60–0.96	0.70	0.54–0.81
Treated	141	0.84	0.73–0.91	NP[c]		NP	
Pugh classification	537	0.80	0.65–0.89	0.89	0.57–0.97	0.71	0.52–0.83
Nontreated	396	0.84	0.60–0.94	0.85	0.46–0.98	0.61	0.48–0.71
Treated	141	0.76	0.52–0.89	0.99	−0.91–1.00	0.98	0.94–0.99
APRI Index	260	0.82	0.66–0.91	0.76	0.35–0.93	0.67	0.42–0.82
Nontreated	155	0.87	0.57–0.97	0.73	0.45–0.92	0.66	0.39–0.82
Treated	105	0.74	0.51–0.87	NP		NP	
Forns index	170	0.86	0.74–0.93	0.87	0.52–0.97	0.73	0.46–0.87
Nontreated	96	0.91	0.73–0.97	0.84	0.35–0.97	0.71	0.42–0.87
Treated	74	0.81	0.59–0.92	NP		NP	

[a] FT AUROC was greater than that with fibrosis staging ($P = 0.01$), ActiTest ($P < 0.0001$), activity grading ($P < 0.0001$), Pugh ($P = 0.006$), APRI ($P = 0.03$), and Forns ($P = 0.04$).

[b] FT AUROC was greater than that with fibrosis staging ($P = 0.046$), ActiTest ($P < 0.0001$), activity grading ($P < 0.001$), and APRI ($P = 0.02$).

[c] NP, not possible because the number of events was too low.

occurred in the moderate group (n = 98); and 22% complications (27 complications) and 17% HCV-related mortality (17 deaths) occurred in the severe group (n = 168) ($P < 0.001$ between the severe vs the 2 other groups for both complications and deaths).

Discussion

Until now, the prognostic markers validated for use in chronic HCV have been the histologic fibrosis staging of biopsy specimens, the Pugh score for patients with cirrhosis *(19–21, 40)*, and hyaluronic acid *(41)*.

Our results indicate that FT has a better 5-year prognostic value than biopsy estimates, regardless of the treatment and risk factors. Even after exclusion of decompensated cirrhotic patients, the prognostic value of FT was at least similar to biopsy. In the multivariate model, biopsy was more predictive than FT for complications but not for overall survival. Because of the applicability, cost, and risks of biopsy, prognostic studies with FT and other noninvasive methods should be the first-line approach.

Although this prognostic study was not specifically designed to validate FT as a true surrogate endpoint of HCV chronic hepatitis severity *(42)*, we observed that FT fulfilled almost all of the 13 criteria of a surrogate endpoint biomarker *(43)*, including specificity and detection limit for fibrosis, with l<5% false positives or false negatives *(8, 9, 18, 26)*. FT was indicative of response to virologic treatment. The virologic treatment response that we observed, with FT improvement and cirrhosis reversal, has been previously observed *(9, 11)*; intra- and interobserver variability of FT has been studied; preanalytical and analytical recommendations have been issued *(8, 26, 44)*; and security algorithms have been designed for detection of false positives or false negatives *(18)*. Serial monitoring of FT is possible, as has been demonstrated in several studies *(10, 11, 45)*.

In comparison, liver biopsy does not satisfy quality criteria as a surrogate endpoint marker because of its complication rate, sampling error, intra- and interobserver variability, expense, and patient reluctance to undergo serial monitoring. The results of our analysis of hard clinical endpoints during this 5-year follow-up demonstrate a higher failure rate of biopsy than FT *(18)*. When a false negative was defined as a patient without baseline cirrhosis (FT or biopsy) who suffered HCV-related complication or death during the 5 years, there were 7 false negatives (true cirrhosis not detected at baseline) attributable to biopsy in this study and only 1 to FT.

We used a quantitative estimate for FT and a discrete estimate for biopsy, but a comparison of AUROCs by use of discrete FT scores vs histology (all in 5 classes from F0 to F4) for the main endpoint (HCV complications) was still significant in favor of FT: 0.96 (95% CI, 0.94%–0.97%) vs 0.91 (95% CI, 0.85%–0.95%), $P = 0.003$, respectively. Validation studies of biomarkers vs biopsy as surrogate markers should be performed by independent groups.

Our study was not conclusive for the use of FT score to determine the need for treatment, but the results supported a previously suggested treatment algorithm based on FT values *(18)*. Because patients with low FT scores were unlikely to develop complications, decisions not to treat such patients were unlikely to be associated with clinical decompensation, at least over a relatively short follow-up period. Patients with moderate FT scores were at low risk, with a 1% risk at 5 years. Although availability of FT test results could influence therapeutic decisions,

during follow-up (1997–2004), most patients were managed according to biopsy results, and therapeutic decisions were never related to the FT results from 1997 to 2002. Interestingly, for nontreated patients with severe FT >0.58, overall survival was significantly lower than controls ($P = 0.02$), whereas for sustained virologic responders with FT> 0.58, overall survival was similar to that of controls. Thus, FT permitted identification of patients with a high mortality risk.

Because the Pugh index was validated in patients with cirrhosis, a group that represented <20% of the included patients, the prognostic superiority of FT over Pugh was expected. The superiority of FT over the Forns and APRI indexes was also expected, because these indexes have lower values for the diagnosis of advanced fibrosis as assessed by AUROCs *(13, 46)*.

One weakness of the present study was that the included population was not a random, community-based population. Baseline comparisons between the included and nonincluded populations *(18)*, however, have not identified obvious causes of bias: data were very similar for variables associated with a risk of bias in prognostic studies.

The patients of the core group were more likely to be responders to HCV treatment, with better response variables: younger, lower body mass index, less steatosis, and more genotype 3. Patients in the core group were more often new patients with an indication of first liver biopsy, which could explain more simultaneous biopsy and FT than in patients of the validation group.

Compared with the nonincluded population, the core group or the validation group likely included more patients who returned more frequently or had other features suggestive of more aggressive disease. The survival in the core group was higher than in a community-based retrospective cohort *(47)*, however, and lower than in another prospective tertiary hospital cohort *(40)*. These 2 studies differed from ours in design, including only untreated patients and looking at follow-up from the time of HCV detection. Although the rate of outcomes in the patients with severe FT represents nearly a 20% cumulative incidence, the low event rate is also a limitation of this study. Our core group, however, had the advantage of including treated and untreated patients with a wide range of fibrosis severity and risk factors, without the exclusion of heavy drinkers or HIV-coinfected patients. After we excluded decompensated patients, fewer events were included, but the main results were similar to those for the entire prospective cohort. Another advantage of this study was that it allowed the observation of similar prognostic values of FT in the validation population of 395 patients.

As previously reported *(47)*, deaths unrelated to HCV, including 3 suicides, were as frequent as HCV-related death. The overall survival of patients with nonsevere fibrosis at baseline was close to that of paired controls in the general population. In patients with severe fibrosis, overall survival was 5% lower than that of the control population, a rate that should be confirmed with longer follow-up.

In conclusion, the FT has prognostic values at least similar to that of liver biopsy, with the advantage of being noninvasive.

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References

1. Afdhal NH. Diagnosing fibrosis in hepatitis C: is the pendulum swinging from biopsy to blood tests? Hepatology 2003;37:972–4.
2. Dienstag J. The role of liver biopsy in chronic hepatitis C. Hepatology 2002;36:S152–60.
3. Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, 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, Leandro G. 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, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology 2003;38:1449–57.
6. Poynard T, Ratziu V, Bedossa P. Appropriateness of liver biopsy. Can J Gastroenterol 2000;14:543–8.
7. Gebo KA, Herlong HF, Torbenson MS, Jenckes MW, Chander G, Ghanem KG, et al. Role of liver biopsy in management of chronic hepatitis C: A systematic review. Hepatology 2002;36:S161–72.
8. Poynard T, Imbert-Bismut F, Munteanu M, Messous D, Myers RP, Thabut D, et al. Overview of the diagnostic value of biochemical markers of liver fibrosis (FibroTest, HCV-Fibrosure) and necrosis (ActiTest) in patients with chronic hepatitis C. Comp Hepatol 2004;3:8.
9. Imbert-Bismut F, Ratziu V, Laurence Pieroni L, Charlotte F, Benhamou Y, Poynard T, et al. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. Lancet 2001;357:1069–75.
10. Poynard T, Imbert-Bismut F, Ratziu V, Chevret S, Jardel C, Moussalli J, et al. Biochemical markers of liver fibrosis in patients infected by hepatitis C virus: longitudinal validation in a randomized trial. J Viral Hepat 2002;9:128–33.
11. Poynard T, McHutchison J, Manns M, Myers RP, Albrecht J. Biochemical surrogate markers of liver fibrosis and activity in a randomized trial of peginterferon alfa-2b and ribavirin. Hepatology 2003;38:481–92.

12. Myers RP, Benhamou Y, Imbert-Bismut F, Thibault V, Bochet M, Charlotte F, et al. Serum biochemical markers accurately predict liver fibrosis in HIV and hepatitis C virus-coinfected patients. AIDS 2003;17:1–5.

13. Thabut D, Simon M, Myers RP, Messous D, Thibaut V, Imbert-Bismut F, et al. Non-invasive prediction of fibrosis in patients with chronic hepatitis C [letter]. Hepatology 2003;37:1220–1.

14. Callewaert N, Van Vlierberghe H, Van Hecke A, Laroy W, Delanghe J, Contreras R. Noninvasive diagnosis of liver cirrhosis using DNA sequencer-based total serum protein glycomics. Nat Med 2004; 10:1–6.

15. Castéra L, Vergniol J, Foucher J, Brigitte Le Bail B, Chanteloup E, Haaser M, 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.

16. Halfon P, Bourliere M, Deydier R, Botta-Fridlund D, Portal I, Renou C, et al. Independent prospective multicenter validation of biochemical markers (FibroTest-ActiTest) for the prediction of liver fibrosis and activity in patients with chronic hepatitis C. Am J Gastroenterol 2006;101:547–55.

17. Rossi E, Adams L, Prins A, Bulsara M, de Boer B, Garas G, et al. Validation of the FibroTest biochemical markers score in assessing liver fibrosis in hepatitis C patients. Clin Chem 2003;49:450–4.

18. Poynard T, Munteanu M, Imbert-Bismut F, Charlotte F, Thabut D, Le Calvez S, et al. Prospective analysis of discordant results between biochemical markers and biopsy in patients with chronic hepatitis C. Clin Chem 2004;50:1344–55.

19. Niederau C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hurter D, et al. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology 1998;28:1687–95.

20. Forns X, Ampurdanes S, Sanchez-Tapias JM, Guilera M, Sans M, Sanchez-Fueyo A, et al. Long-term follow-up of chronic hepatitis C in patients diagnosed at a tertiary-care center. J Hepatol 2001; 35:265–71.

21. Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I, et al. Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. J Hepatol 1998;28:930–8.

22. The METAVIR cooperative group. Inter- and intra-observer variation in the assessment of liver biopsy of chronic hepatitis C. Hepatology 1994;20:15–20.

23. 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.

24. US Patent 6 631 330.

25. Halfon P, Imbert-Bismut F, Messous D, Antoniotti G, Benchetrit D, Cart-Lamy P, 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.

26. Imbert-Bismut F, Messous D, Thibaut V, Myers RB, Piton A, Thabut D, 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.

27. Munteanu M, Messous D, Thabut D, Imbert-Bismut F, Jouys M, Massard J, et al. Intra-individual fasting versus postprandial variation of biochemical markers of liver fibrosis (FibroTest) and activity (ActiTest). Comp Hepatol 2004;3:3.

28. Imbert-Bismut F, Messous D, Raoult A, Poynard T, Bertrand JJ, Marie PA, et al. Results transferability on RXL, ARX, X-Pand, BN2 (Dade Behring) and modular DP (Roche Diagnostics) analysers: application to component assays of FibroTest and ActiTest. Ann Biol Clin (Paris) 2005;63:305–13.

29. Rosenthal-Allieri MA, Peritore ML, Tran A, Halfon P, Benzaken S, Bernard A. Analytical variability of the FibroTest proteins. Clin Biochem 2005;38:473–8.

30. Ferard G, Piton A, Messous D, Imbert-Bismut F, Frairi A, Poynard T, et al. Intermethod calibration of alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) results: application to FibroTest and ActiTest scores. Clin Chem Lab Med 2006;44: 400–6.

31. Pugh R, Murray-Lyon I, Dawson J, Pietroni M, Williams R. Transection of the oesophagus for bleeding oesophagus varices. Br J Surg 1973;60:646–9.

32. Wai CT, Greenson JK, Fontana RJ, Kalbfleish JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38:518–26.

33. Forns X, Ampurdanes S, Llovet JM, Aponte J, Quinto L, Martinez-Bauer E, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 2002; 36:986–92.

34. Hintze JL. NCSS 2003 User Guide. Kaysville, UT: Number Cruncher Statistical Systems, 2003.

35. Vallin J, Meslé F. Tables de Mortalité Françaises pour les XIXe et XXe Siècles et Projections pour le XXIe Siècle. Institut National d'Etudes Démographiques, 2001.

36. Ederer F, Heise H. Instructions to IBM 650 Programmers in Processing Survival Computations. Methodological Note No. 10, End Results Evaluation Section. Bethesda, MD: National Cancer Institute, 1959.

37. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837–45.

38. McClish DK. Analyzing a portion of the ROC curve. Med Decis Making 1989;9:190–5.

39. Zhou X, Obuchowski N, McClish D. Statistical Methods in Diagnostic Medicine. New York: John Wiley & Sons, 2002.

40. Khan MH, Farrell GC, Byth K, Lin R, Weltman M, George J, et al. Which patients with hepatitis C develop liver complications? Hepatology 2000;31:513–20.

41. Guechot J, Serfaty L, Bonnand AM, Chazouilleres O, Poupon RE, Poupon R. Prognostic value of serum hyaluronan in patients with compensated HCV cirrhosis. J Hepatol 2000;32:447–52.

42. Temple RJ. A regulatory authority's opinion about surrogate endpoints. In: Nimmo WS, Tucker GT, eds. Clinical Measurement in Drug Evaluation. New York: John Wiley and Sons, Inc., 1995: 1–2.

43. Kelloff GJ, Sigman CC. New science-based endpoints to accelerate oncology drug development. Eur J Cancer 2005;41:491–501.

44. Ferard G, Imbert-Bismut F, Messous D, Piton A, Ueda S, Poynard T, et al. A reference material for traceability of aspartate aminotransferase (AST) results. Clin Chem Lab Med 2005;43:549–53.

45. D'Arondel C, Munteanu M, Moussalli J, Thibault V, Naveau S, Simon A, et al. A prospective assessment of an "a la carte" regimen of PEG interferon alfa 2b and ribavirin combination in patients with chronic hepatitis C using biochemical markers. J Viral Hepat 2006;13:182–9.

46. Le Calvez S, Thabut D, Messous D, Munteanu M, Ratziu V, Imbert-Bismut F, et al. The predictive value of FibroTest vs APRI for the diagnosis of fibrosis in chronic hepatitis C [letter]. Hepatology 2004;39:862–3.

47. Rodger AJ, Roberts S, Lanigan A, Bowden S, Brown T, Crofts N. Assessment of long-term outcomes of community-acquired hepatitis C infection in a cohort with sera stored from 1971 to 1975. Hepatology 2000;32:582–7.