

Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study

Françoise Imbert-Bismut, Vlad Ratziu, Laurence Pieroni, Frederic Charlotte, Yves Benhamou, Thierry Poynard, for the MULTIVIRC group

Summary

Background Liver biopsy is thought mandatory for management of patients with hepatitis C virus (HCV) infection, especially for staging fibrosis. We aimed, in our prospective study, to assess the predictive value of a combination of basic serum biochemical markers for diagnosis of clinically significant fibrosis (including early stages).

Methods We assessed liver-biopsy patients with detectable HCV by PCR, for eligibility, and took a blood sample on the day of the procedure. The analysis was done in a first-year period for 205 patients and then tested in a second period on 134 patients. We devised a fibrosis index that included the most informative markers (combined with age and sex) for the first-year group. 11 serum markers were assessed as well as fibrosis stage: F0=no fibrosis and F1=portal fibrosis; and for clinically significant fibrosis, F2=few septa, F3=many septa, and F4=cirrhosis. Statistical analysis was by logistic regression, neural connection, and receiver-operating characteristic (ROC) curves.

Findings First-year and second-year patient-group characteristics and biochemical markers did not differ. The overall frequency of clinically significant fibrosis was 40% (138 patients). The most informative markers were: α_2 macroglobulin, α_2 globulin (or haptoglobin), γ globulin, apolipoprotein A₁, γ glutamyltranspeptidase, and total bilirubin. The areas (SD) under the ROC curves for the first-year (0.836 [0.430]) and second-year groups (0.870 [0.340]) did not differ ($p=0.44$). With the best index, a high negative predictive value (100% certainty of absence of F2, F3, or F4) was obtained for scores ranging from zero to 0.10 (12% [41] of all patients), and high positive predictive value (>90% certainty of presence of F2, F3, or F4) for scores ranging from 0.60 to 1.00 (34% [115] of all patients).

Interpretation A combination of basic serum markers could be used to substantially reduce the number of liver biopsies done in patients with chronic HCV infection.

Lancet 2001; **357**: 1069–75

Departments of Biochemistry (F Imbert-Bismut PhD, L Pieroni PhD), **Hepatogastroenterology** (V Ratziu MD, Y Benhamou MD, T Poynard MD), and **Pathology, Hospitalier Pitié-Salpêtrière**, (F Charlotte MD); and **Laboratoire d'Immunologie des Tumeurs, Faculté des Sciences Pharmaceutiques et Biologiques de Paris Université René Descartes, Paris, France** (V Ratziu, T Poynard)

Correspondence to: Prof Thierry Poynard, Service d'Hépatogastroentérologie, Groupe Hospitalier Pitié-Salpêtrière, 75651 Paris, Cedex 13, France (e-mail: tpoynard@teaser.fr)

Introduction

Liver biopsy is thought mandatory for management of patients infected by hepatitis C virus (HCV), particularly to stage fibrosis.^{1,2} However, after biopsy 30% of patients feel pain, 0.3% have severe complications, and 0.03% die.^{3,4} Several markers have substantial predictive values for diagnosis of cirrhosis,^{4–10} but none are available for diagnosis of earlier stages—eg, with few septa (the start of bridging fibrosis). No prospective studies have been done in a large population infected only by HCV. We aimed to prospectively assess the predictive value of a combination of basic serum biochemical markers for the diagnosis of clinically significant fibrosis (ranging from a few septa to cirrhosis) and necroinflammatory activity (necrosis and inflammation). If markers with high positive or negative predictive values of important fibrosis can be obtained, fewer liver biopsies would need to be done and thus the cost and risk of liver biopsy would be lessened.^{3,4}

Methods

Patients

From August, 1997, to March, 2000, all liver-biopsy patients who gave informed consent and with detectable HCV by PCR were assessed for eligibility and had a blood sample taken on the day of biopsy. Patients belonged to a single centre cohort, Cohorte Hépatite C Pitié-Salpêtrière (DOSVIRC). This cohort included all patients with HCV infection (defined by a positive serological test by at least a second-generation ELISA) attending the liver and gastrointestinal unit of Pitié-Salpêtrière Hospital, Paris, France.¹¹ A questionnaire of 129 items was completed for every patient and included: sociodemographic data; risk factors; clinical, biological, virological, and treatment information from each visit; and histological data obtained at liver biopsy. The duration of HCV infection was estimated from transfusion date or first exposure to other parenteral sources, but it could not be calculated for patients with sporadic infection or those in whom the source of infection was unknown. Exclusion criteria were coinfection with HIV, hepatitis B virus, or other liver disease, and non-interpretable liver biopsy. The analysis was done in a first-year period on 205 patients and then tested in a second period on 134 patients.

Serum markers

11 markers were assessed: α_2 macroglobulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ glutamyltranspeptidase (GGT), total bilirubin, albumin, α_1 globulin, α_2 globulin, β globulin, γ globulin, and apolipoprotein A₁. α_2 globulins mainly consisted of α_2 macroglobulin and haptoglobin; a retrospective assessment of haptoglobin was done for the two periods. Interleukin 10 (IL-10), tumour growth factor β 1 (TGF β 1), hepatocyte growth factor, apolipoprotein A₂, and apolipoprotein B were also assessed in the second period.

AST, ALT, GGT, and total bilirubin were measured by autoanalyser (Hitachi 917 Automate; Mannheim, Germany) and Roche Diagnostics reagents (Mannheim, Germany). Albumin was assessed by bromocresol green method.¹² Serum protein electrophoresis for α_1 globulin, α_2 globulin, β globulin, and γ globulin was done in an automatic system (Hydrasys and Hyrys, Sebia; Issy-Les-Moulineaux, France). Apolipoprotein A₁, apolipoprotein A₂, apolipoprotein B, α_2 macroglobulin, and haptoglobin were measured in serum samples with an automatic nephelometer (BNII, Dade Behring; Marburg, Germany). Plasma TGF β 1 and hepatocyte growth factor concentrations were measured with Quantikine human TGF β 1 immunoassay and Quantikine human hepatocyte growth factor immunoassay, respectively (R and D Systems; Minneapolis, MN, USA). Latent TGF β 1, was activated to the immunoreactive form by acid and then neutralised. Plasma IL-10 was measured with an immunoassay kit (Beckman Coulter Immunotech; Marseille, France). To prevent contamination with platelet-derived TGF β 1, blood samples were centrifuged 1 h after they were obtained, and serum samples were stored at -80°C until assays were done (<1 year for cytokines).

Histological staging and grading

Histological features of liver specimens were analysed with the METAVIR group scoring system.^{13,14} Liver biopsy specimens of more than 10 mm in length were fixed, paraffin-embedded, and stained with at least haematoxylin eosin safran—and Masson's trichrome or picosirius red for collagen. Every biopsy specimen was staged on a scale of F0 to F4: F0=no fibrosis, F1=portal fibrosis without septa, F2=few septa, F3=numerous septa without cirrhosis, and F4=cirrhosis. Histological activity, a measure of intensity of necroinflammatory lesions, was graded as follows: A0=no histological activity, A1=mild activity, A2=moderate activity, and A3=severe activity. The METAVIR scoring system was assessed by one pathologist (FC), who was unaware of patient characteristics.

Statistical analysis

Statistical analysis was by logistic regression, neural connection, and receiver-operating characteristic (ROC) curves.^{15,16} Analysis was done in the first year and then tested in the second period. In accordance with the METAVIR scoring system, patients were divided into several groups. The main endpoint was the identification of patients with substantial fibrosis (F2, F3, or F4) versus those without (F0 or F1). F2, F3, and F4 categories were grouped together because F2 is generally chosen as a threshold for treatment of chronic HCV infection.¹ In secondary analyses, patients were also grouped by activity grades: those without much activity (A0 or A1), and patients with substantial activity (A2 or A3). Patients were also classified into three overall severity groups: without important histological features ($A < 2$ and $F < 2$); important lesions ($A \geq 2$ and $F \geq 2$, or both); and widespread fibrosis or cirrhosis (F3 or F4).

First, factors that differed significantly between these groups were identified by univariate analyses: χ^2 , student t test, Mann-Whitney, and variance analysis with the Bonferroni all-pair-wise multiple comparison. The independent discriminative value of markers for the diagnosis of fibrosis was then assessed by logistic regression analysis. The third step was to construct an index that combined the independent factors. The best index for discrimination was the logistic regression function that combined the most discriminatory

independent factors. Diagnostic values of indices and isolated factors were assessed by sensitivity, specificity, positive and negative predictive values, and ROC curves. Predictive values were similar between the two periods but are presented only for the second. Predictive values were assessed for the prevalence of clinically significant fibrosis. Comparison of overall diagnostic value of markers was by area under the ROC curves. Analyses were done separately for the different overall severity groups.

An analysis of only the six most significant markers was done to reduce the number of factors. During the analyses, we noted that α_2 globulins had an independent diagnostic value if α_2 macroglobulin was included. Therefore, we retrospectively assessed the diagnostic value of haptoglobin, which is the second main component of α_2 globulins. Finally, we constructed an index that combined haptoglobin and four other identified markers and excluded protein electrophoresis components.

Neural connection was by the Multi-Layer Perceptron (Neural Connection version 1.0)¹⁶ which uses an incremental learning technique. To train the application, half of the patients were randomly allocated to a training group. First, some examples from this group were put into the Multi-Layer Perceptron. The best network produced was passed to the second stage and used as a starting point for training. Second, a larger sample of data was used to train the network, and again the best network was passed on to the next stage. The procedure was repeated for four stages. Afterwards, 25% of patients (validation group) were used to monitor neural network performance and finally, the remaining 25% of patients (test group) were used to measure the performance of the trained application.

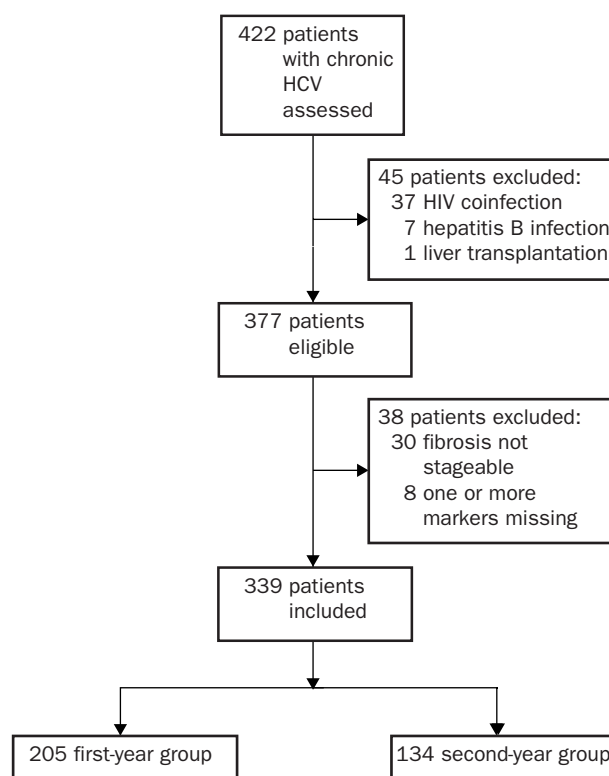


Figure 1: **Study design**

Independent markers were identified during the first year and their predictive values validated during the second period. HCV=hepatitis C virus.

IL-10, TGF β 1, hepatocyte growth factor, apolipoprotein A₂, and apolipoprotein B were assessed in the second period only, to explain the value of markers identified in the first period. However, their diagnostic values were assessed by the same methods as the other markers.

Results

Participants

We assessed 422 patients with chronic HCV infection for eligibility (figure 1). We excluded 45 because of: HIV coinfection (37), hepatitis B virus coinfection (seven), and transplantation (one). We could not stage fibrosis in 30 of the remaining 377 patients, and at least one of the 11 markers was missing in eight, which left 339 included patients. Patient characteristics and biochemical markers did not differ between first-year and second-year samples (table 1). The overall frequency of clinically significant fibrosis was 40% (138 patients).

Diagnosis of fibrosis

Table 2 shows diagnostic values (area under ROC curves) of biochemical markers and their independent association with fibrosis (logistic regression). Because ALT and AST were highly correlated ($r=0.88$), only ALT was used. The fibrosis indices combined ten or the six most informative markers (α_2 macroglobulin, α_2 globulin, total bilirubin, γ globulin, apolipoprotein A₁, and GGT), or five markers (α_2 globulin and γ globulin excluded; haptoglobin included). All indices had high diagnostic values in both

year groups, whether groups were analysed separately or together (table 2). The ROC curves for the three indices were very similar (figure 2). The areas (SD) under the curves did not differ: 0.851 (0.370), 0.847 (0.370), and 0.837 (0.370), respectively. Figure 3 shows the box plots of the five-marker and six-marker fibrosis indices (in which scores range from zero to 1.00), for each fibrosis stage. With the six-marker index, a high negative predictive value (>90% certainty of absence of F2, F3, or F4) was obtained for scores from zero to 0.20. Of these 119 patients with low scores (35% of total) there were 13 false negatives: four F2, A0; six F2, A1; and three F2, A2. A high positive predictive value (>90% certainty of presence of F2, F3, or F4) was obtained for scores from 0.80 to 1.00. Of these 50 patients with high scores (15% of total), there were five false positives: two F1, A1, and three F1, A2.

When analysis was done on the second-year group only (table 3), and with predictive values greater than or equal to 90% deemed acceptable, biopsies could have been avoided in 62 patients of 134 (46%) with scores below 0.10 (16 patients, all F0 or F1) or above 0.60 (46 patients, only four F1). Neural connection methods gave similar results to logistic regression. The number of patients correctly classified (whether with important fibrosis or not) by Multilayer Perceptron with α_2 macroglobulin, haptoglobin, GGT, total bilirubin, apolipoprotein A₁, age, and sex was 130 of 163 (80%) for the training group, 61 of 82 (74%) for the validation group, and 59 of 81 (73%) for the test group.

In the second-year group, the addition of IL-10, TGF β 1, apolipoprotein A₂, and apolipoprotein B increased the area (SD) under the curve slightly to 0.889 (0.340), but did not differ ($p=0.60$) from the six-marker fibrosis index. None of the extra markers added significant diagnostic value to the indices by logistic regression or neural connection (data not shown).

43 patients had ALT lower than 35 IU/L, and ten of those had important fibrosis. The diagnostic value of the six-marker fibrosis index was still high, with an area under the ROC curve of 0.758 (0.590). Two patients with a score greater than 0.80 had cirrhosis. Among the 29 patients with scores lower than 0.20, 25 had no notable fibrosis.

Characteristics	First year	Second year	Total
Number of patients	205	134	339
Age at biopsy (years)	47 (14)	48 (13)	47 (13)
Male	108 (53%)	88 (66%)	196 (58%)
Female	97 (47%)	46 (34%)	143 (42%)
Fibrosis stage			
No fibrosis (F0)	36 (18%)	20 (15%)	56 (17%)
Portal fibrosis (F1)	91 (44%)	54 (40%)	145 (43%)
Few septa (F2)	40 (20%)	28 (21%)	68 (20%)
Many septa (F3)	18 (9%)	10 (7%)	28 (8%)
Cirrhosis (F4)	20 (10%)	22 (16%)	42 (12%)
Necroinflammatory activity grade			
None (A0)	52 (25%)	17 (13%)	69 (20%)
Mild (A1)	85 (41%)	80 (60%)	165 (49%)
Moderate (A2)	63 (31%)	33 (25%)	96 (28%)
Severe (A3)	5 (2%)	4 (3%)	9 (3%)
Mean iron score	1.2 (2.4)	1.4 (2.6)	1.3 (2.5)
Steatosis	25 (12%)	20 (15%)	44 (13%)
Markers (normal range)			
AST IU/L (17–27 female; 20–32 male)	71 (65)	84 (91)	77 (77)
ALT IU/L (11–26 female; 16–35 male)	108 (96)	122 (139)	114 (115)
Total bilirubin μ mol/L (1–21)	11 (7)	13 (28)	12 (19)
Albumin g/L (30–50)	44 (4)	44 (6)	44 (5)
GGT U/L (7–32 female; 11–49 male)	81 (126)	99 (176)	88 (148)
α_2 macroglobulin g/L (female 1.6–4.0; male 1.4–3.3)	2.6 (0.9)	2.5 (0.9)	2.6 (0.9)
α_1 globulin g/L (1–3)	1.9 (0.4)	1.8 (0.4)	1.9 (0.4)
α_2 globulin g/L (4–9)	7.6 (1.5)	6.8 (1.5)	7.3 (1.5)
β globulin g/L (4–10)	6.8 (1.1)	7.0 (1.1)	6.9 (6.9)
γ globulin g/L (5–12)	14.9 (4.7)	14.6 (5.3)	14.8 (4.9)
Apo A1 g/L (1.2–1.7)	1.5 (0.33)	1.4 (0.33)	1.5 (0.33)
Apo B g/L (0.5–1.4)	NE	0.9 (0.27)	NE
Apo A ₂ g/L (0.3–0.5)	NE	0.3 (0.09)	NE
IL-10 μ g/L (<16)	NE	78 (208)	NE
TGF β 1 (35–64) pg/L	NE	31 (11)	NE
Haptoglobin g/L (0.35–2.00)*	0.97 (0.59)	0.89 (0.54)	0.94 (0.57)
HGF pg/L (319–1475)	NE	1181 (597)	NE

Data are mean (SD) or proportion. AST=aspartate aminotransferase. ALT=alanine aminotransferase. GGT= γ glutamyl transpeptidase. Apo=apolipoprotein. IL=interleukin. TGF=tumour growth factor. HGF=hepatocyte growth factor. NE=not established. *For haptoglobin, values were missing for eight patients in the first period and one in the second.

Table 1: Characteristics of included patients

Markers	First year		Second year	
	Area (SD) under ROC curve	p (logistic regression)	Area (SD) under ROC curve	p (logistic regression)
AST	0.773 (0.570)	0.13	0.679 (0.580)	0.35
α_2 macroglobulin	0.749 (0.570)	<0.0001	0.740 (0.460)	<0.0001
ALT	0.725 (0.570)	0.09	0.564 (0.580)	0.11
Haptoglobin (decrease)†	0.704 (0.570)	0.02	0.654 (0.580)	0.006
γ globulin	0.680 (0.570)	0.16	0.670 (0.460)	0.59
GGT	0.672 (0.570)	0.03	0.705 (0.460)	0.01
Total bilirubin	0.611 (0.570)	0.69	0.726 (0.460)	0.008
Apo A ₁ (decrease)	0.554 (0.570)	0.14	0.647 (0.580)	0.12
Albumin (decrease)	0.514 (0.570)	0.12	0.662 (0.460)	0.53
α_1 globulin	0.518 (0.570)	0.30	0.577 (0.580)	0.80
α_2 globulin (decrease)	0.508 (0.570)	0.007	0.518 (0.580)	0.03
β globulin	0.475 (0.570)	0.75	0.601 (0.580)	0.45
Logistic function‡				
10 markers	0.856 (0.430)	<0.0001	0.885 (0.340)	<0.0001
6 markers	0.836 (0.430)	<0.0001	0.870 (0.340)	<0.0001
5 markers	0.827 (0.430)	<0.0001	0.851 (0.340)	<0.0001

AST=aspartate aminotransferase. ALT=alanine aminotransferase. GGT= γ glutamyltranspeptidase. Apo=apolipoprotein. ROC=receiver-operating characteristic. *Few F2 many septa F3, cirrhosis F4. †Haptoglobin was retrospectively assessed. Values were missing for eight patients in the first period and one in the second. p value is for logistic regression when haptoglobin replaced α_2 globulin. ‡Function included age and sex.

Table 2: Diagnostic value of biochemical markers for clinically significant fibrosis*

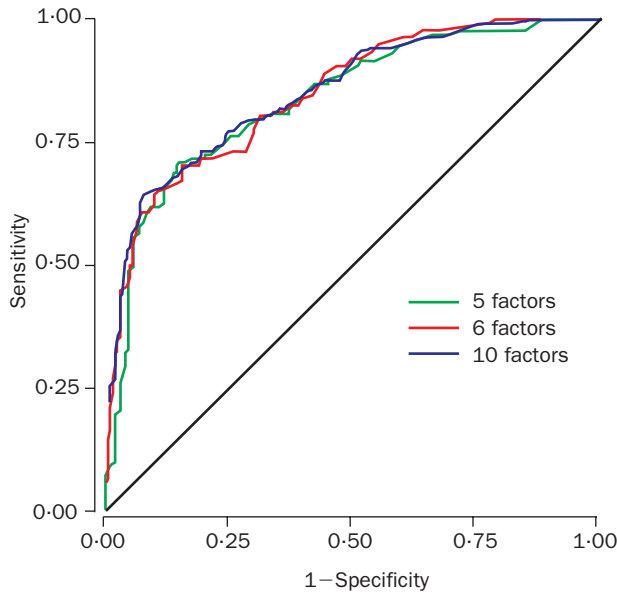


Figure 2: ROC curves of fibrosis indices combining five, six, or ten biochemical factors, and age and sex
ROC=receiver-operating characteristic.

For diagnosis of cirrhosis or widespread fibrosis, the six-marker fibrosis index had a very large area (SD) under the ROC curve, 0.923 (0.370). A high negative predictive value (>90% certainty of absence of F3 or F4) was obtained for scores from zero to 0.80. For scores greater than 0.80, there was a high positive predictive value (>85% certainty of presence of F3 or F4).

Diagnosis of necroinflammatory activity

The best diagnosis of clinically significant activity (A2 or A3) was with a logistic regression that combined ALT, α_2 macroglobulin, β globulin, γ globulin, apolipoprotein A₁, and GGT ($R^2=0.243$, $p<0.0001$). The necro-inflammatory index that combined these six markers ranged from zero to 1.00. 64 patients without activity (A0) had significantly lower mean (SD) index scores than 161 patients with mild activity (A1), 0.13 (0.13) versus 0.26 (0.20), respectively, $p<0.0001$. Both these scores were lower than in 93 patients with moderate activity (A2), 0.50 (0.24) (A0 or A1 vs A2, $p<0.0001$) and seven with severe activity (A3) 0.59 (0.17) (A3 vs A0 or A1, $p<0.0001$). The best logistic regression for diagnosis of clinically significant fibrosis (F2, F3, or F4) or substantial activity (A2 or A3) combined the same six markers as for such fibrosis alone plus ALT ($R^2=0.297$, $p<0.0001$).

Cytokines, fibrosis stages, and biochemical markers

TGF β 1 fell in association with fibrosis stage (figure 4). Stages F0, F1, and F2 had significantly higher values than F4 (Bonferroni all-pairwise multiple comparison; $p<0.0001$). TGF β 1 was positively associated with haptoglobin ($r=0.39$, $p<0.0001$) and negatively with α_2 macroglobulin ($r=-0.20$, $p=0.02$), bilirubin ($r=-0.32$, $p<0.0001$) and GGT ($r=-0.20$, $p=0.01$). Hepatocyte growth factor increased with fibrosis stage (figure 4). Stages F0 and F1 had significantly lower values than F4 ($p=0.02$). Hepatocyte growth factor was associated with α_2 macroglobulin ($r=0.45$, $p=0.006$) and GGT ($r=0.54$, $p<0.0001$). IL-10 was not correlated with fibrosis stage but only with γ globulin ($r=0.20$, $p=0.01$). α_2 macroglobulin was significantly lower at F0 and F1 than F2, F3, and F4 ($p<0.0001$) (figure 3). F2 was lower than

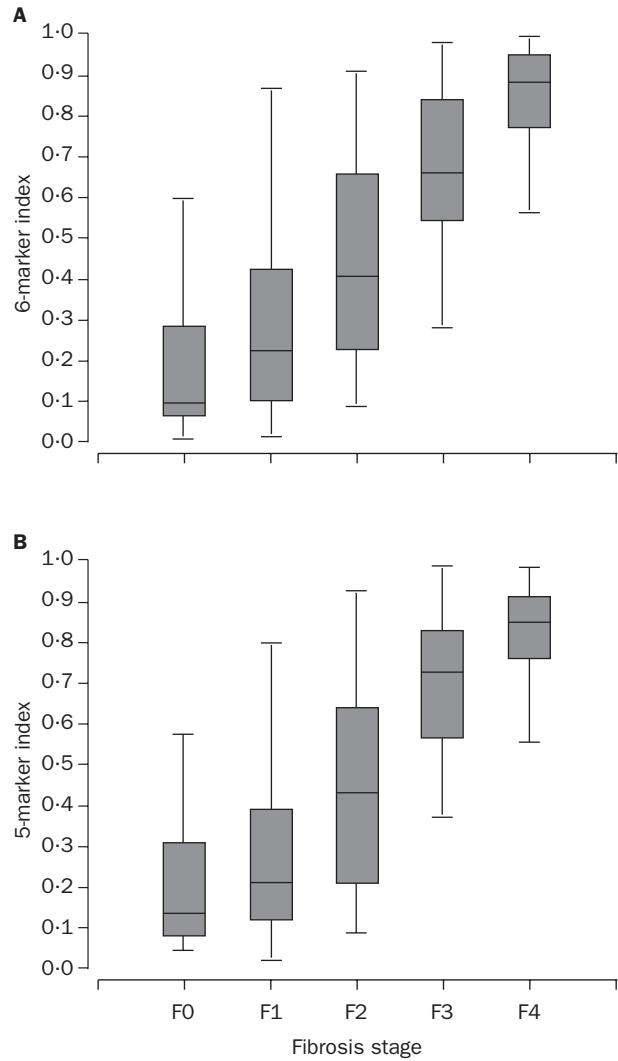


Figure 3: Fibrosis index score for each fibrosis stage
A: 6-marker index: F0 n=56; F1 n=145; F2 n=68; F3 n=28; F4 n=42.
B: 5-marker index: F0 n=55; F1 n=139; F2 n=64; F3 n=26; F4 n=41.
The top and bottom of the box are the 25th and 75th percentiles. The length of the box is thus the IQR. The line through the middle of the box is the median. The upper error bar is the largest observation ≤ 75 th percentile plus $1.5 \times IQR$. The lower error bar is the smallest observation that is ≥ 25 th percentile minus $1.5 \times IQR$. Bonferroni all-pairwise multiple comparison; $p<0.0001$ between all stages.

F3 ($p=0.0009$) and F4 ($p=0.01$). Haptoglobin was significantly higher at F1 than F2 ($p=0.0009$), F3 ($p=0.01$), and F4 ($p<0.0001$) (figure 3).

Cut-off of fibrosis score	Sensitivity	Specificity	Likelihood ratio	Predictive values for an observed prevalence=0.45*	
				Positive	Negative
0.10	1.00	0.22	1.3	0.50	1.00
0.20	0.92	0.46	1.7	0.58	0.87
0.30	0.87	0.59	2.1	0.63	0.85
0.40	0.78	0.69	2.5	0.67	0.80
0.50	0.75	0.85	5.0	0.80	0.80
0.60	0.70	0.95	12.9	0.91	0.76
0.70	0.62	0.95	15.2	0.92	0.66
0.80	0.38	0.97	14.2	0.92	0.62
0.90	0.27	0.97	9.9	0.90	0.55

*60 of 134 second-year-group patients (45%) had clinically significant fibrosis.

Table 3: Sensitivity, specificity, and predictive value of the six-marker fibrosis index—second-year group

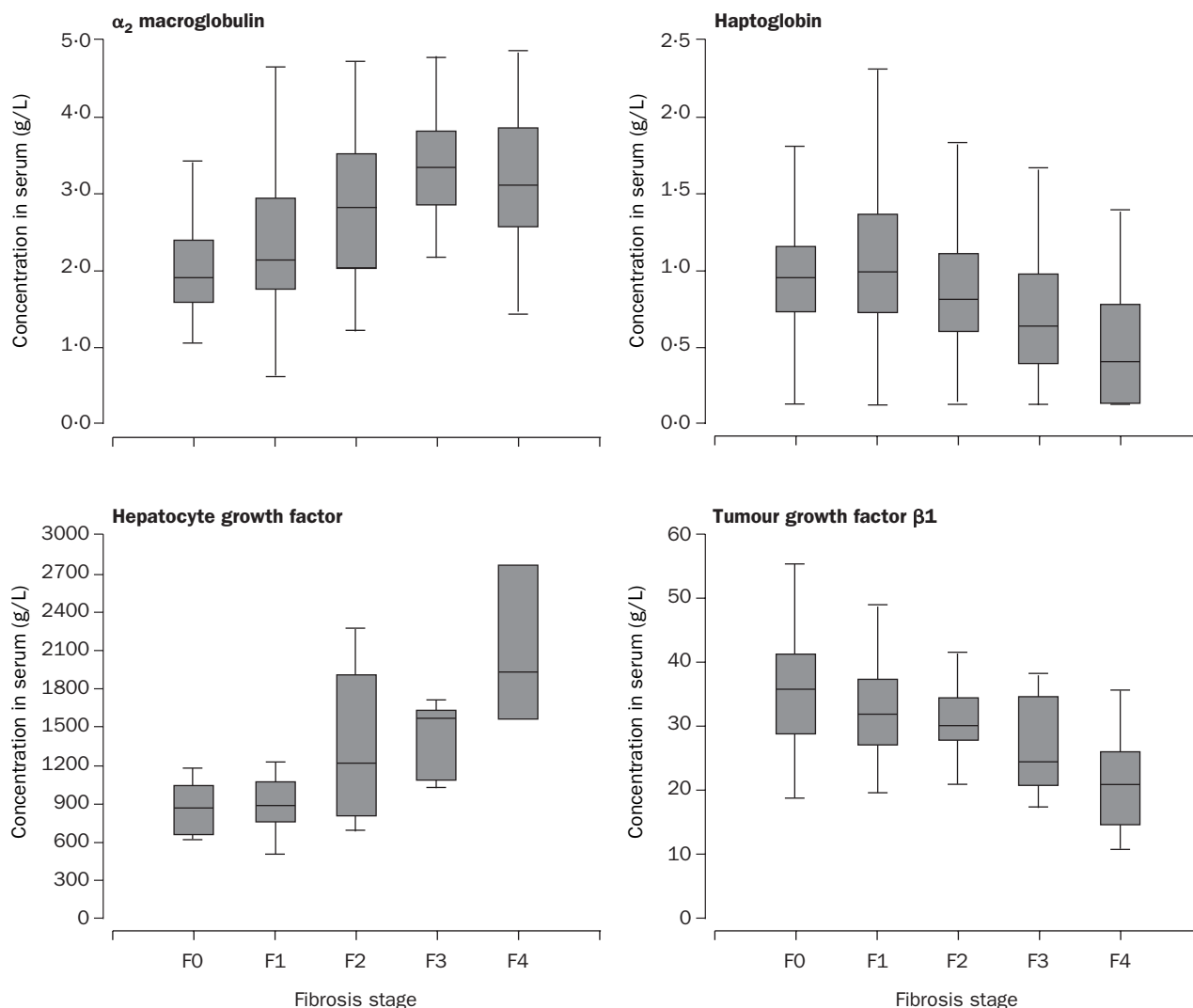


Figure 4: Relation between fibrosis stage and α_2 macroglobulin, haptoglobin, hepatocyte growth factor, and tumour growth factor $\beta 1$

Discussion

Our results show that a combination of five or six basic biochemical markers can have high positive or negative predictive value for diagnosis of clinically significant fibrosis, even at the early stage of a few septa. The most informative markers were, in decreasing rank: α_2 macroglobulin, haptoglobin, GGT, γ globulin, total bilirubin, and apolipoprotein A₁.

α_2 globulin is mainly composed of α_2 macroglobulin and haptoglobin. Fibrosis was associated with an increase of α_2 macroglobulin and a decrease of haptoglobin, which masked the diagnostic value of α_2 globulin in univariate analysis. We have previously noted a significant diagnostic value of increased α_2 macroglobulin for fibrosis staging in patients with alcoholic liver disease,⁶ which has been confirmed.⁹ α_2 macroglobulin is an acute-phase protein and is produced at sites of inflammation and liver fibrosis by hepatocytes, stellate cells, and granuloma cells.¹⁷⁻¹⁹ Moreover, α_2 macroglobulin is related to fibrosis since it is a feature of stellate cell activation.²⁰ It is also a proteinase inhibitor, and increased synthesis can inhibit catabolism of matrix proteins and enhance fibrotic processes in the liver.¹⁷⁻²⁰ Haptoglobin was strongly and negatively associated with fibrosis, as already noted;^{21,22} this association was not related to haemolysis, hypersplenism, or hepatic insufficiency. Furthermore, we did not find an

association with unconjugated bilirubin (data not shown).

These opposing correlations with fibrosis of α_2 macroglobulin (positive) and haptoglobin (negative) could be explained by the different roles of hepatocyte growth factor and TGF $\beta 1$ in fibrogenesis and acute phase response.²³⁻²⁵ As seen in experimental fibrosis, increase in hepatocyte growth factor could account for the unexpected fall in reduction in TGF $\beta 1$, rise of α_2 macroglobulin, and decrease of haptoglobin. Transduction with hepatocyte growth factor gene suppresses increase of TGF $\beta 1$,²³ and the factor stimulates synthesis of α_2 macroglobulin²⁴ and reduces synthesis of haptoglobin.^{24,25}

GGT is associated with fibrosis and has been used with prothrombin and apolipoprotein A₁ as a serum marker.^{5,6,26} Its diagnostic value in our study was independent of other factors, especially transaminases and bilirubin. GGT and bilirubin were both associated with hepatocyte growth factor. Early cholestasis or an increase of epidermal growth factor could be one explanation for the GGT increase with severity of fibrosis.²⁷

γ globulin serum concentration is associated with cirrhosis and portosystemic shunts.²⁸ In our study, although lower than in patients with cirrhosis, it was already higher in patients with non-cirrhotic fibrosis than in those with scores of F1 or F0.

Apolipoprotein A₁ serum concentration is associated with fibrosis and used with prothrombin and GGT as a serum marker.^{5,6,26} It is trapped on extracellular matrices.²⁹ Apolipoproteins, especially A₂,³⁰ also interact with HCV capsid proteins. Neither apolipoprotein A₂ nor apolipoprotein B significantly added to the diagnostic value of apolipoprotein A₁ alone. Albumin had no independent diagnostic value, probably because we had excluded patients with severe cirrhosis.

Finally, serum cytokines did not add much diagnostic value to the biochemical markers, which are easier and cheaper to measure than cytokines. Measurements of electrophoresis compounds (α_2 globulins and γ globulins) are outdated semi-quantitative assessments. Their replacement by haptoglobin in a five-marker index did not significantly alter predictive value.

Extra-cellular matrix component markers (procollagen III peptide, hyaluronate, collagens, or collagenases) have diagnostic value, but are not significantly greater than other biochemical markers or prothrombin time⁵⁻¹⁰ when measured in serum. We did not include prothrombin time or platelets because we wanted a combination of inexpensive biochemical markers, which could be easily and automatically analysed.

We are confident that our study samples are representative of most patients. Clinical, histological, and biochemical characteristics of our prospective population were stable during the 33 months of the study and similar to those for populations in recent large randomised trials.³¹ We did not include patients with obvious decompensated cirrhosis. Inclusion of patients with severe liver disease would have artificially improved the predictive values of the logistic function. On the other hand, we included patients with very slight histological features (17% without fibrosis), and 43 (13%) with ALT lower than 35 IU/mL, who would usually not be included in randomised trials.

Results were similar with logistic regression or neural connection. We preferred the logistic regression method, because with the neural connection the weight accorded to each factor is not certain. Whatever the statistical method, sampling variation poses potential difficulties, especially in early stages of disease when fibrosis might be unevenly distributed. Therefore, our biochemical markers might provide a more accurate description of fibrogenic events that occur across the whole liver. Our study was cross-sectional, and such markers should be longitudinally assessed. Use of our fibrosis score is uncertain in comorbid conditions such as renal dysfunction, excessive alcohol intake, and in other forms of fibrotic liver diseases.

Although our results need to be repeated by another centre, we think that the number of biopsies in the management of chronic HCV infection could be reduced (by up to 46% according to our result in the second-year period). Diagnostic value of our fibrosis index was highly reproducible between the two year groups. Patients are treated according to fibrosis stage and grade (1-4). If a decision not to treat were made without biopsy, with a fibrosis score of less than 0.20, only 13 of 119 patients would have been false negatives. Of these 13, none had cirrhosis or extensive fibrosis (F3 or F4) and only three had moderate activity. If a treatment decision without biopsy would have been made with a fibrosis score of greater than 0.80, only five patients of 50 would have been false positives. Of these, three had moderate activity, which justified treatment despite having only portal fibrosis.² Two of these three patients underwent a transvenous liver biopsy, which showed raised

portocaval gradients of 19 and 13 mm Hg. Therefore, these patients probably had substantial fibrosis. In this instance, two patients (4%) would have been unnecessarily treated. Therefore, our index can detect most patients who have moderate and severe histological activity but who do not have clinically significant fibrosis.

Other algorithms for fibrosis diagnosis should be compared with our fibrosis index.³² In clinical practice we plan to use this index to make decisions about treatment in patients who have contraindications to, or who refuse, liver biopsy. We also plan to prospectively test the hypothesis that use of this index, and a resulting reduction of liver biopsies, could be cost effective in management of HCV infection.

Contributors

F Imbert-Bismut and Laurence Pieroni did the biochemical assays and assessed quality control. V Ratziu and Y Benhamou recorded data and were responsible for execution of the study. F Charlotte read liver biopsies. T Poinard initiated and designed the study, analysed data, and devised the fibrosis scores. All investigators helped to write the manuscript.

MULTIVIRC group (Paris): Christine Schall (administration); Frédéric Charlotte (anatomopathologie); Bruno Riou, Pierre Coriat (anesthésie-réanimation); Bernhard Hainque, Françoise Imbert-Bismuth, Laurence Pieroni, Annie Piton, Lina Khalil (biochimie); Françoise Mercadier (Centre de Transfusion Sanguine); Alain Pavie, Richard Dorent, Iradj Gandjbakhch (chirurgie cardiaque); Olivier Deckmyn, Jean René Vidaud (computer unit); Cecile Blot, Muriel Lechevallier, Hélène Vilette (data management); Olivier Chosidow, Camille Francès (dermatologie); André Grimaldi (diabétologie); Philippe Giral, Eric Bruckert (endocrinologie); Pierre Opolon, Thierry Poinard, Yves Benhamou, Vlad Ratziu, Joseph Moussalli, Corinne Regimbeau, Vincent Di Martino, Marie Bochet, Brigitte Bernard, Julien Taïeb, Dominique Thabut, Mercedes Torres, Luminita Bonyhai, Marie Hélène Sayeg, Hélène Scapa, Marie Hélène Desailles (hépatogastroentérologie); Pascale Ghillani, Lucille Musset (immunochimie); Brigitte Autran, Patrice Debré, Ionnis Theodorou, Guy Gorochov (immunologie cellulaire); Françoise Bricaire, Martin Danis, Christine Katlama (maladies infectieuses); Patrice Cacoub, Olivier Bousquet, Serge Herson, Sophie Pelletier, Jean-Charles Piette (médecine Interne); André Aurengo, Laurence Leenhardt (médecine nucléaire); Gilbert Deray, Sylvie Mouquet (néphrologie); Jean-Marc Leger, Olivier Lyon-Caen (neurologie); Eric Antoine, David Khayat (oncologie médicale); Marie-Hélène Fievet, Alain Thuillier (pharmacie); Olivier Lucidarme, Philippe Grenier (radiologie); Pierre Bourgeois, Nathalie Wrona (rhumatologie); Laurent Hannoun, Dominique Borie, Dominique Heraut (transplantation hépatique et chirurgie); Olivier Bitker (transplantation rénale); Jean-Marie Hureau, Vincent Thibaut (virologie); Michel Vidaud, Martine Olivi, Franck Bonvoust (ESA CNRS 8067 et Faculté de Pharmacie).

Acknowledgments

This study was supported by grants (ARC5117) from Association pour la Recherche sur le Cancer.

References

- 1 European Association for the Study of the Liver. Consensus statement. EASL International Consensus Conference on Hepatitis C. *J Hepatol* 1999; **30**: 956-61.
- 2 Poinard T, Ratziu V, Benhamou Y, Di Martino VD, Bedossa P, Opolon P. Fibrosis in patients with chronic hepatitis C: detection and significance. *Semin Liver Dis* 2000; **20**: 47-55.
- 3 Cadanel JF, Rufat P, Degos F. Practices of liver biopsy in France: results of a prospective nationwide survey. *Hepatology* 2000; **32**: 477-81.
- 4 Poinard T, Ratziu V, Bedossa P. Appropriateness of liver biopsy. *Can J Gastroenterol* 2000; **14**: 543-48.
- 5 Teare JP, Sherman D, Greenfield SM, et al. Comparison of serum procollagen III peptide concentrations and PGA index for assessment of hepatic fibrosis. *Lancet* 1993; **342**: 895-98.
- 6 Naveau S, Poinard T, Benattar C, Bedossa P, Chaput JC. Alpha-2-macroglobulin and hepatic fibrosis: diagnostic interest. *Dig Dis Sci* 1994; **39**: 2426-32.
- 7 Guechot J, Laudat A, Loria A, Serfaty L, Poupon R, Giboudeau J. Diagnostic accuracy of hyaluronan and type III procollagen amino-terminal peptide serum assays as markers of liver fibrosis in chronic viral hepatitis C evaluated by ROC curve analysis. *Clin Chem* 1996; **42**: 558-63.

- 8 Poynard T, Bedossa P. Age and platelet count: a simple index for predicting the presence of histological lesions in patients with antibodies to hepatitis C virus. METAVIR and CLINIVIR Cooperative Study Groups. *J Viral Hepat* 1997; **4**: 199–208.
- 9 Oberti F, Valsesia E, Pilette C, et al. Noninvasive diagnosis of hepatic fibrosis or cirrhosis. *Gastroenterology* 1997; **113**: 1609–16.
- 10 Wong VS, Hughes V, Trull A, Wight DG, Petrik J, Alexander GJ. Serum hyaluronic acid is a useful marker of liver fibrosis in chronic hepatitis C virus infection. *J Viral Hepat* 1998; **5**: 187–92.
- 11 Poynard T, Bedossa P, Opolon P, for the OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet* 1997; **349**: 825–32.
- 12 Dumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta* 1971; **31**: 87–96.
- 13 The French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. *Hepatology* 1994; **20**: 15–20.
- 14 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.
- 15 Hintze JL. NCS 97 User's Guide-1. Kaysville, UT: Number Cruncher Statistical Systems, 1997.
- 16 SPSS. Neural connection 1.0 user's guide. Chicago, IL, 1995.
- 17 Tiggelman AM, Boers W, Moorman AF, et al. Localization of alpha 2-macroglobulin protein and messenger RNA in rat liver fibrosis: evidence for the synthesis of alpha 2-macroglobulin within *Schistosoma mansoni* egg granulomas. *Hepatology* 1996; **23**: 1260–67.
- 18 Tiggelman AM, Linthorst C, Boers W, Brand HS, Chamuleau RA. Transforming growth factor-beta-induced collagen synthesis by human liver myofibroblasts is inhibited by alpha2-macroglobulin. *J Hepatol* 1997; **26**: 1220–28.
- 19 Meisse D, Renouf S, Husson A, Lavoinne A. Cell swelling increased the alpha2-macroglobulin gene expression in cultured rat hepatocytes. *FEBS Lett* 1998; **422**: 346–48.
- 20 Kawser CA, Iredale JP, Winwood PJ, Arthur MJ. Rat hepatic stellate cell expression of alpha-2-macroglobulin is a feature of cellular activation: implications for matrix remodelling in hepatic fibrosis. *Clin Sci* 1998; **95**: 179–86.
- 21 Bacq Y, Schillio Y, Brechot J-F, De Muret A, Dubois F, Metman E-H. Decrease of haptoglobin serum level in patients with chronic viral hepatitis C. *Gastroenterol Clin Biol* 1993; **17**: 364–69.
- 22 Louagie HK, Brouwer JT, Delanghe JR, De Buyzere ML, Leroux-Roels GG. Haptoglobin polymorphism and chronic hepatitis C. *J Hepatol* 1996; **25**: 10–14.
- 23 Ueki T, Kaneda Y, Tsutsui H, et al. Hepatocyte growth factor gene therapy of liver cirrhosis in rats. *Nat Med* 1999; **5**: 226–30.
- 24 Guillen MI, Gomez-Lechon MJ, Nakamura T, Castell JV. The hepatocyte growth factor regulates the synthesis of acute-phase proteins in human hepatocytes: divergent effect on interleukin-6-stimulated genes. *Hepatology* 1996; **23**: 1345–52.
- 25 Moshage H. Cytokines and the hepatic acute phase response. *J Pathol* 1997; **181**: 257–66.
- 26 Poynard T, Aubert A, Bedossa P, et al. A simple biological index for detection of alcoholic liver disease in drinkers. *Gastroenterology* 1991; **100**: 1397–402.
- 27 Edwards AM, Lucas CM, Baddams HM. Modulation of gamma-glutamyltranspeptidase in normal rat hepatocytes in culture by cell density, epidermal growth factor and agents which alter cell differentiation. *Carcinogenesis* 1987; **8**: 1837–42.
- 28 Sakumoto I, Kikuchi N, Takane T, Yoshida H, Hosaka H. Studies on the relationship between the histological changes and liver cell function or plasma proteins in hepatic diseases. *Gastroenterol Jpn* 1976; **11**: 224–36.
- 29 Paradis V, Laurent A, Mathurin P, et al. Role of liver extracellular matrix in transcriptional and post-transcriptional regulation of apolipoprotein A-I by hepatocytes. *Cell Mol Biol* 1996; **42**: 525–34.
- 30 Sabile A, Perlemuter G, Bono F, et al. Hepatitis C virus core protein binds to apolipoprotein AII and its secretion is modulated by fibrates. *Hepatology* 1999; **30**: 1064–76.
- 31 Poynard T, Marcellin P, Lee SS, et al. Randomised trial of interferon α 2b plus ribavirin for 48 weeks or for 24 weeks versus interferon α 2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. *Lancet* 1998; **352**: 1426–32.
- 32 McRosenberg W, Burt A, Becka M, Voelker M, Arthur MJ. European Liver Fibrosis Consortium. Automated assays of serum markers of liver fibrosis predict histological hepatic fibrosis. *Hepatology* 2000; **32** (abstr): 183.