Non invasive markers of liver fibrosis in hepatitis C

M. Adler[1], B. Frotscher[2], Ph. Thiry[2], Th. Gustot[1]

(1) Medico-Surgical Department of Gastroenterology and Hepatopancreatology ; (2) Department of Clinical Chemistry, Hôpital Erasme, Brussels, Belgium.

Introduction

Liver fibrosis reflects a loss of homeostasis between fibrogenesis and matrix degradation (1).

In the extracellular space, matrix degradation occurs as a consequence of the action of enzymes called matrix metalloproteinases (MMPs), themselves inhibited by tissue inhibitors (TIMP 1-4) which are protease inhibitors. Chronic liver injury, whatever the cause (alcohol, virus, non alcoholic liver disease, biliary disease,…), lead to hepatic stellate cells activation, producing a fibrogenic environment within the liver, leading to extensive fibrosis and cirrhosis, through a combination of extracellular matrix (including collagens, non collagenous glycoproteins and proteoglycans) overproduction, diminished MMP activation and inhibition of active MPPs by TIMPs.

The gold standard for the assessment of liver fibrosis is liver biopsy. However, it is an invasive procedure with a 0.3 to 0.5% risk of major complications and 0.03 to 0.1% risk of mortality (2-4). Other drawbacks include the sampling error associated with a potential underestimation of cirrhosis in 20% of the cases, particularly in the setting of macronodular cirrhosis (5), its intra or interpathologists discordance in 20 to 30 % of the cases (6) and price (220 in Belgium and 1,500 to 2,000 $ in the US) (7). Bedossa *et al*. have recently demonstrated that a length of biopsy of at least 25 mm is necessary to evaluate accurately liver fibrosis (8). There is thus a clinical need for non-invasive markers of liver fibrosis, both for the diagnosis of significant fibrosis (which is associated with a risk of cirrhosis) and to monitor the effects of treatments on fibrogenesis and fibrolysis. The rationale for these tests is that they offer the advantage of measuring liver function or use mathematical formulas that take into account liver function, often in combination with markers that are fibrosis-specific.

Fibrosis assessment using non invasive markers

Non-invasive surrogate markers of liver fibrosis are direct or indirect.

Among the direct markers of fibrosis, extracellular matrix molecules such as hyaluronic acid (a proteoglycan produced by hepatic stellate cells and degraded by sinusoidal endothelial cells) or degrading enzymes of extracellular matrix proteins including MMPs and their inhibitors (TIMPs) have been shown to be useful and practical (9-11). However, their diagnostic performance varies greatly from one study to another with a wide range of sensitivity (58 to 94% for procollagen III propeptide, 74 to 83% for MMP2, 79 to 86% for hyaluronic acid) and specificity (58 to 95% for procollagen III propeptide, 96 to 100% for MMP2 and 86 to 89% for hyaluronic acid). Patel *et al.* (12) suggest that a combined score including hyaluronic acid, TIMP-1 and α2-macroglobulin is able to differentiate hepatitis C patients with minimal fibrosis from those with significant fibrosis with a 82% diagnostic performance

Indirect markers of fibrosis, although more indicative of liver dysfunction than reflecting fibrogenesis or fibrolysis, are reasonable cheap, reproducible, easily obtained and have been validated for the non-invasive staging of fibrosis.

Prothrombin time considered alone has a reasonable good (86%) diagnostic performance for the diagnosis of cirrhosis with a cut-off value of 80% (13). It has been suggested (14) that ALAT above normal value has good predictive value for > F1 fibrosis in the setting of chronic hepatitis C. However, normal values for transaminases vary from one laboratory to another and the so-called standardization of transaminases using the upper normal limit given by laboratories is hazardous (15). Transaminases values depend on age, sex and BMI (16).

Correlations between age and platelet count and fibrotic severity has been observed in France (17,18) and US investigators (19) have confirmed that platelet count in combination with AST/ALT ratio can be used to predict severe hepatic fibrosis in hepatitis C patients who have no history of excessive alcohol intake.

Personal experience (20) based on the comparison between patients with cirrhosis and non-cirrhotic liver disease reveal that the 3 best biochemical parameters are, in decrescent order, cholinesterase, prealbumin and prothrombin time. The respective receiver operating characteristic (ROC) curves, optimal cut-off values, specificity, sensitivity and efficiency in comparison with other tests, including liver function tests such as the aminopyrine breath test and the monoethylglycinexylidide (MEGX) test are depicted in Table I.

Address for correspondence : M. Adler, Service Médico-Chirurgical de Gastro-Entérologie et d'Hépato-Pancréatologie, Hôpital Erasme, 808 route de Lennik, 1070 Brussels, Belgium. E-mail : madler@ulb.ac.be.

Table I. — **Univariate analysis of several biochemical and functional indicators of liver cirrhosis**

Test	X^2	*ROC AUC*	*Cut-off value*	*Spec. %*	*Sens. %*	*Effic.*
Cholinesterase IU/l	59.5	0.93	3.7	95.7	81.5	88.6
Prealbumin mg/dl	59.0	0.93	14.0	82.6	87.7	85.2
Prothrombin time %	58.2	0.92	75.0	82.6	92.3	87.5
Bile acids µmoles/l	58.1	0.92	21.1	89.1	84.6	86.0
Aminopyrine breath test %	54.9	0.91	3.7	80.5	90.7	85.6
Bilirubin mg/dl	45.9	0.87	1.2	87.0	77.0	82.0
Augmented partial thromboplastin time %	44.0	0.87	31.0	71.7	87.6	79.7
Albumin g/dl	39.9	0.85	3.9	80.0	78.5	79.5
Procollagen III peptide U/ml	33.3	0.82	1.4	82.6	70.8	76.7
Platelets 10 E3/mm^3	32.7	0.79	155	89.1	72.3	80.7
Laminin U/ml	29.8	0.80	2.2	67.4	92.3	79.9
MEGX ng/ml	28.3	0.78	37.0	71.7	81.5	76.6
Gamma globulin g/dl	29.1	0.80	1.5	91.3	61.5	76.4
Hemoglobin g/dl	26.9	0.77	91%	71.7	80.0	75.0
Angiotensin conversion enzyme pg/ml	21.7	0.76	50.0	80.4	67.6	74.0

X^2 = Wilcoxon chi-square, ROC AUC = area under the curve of the ROC curve, Spec. = specificity, Sens. = sensitivity, Effic. = efficiency.

Combination of markers into a score seems to increase their diagnostic performance. The value of several scores for the diagnosis of significant fibrosis in hepatitis C is depicted in Table II. Indices such as age, aminotransferases, platelet count, cholesterol (7,17,21, 22) used in combination have an area under the curve (AUC) for significant fibrosis ranging from 0.82 to 0.89 (more than 0.8 being considered as an accepted value for a useful clinical test).

External validation of the AST-Platelets Ratio Index (23) and the Forn's index (24) fail to confirm a continuous relationship between fibrosis stage and the index, this in contrary to the Fibrotest. Forn's index validated by Patel *et al.* (24) was useful in only one third of the patients. Myers (17), an investigator of the Groupe Hospitalier Pitié compared the Fibrotest with prothrombin time, platelets and the age-platelet index indicated greater predictive value of the former test for the estimation of significant hepatitis C-related fibrosis.

Most importantly these scores include biochemical tests which have poor sensitivity for detecting early fibrosis, they are not exclusively influenced by liver dysfunction and are poorly standardized between laboratories his leading to interlab variability.

A new technique for analysing profiles of serum protein N-glycans, called glycomics, has been developed by a Belgian group (25) and the glycocirrhotest distinguishes patients with compensated cirrhosis by measuring two easily detectable peaks in the DNA-sequences-based profile of serum protein N-glycans.

On the contrary, the Fibrotest, initially developed by the French group of the "Groupe Hospitalier Pitié Salpêtrière" (26) has the originality to propose indirect biochemical tests easily obtained routinely and automated using minimal equipment.

It includes bilirubin, γGT, α2 macroglobulin, apolipoprotein A1, haptoglobin, age and sex included in a binary regression model.

The Fibrotest has been mostly studied in patients with hepatitis C.

The AUC, negative and positive predictive values of the Fibrotest to estimate minimal (< F2) versus significant (≥ F2) fibrosis, together with the prevalence of F2 fibrosis and the applicability of the test in the context of hepatitis C are depicted in Table III.

We have to notice that the first 6 publications (26,27,28,17,29,30) come from the same group and the last 2 (one from Australia (31) and one from France (32)) can be considered as an external validation. In some studies internal validation has been performed, comparing a control series and a tested series. AUC ranges from 0.74 to 0.87, whereas negative and positive predictive values for the prediction of absence or presence of significant fibrosis ranges from 85 to 100% and from 70 to 91% respectively.

With the Fibrotest, liver biopsy could be avoided in about 50% of the cases.

Most of the discordant results are explained by Poynard (33) by the sampling variability of the liver biopsy. Concordance is better when the size of the liver biopsy is above 15 mm, and when at least 6 portal tracts can be analysed by the pathologist.

Apolipoprotein A1, haptoglobin and α2 macroglobulin can be measured using a single autoanalyzer, have minimal interlaboratory variability (34,35) and the measurements are not influenced by food intake (36).

Apolipoprotein A1 is synthetized by the liver and its function is to transport cholesterol. In case of liver fibrosis, its liberation is slowed and its blood concentration decreases.

Haptoglobin is synthetized by the liver. It decreases in case of liver inflammation and scar formation because its secretion is influenced by the hepatocyte growth factor which is activated by inflammation and fibrosis. This cytokine has an inverse effect on α2 macroglobulin which is produced in excess during liver necroinflammation and

Table II. — **Combined scores indicating significant liver fibrosis in hepatitis C**

	Forns (21), n = 351,125	Myers (17), n = 323	Wai (22), n = 192,72	Saadeh (7), n = 116
% ≥ F2	24, 26	41	47	29
AUC ROC	0.86, 0.81		0.82,0.88	0.84
NPV (< F2) %	96 (< 4.2)	86 (< 0.2)	86, 90 (≤ 0.5)	Sens 85% (≤ 3)
PPV (≥ F2) %	66 (> 6.9)	69 (> 7)	88, 91 (> 1.5)	Spec 100% (> 7)
Applicability %	51		50	23
	Age, chol, plt, ggt	Age, plt	AST (ULN) X100/plt	INR, ALT/AST, plt

≥ F2, < F2 = superior or equal, less than F2 fibrosis stage according to Metavir classication ; AUC ROC = area under the curve of the receiver operating curves ; NPV = negative predictive value ; PPV = positive predictive value, chol = cholesterol ; plt = platelets ; AST = aspartate aminotransferases ; INR = international normalized ratio ; ALT = alanine aminotransferase ; sens = sensitivity ; spec = specificity ; numbers in parenthesis indicate the cut-off value ; the two digits separated by a comma indicate the control and the tested series.

Table III. — **Internal validation of the Fibrotest in several series**

	Imbert – Bismut (26) n = 205, 134	Myers (27) n = 211	Poynard (28) n = 165	Myers (17) n = 323	Myers (29) n = 130	Thabut (30) n = 476	Rossi (31) n = 125	Halfan (32) n = 259
% ≥ F2	39, 44	54	29	41	44	38	40	40
AUC ROC	0.83, 0.87	0.8	0.74	0.83	0.8	0.84	0.74	0.80
NPV (< F2) %	100 (< 0.2)	87 (≤ 0.2)		87 (≤ 0.2)	93 (≤ 0.2)		85 (< 0.1)	
PPV (≥ F2) %	91 (> 0.6)	70 (> 0.8)		88 (> 0.7)	86 (> 0.6)	90 (> 0.6)	78 (> 0.6)	
Applicability %	46				55		46	

≥ F2, < F2 superior or equal, less thanF2 fibrosis stage according to Metavir classication ; AUC ROC = area under the curve of the receiver operating curves ; NPV = negative predictive value ; PPV = positive predictive value, chol = cholesterol ; plt = platelets ; AST = aspartate aminotransferases ; INR = international normalized ratio ; ALT = alanine aminotransferase; sens = sensitivity; spec = specificity; numbers in parenthesis indicate the cut-off value; the two digits separated by a comma indicate the control and the tested series.

scar formation. It induces production of collagen, being a potent protease inhibitor (37).

Bilirubin is a protein derived from degradation of red blood cells and it is cleared by the liver into bile.

Gammaglutamyl transpeptidase is synthetized by the liver. Its production is increased by hepatocyte growth factor.

The Actitest which combines the 5 markers used for the Fibrotest plus the transaminase ALT is an estimate of necroinflammatory activity (from A0 to A3) and ranges from 0 to 1.

Monitoring of fibrosis progression or regression

Non-invasive markers of fibrosis have a promising additional interest in the follow-up of the liver disease treated or not with antiviral treatment.

In a series of HCV+ ve patients treated with interferon alone (28), the Fibrotest evolution with time had a better profile compared to relapsers and non-responders, whereas no significant differences were observed for hyaluronic acid. Patel's study (38) in patients treated by mono or bi-therapy also demonstrated that hyaluronic acid cannot replace liver biopsy for the serial assessment of fibrosis.

The value of the Fibro-Acti test, as surrogate marks for liver biopsy in patients with chronic hepatitis C treated by Pegingerferon and ribavirin, both for the initial evaluation and for the follow-up has been demonstrated recently by Poynard (39). This test has to be performed outside the period of treatment as bilirubin and haptoglobin are influenced by ribavirin induced hemolysis.

Changes in antipyrine clearance and platelet count are more sensitive than conventional tests for indicating fibrotic change over time (40). Several factors limit the widespread use of quantitative liver function tests in this setting. However, a drop of the platelet count of more than 4×10^9 cells/L is indicative a worsening fibrosis.

We may conclude that biochemical scores constitute undoubtedly a breakthrough in the management of patients with chronic liver disease, particularly, at the present time, for the non-invasive estimation of the degree of fibrosis in patients with HCV +ve hepatitis. However, the actual 80-85% diagnostic performance should be improved, up to 90% and its value, whatever the cause of liver disease, in particular because lesions of multiple origin are often present in the same patient.

At this time, the Fibrotest, being simple, cheap, largely available and validated both internally and externally is an interesting complement to liver biopsy and can be recommended for patients unwilling to accept the procedure or for whom there is a contra-indication to perform it.

There is still a need to evaluate the use of this model or other refined markers of liver fibrosis in following-up disease progression, particularly in patients with high degree of fibrosis and cirrhosis whether the patient has been treated or not, obviating the necessity of repeating liver biopsies.

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