

# Noninvasive Biochemical Markers of Liver Fibrosis

Mircea Grigorescu

3<sup>rd</sup> Medical Clinic, University of Medicine and Pharmacy, Cluj-Napoca

## Abstract

The assessment of liver fibrosis provides useful information not only for diagnosis but also for therapeutic decision. Although needle biopsy of the liver is the gold standard for fibrosis assessment, it has some technical limitations and risks. This has led to the development of noninvasive biochemical markers of liver fibrosis: direct markers which reflect extracellular matrix turnover and indirect markers which reflect alterations in hepatic function. Markers associated with matrix deposition or degradation and some cytokines implied in fibrosis may be used as individual markers or as combination of markers to generate an algorithm to evaluate the stage of fibrosis. Also, fibrosis may be predicted by using indirect markers as a single routine laboratory test or multicomponent indirect fibrosis tests.

Serum markers are of great value not only in patients at risk for liver biopsy, but also as a part of the assessment of patients with chronic liver disease avoiding the invasive methods.

## Key words

Liver fibrosis – fibrosis assessment – noninvasive methods – direct markers – indirect markers

## Rezumat

Evaluarea fibrozei hepatice furnizează informații cu valoare diagnostică și pentru adoptarea deciziei terapeutice. Deși biopsia hepatică rămâne “standardul de aur” pentru evaluarea fibrozei, aceasta este grevată de limite tehnice și riscuri. Aceasta a condus la dezvoltarea de markeri biochimici neinvazivi: markeri direcți, care reflectă turnoverul matricei extracelulare și markeri indirecți, care reflectă modificări ale

funcției hepatice. Markerii asociați cu depunerea sau degradarea matricei extracelulare și unele citokine implicate în fibroză pot fi utilizați ca markeri individuali sau în combinații pentru a genera un algoritm care să poată evalua stadiul fibrozei. De asemenea, fibroza poate fi evaluată prin utilizarea markerilor indirecți, teste de rutină de laborator sau teste indirecte compozite.

Markerii serici au o valoare deosebită nu numai pentru pacienții cu risc pentru biopsia hepatică, dar de asemenea ca parte a evaluării pacienților cu boli hepatice cronice, evitând utilizarea metodelor invazive.

## Introduction

Fibrosis is a nonspecific response to injuries which implies the synthesis of an extracellular matrix (ECM). ECM represents a group of macromolecules, including collagens, non-collagen-glycoproteins, matrix bound growth factors, glycosaminoglycans, proteoglycans and matrix proteins (1).

In the fibrotic liver there are quantitative and qualitative ECM changes. The total collagen content in the liver increases 3-10 fold and qualitative changes include: an increase in fibril-forming collagens (type I, III, IV), non-fibril forming collagens (type IV, VI), glycoproteins (fibronectin, laminin, SPARC, osteonectin, tenascin, von Willebrand factor), proteoglycans and glycosaminoglycans (perlecan, decorin, aggrecan, lumican, fibromodulin) (2-4).

The result is the replacement of the low density basement membrane-like matrix with the high density matrix of the interstitial type, which impairs the metabolic and synthesis function of hepatocytes, hepatic stellate cells (HSC), and endothelial cells (3).

Hepatic stellate cell activation is the central event leading to hepatic fibrosis. Activation of HSC implies two steps: initiation (“preinflammatory stage”) and perpetuation which involves also several changes: proliferation, chemotaxis, fibrogenesis, contractility, matrix degradation, retinoid loss, WBC chemoattractants and cytokine release (1,3,5).

The presence of specific cytokines, chemokines or other biologic active mediators is required for each step.

ECM is an active tissue, implying not only matrix synthesis, but also matrix degradation in a dynamic process which lead to the ECM remodelling. Thus, although fibrosis in the liver may be a progressive process leading to cirrhosis, fibrosis is also a potentially reversible process, at least in early stages (6-8).

The assessment of fibrosis in hepatic diseases provides much information and has a high value, not only for the diagnosis and prognosis of disease, but also for the therapeutic decision and for the monitoring of the natural history or the evolution under treatment.

The clinician has many possibilities, with unequal value for evaluating fibrosis (Table I).

**Table I** Methods to assess liver fibrosis

<b>Invasive methods</b>
Classical histological evaluation
Semiquantitative scoring systems
Computer-assisted interactive quantitative analysis
Immunohistochemical studies
<b>Noninvasive methods</b>
Serological assays
- indirect markers
- direct markers
- cytokine profile
Imaging methods
- ultrasonography
- computed tomography
- magnetic resonance imaging
- elastography
Genetic studies

Despite the impressive development of potential diagnostic tests for fibrosis assessment, needle biopsy of the liver remains the gold standard and provides much useful information. Unfortunately, liver biopsy has some limitations, including sample errors and it is only about 80% accurate in fibrosis staging: it may miss advanced fibrosis in 30% of patients (9-12) (Table II).

**Table II** Needle biopsy of the liver - value and limitations

Value	Limitations
Gold standard for diagnosis	Potential complications, including mortality
- confirmatory diagnostic value	Significant sampling errors
- etiological suggestion	High cost
- differential diagnosis	Subjective nature of the analysis with intra and interobserver variation
Assessment of grade and stage	
Therapeutic decision (eligibility)	
Evaluation of treatment efficacy	
Valuable standard for subsequent comparison in follow-up of untreated and treated patients	

There are several technical requirements for fibrosis staging which include an adequately sized biopsy, a non-fragmented sample, perfect histological techniques, and an impeccable connective tissue stain (11).

The size of sample is essential for diagnosis: 20 mm long, 14 mm wide, containing 11 to 15 complete portal tract (the concept of *minimum number of complete portal tracts*) (12).

Immunohistochemical analysis of ECM provides information in monitoring the stage of the fibrotic process: tenascin deposition is a marker of active fibrogenesis and of an early event, while vitronectin is a marker of mature fibrotic tissue which has a lower chance of regression (13).

The limitations of liver biopsy have led to the identification of alternative possibilities to assess liver fibrosis, mainly by noninvasive methods.

This lack of standard laboratory serum analysis, imaging methods or virologic assays which can distinguish patients who are at risk of progressive fibrosis has led to the identification of reliable markers of liver fibrosis which make possible not only the diagnosis of fibrosis but also the assessment of fibrosis progression.

This approach would have a real contribution for at least four reasons:

- the assessment of actual stage of fibrosis has a prognostic value for the likelihood of response to antiviral treatment, since advanced fibrosis (F3/F4 Metavir) has a lower response rate;

- the noninvasive estimation of fibrosis stage could be a good indicator for the decision to start or to delay the antiviral treatment;

- by taking into account the factors which interfere with the rate of progression of fibrosis, the clinician could predict the time to development of cirrhosis;

- the iterative determination of biological markers could be an easy, noninvasive and nonexpensive method to monitor the progression of fibrosis in the natural evolution or the regression under treatment (14).

The great majority of studies have investigated the diagnostic value of serum markers of liver fibrosis. The requirements for an ideal marker are shown in Table III.

**Table III** Characteristics of an ideal marker of liver fibrosis (15-17)

Liver specific
Noninvasive
Easy to perform
Measurable by sensitive, reproducible and fast methodology
Serum levels independent of alterations in liver, renal or reticuloendothelial function
Capacity to reflect one or more of the following processes:
- stage of fibrosis;
- activity of matrix deposition;
- activity of matrix removal.
Possibility to follow the progression or regression of fibrosis in natural evolution or under treatment

From the practical point of view, the aim of biochemical noninvasive investigation is to discriminate between

patients with no or insignificant fibrosis (F0 to F1 Metavir) and those with significant fibrosis (F2 to F4 Metavir) without requiring liver biopsy.

The accuracy of a test is given as the area under the curve (AUC) of the receiver operator characteristic (ROC). An *ideal* marker would have an AUC of 1.0 and thus a 100% sensitivity and specificity. The great majority of proposed biochemical markers have an AUC between 0.80-0.85 and have value not for staging the disease, but rather for differentiating insignificant (F0/F1) from significant fibrosis (F2-F4 Metavir). Moreover, the indeterminate results occur mainly in patients who have F1 to F2 disease. Taking into account that the value of a biomarker is validated against the biopsy, which has an accuracy of about 80%, it is improbable that a biomarker has a better performance than liver biopsy for staging fibrosis (16, 18).

Serum markers of liver fibrosis may be divided in two categories:

- *direct markers*, which reflect ECM turnover and
- *indirect markers*, which reflect alterations in hepatic function, but not directly ECM metabolism (16, 19).

Other authors prefer the terms:

- *biomarkers*, which directly reflect the biologic process of fibrosis and can be measured and
- *surrogate markers*, which may correlate with fibrosis, but do not directly reflect the pathophysiologic events leading to fibrosis (14).

### Direct serological markers of liver fibrosis

These markers are supposed to be directly involved in the deposition and removal of ECM, i.e. in fibrogenesis and fibrolysis. They include markers of matrix metabolism as well as cytokines. Fibrosis markers can be classified according to their molecular structure (Table IV).

**Table IV** Fibrosis markers (16)

Collagens
- procollagen I C peptide (PICP)
- procollagen III N peptide (PIIINP)
- type IV collagen and its fragments (NC1 and PIVNP)
Glycoproteins and polysaccharides
- hyaluronic acid (HA)
- laminin
- tenascin
- YKL-40
Collagenases and their inhibitors
- metalloproteinases (MMPs)
- tissue inhibitors of metalloproteinases (TIMPs)
Cytokines
- TGF- $\beta$ 1
- PDGF

It is very difficult to make a clear delimitation between markers of ECM deposition and degradation. Serum levels of direct markers reflect simultaneously both processes as well as the total mass of ECM undergoing remodelling (16).

There are strong arguments for this supposition:

- the levels of direct markers are elevated in disease with rapidly progressing fibrosis severe alcoholic hepatitis or active hepatitis;
- the levels of these markers have a decreasing tendency in response to treatment of the disease, before reduction in the stage of fibrosis;
- there is an independent correlation between serum direct markers and the stage of fibrosis in chronic liver diseases (20-24).

Also, there is a good correlation between different direct fibrosis markers, suggesting that they investigate a similar process.

The proposal to assess simultaneously markers of matrix deposition and degradation by using a different combination of these markers in an attempt to evaluate the whole process of matrix remodelling has added little diagnostic accuracy (16).

The assessment of direct markers could be useful for:

- staging liver disease and for
- assessing the effect of treatment and predicting disease progression (16).

### Direct individual markers in staging liver disease

#### Markers associated with matrix deposition

Several studies have investigated the value of procollagen peptides. During synthesis of collagen, procollagen suffers an enzymatic cleavage at both the carboxy – and aminoterminal ends by two different enzymes: procollagen-C–proteinase and procollagen–N–proteinase. The peptides released into the serum: procollagen type I carboxy – terminal peptide and procollagen type III amino-terminal peptide can be used as a measure of matrix deposition (25,26).

#### *Procollagen type I carboxy terminal peptide (PICP)*

PICP has little value in the diagnosis of chronic hepatitis and is elevated in cirrhosis, quantifying disease severity or indicating the alcoholic etiology (27).

#### *Procollagen type III amino-terminal peptide (PIIINP)*

Serum levels of P III NP were extensively studied alone or in combination with different other markers and the results showed the correlation between their levels and histological stage of hepatic fibrosis in alcoholic liver disease, viral hepatitis and primary biliary cirrhosis (26-29).

When refining the methods of assessment by using two assays methods of PIIINP (for col 1-3 : collagen synthesis and for col 1: collagen degradation), some authors found a significant correlation between serum PIIINP (col 1-3 and col-1) and histological changes: fibrosis, periportal necrosis and histological activity index (30).

#### *Serum type IV collagen*

Type IV collagen is an important component of ECM. Unlike type I and III collagens, which are processed by proteolysis, type IV collagen is deposited intact in the matrix and the serum component of type IV collagen reflects matrix

degradation (31). The assay of fragments of type IV collagen in serum (carboxyterminal cross-linking domain – NC1 and aminoterminal 7S domain of procollagen type IV – PIVNP) are used most frequently in practice (32-34). Irrespective of the methods used for determination, serum levels of type IV collagen had a positive correlation with the degree of hepatic fibrosis in patients with chronic viral hepatitis, alcoholic liver disease and were sensitive indicators of the presence of cirrhosis in haemochromatosis (15,16).

In hepatitis C, a cut-off value was established for diagnosing stages greater than F2 (110 ng/ml) and for predicting stage F3 (130 ng/ml) (35).

#### *Laminin*

A major non-collagenous glycoprotein synthesized by HSC, laminin is deposited in the basement membrane of the liver and increases during fibrosis around the vessels, in the perisinusoidal spaces and the portal tract.

Serum laminin levels and pepsin-resistant fragment of laminin (laminin P1) are elevated in chronic liver diseases irrespective of etiology: viral or alcoholic and reflect an increase in perisinusoidal fibrosis (36,37).

Some studies suggest that the serum levels of laminin correlate with the severity of fibrosis and liver inflammation in chronic hepatitis C, and are superior to serum ALT in reflecting liver injury (37), particularly in cirrhosis (16). Also, these studies showed a good correlation of serum laminin with Child-Pugh's score, complications of liver cirrhosis, portal pressure and hepatic vein portal gradient (38).

#### *Hyaluronic acid*

Hyaluronic acid (HA) is a glycosaminoglycan, component of the ECM, synthesized by HSC. In normal circumstances the endothelial cells of the liver sinusoid are the site of HA uptake and degradation (39). Increased levels of HA are due to decreased hepatic removal, increased production or both.

High levels of serum HA have been detected in patients with liver diseases of different etiologies and particularly in those with cirrhosis (40-41).

Serum levels of HA have been shown to be related not only to the stage of fibrosis (41) but also to the degree of necroinflammation (31).

The assessment of both laminin and HA concentration has a good prognostic value for complications of liver cirrhosis: hepatic encephalopathy stage III and IV, refractory ascites, portal vein thrombosis (38).

Serum HA at a level of < 60 mg/l excludes vein significant fibrosis or cirrhosis with a positive predictive value (PPV) of 93% and 99% respectively and has an important role in identification of early fibrosis, thus reducing the need for biopsy in this subgroup of patients (24).

At the cut-off value of 85 mg/l serum HA had a sensibility of 64.5% and specificity of 91.2% for discriminating patients with extensive liver fibrosis from those with no or mild fibrosis. At the cut-off value of 110mg/l the sensitivity was 79.2% and specificity 89.4% for discriminating patients with from those without liver cirrhosis (42).

It appears that as an isolated marker, HA is the most useful diagnostic tool for both staging and grading in patients with chronic C virus infection (31).

#### *YKL-40 (chondrex)*

YKL-40 is a mammalian member of a chitinase family (18-glycosylhydrolases). YKL-40 is produced in a wide variety of cell types and especially in cells located in tissues with increased remodelling/degradation or inflammation of the ECM. The cellular source in the liver is supposed to be activated HSC (43). Its physiological function is unknown, but YKL-40 may contribute to tissue remodelling, acts as a growth factor for fibroblasts, acts synergistically with insulin-like growth factor, as a chemoattractant for endothelial cells and has a role in angiogenesis (43-44). In liver diseases, serum levels of YKL-40 were closely related to the degree of fibrosis histologically documented, the highest values being found in severe fibrosis (43).

In chronic HCV infection, serum levels greater than 284.8 ng/ml predict cirrhosis with a sensitivity of 80% and specificity of 71% and have a negative predictive value (NPV) of 78% (45). Unlike PIVNP and HA, serum YKL-40 is significantly elevated in the subset of alcoholic cirrhotic patients who have also alcoholic hepatitis and is the best of these serological markers in discriminating between patients with mild fibrosis and those with no fibrosis (43,46).

#### **Markers associated with matrix degradation**

Products of matrix degradation result from the activity of a family of enzymes: matrix metalloproteinases (MMPs).

Synthesized intracellularly and secreted as pro-enzymes, MMPs are activated by a proteolytic cleavage by membrane-type matrix metalloproteinase 1 (MT1-MMP) or plasmin and inhibited by binding to specific tissue inhibitors of metalloproteinase (TIMPs).

Considering their substrate specificity there are five categories of MMPs: interstitial collagenases (MMP-1, -8, -13), gellatinases (MMP-2, -9 and fibroblast activation protein), stromelysins (MMP-3, -7, -10, -11), membrane type (MMP-14, -15, -16, -17, -24, -25) and metalloelastase (MMP-12) (6,47,48).

The MMPs and their inhibitors are involved in the control of matrix degradation (47).

In chronic liver disease, the investigations have centered on MMP2 (gellatinase or 72 kDA type IV collagenase), membrane-type metalloproteinase-1 or -2 which activate latent MMP2 and TIMP-1 and TIMP-2.

MMP-1 shows a substrate specificity for interstitial collagen type I and III, while MMP-2 has as substrate collagen type IV, V, VII, X elastin and fibronectin.

TIMPs can irreversibly bind the proenzyme or active forms of MMPs and inactivate them. Excess production of TIMPs relative to MMPs may be an important factor for progression of liver fibrosis (49).

HSC are the principal source of MMP-2 in the human liver and activation of MMP-2 require interaction with

hepatocytes. TIMP-1 is produced by HSC and hepatocytes (6,47,49).

Regarding the diagnostic value of MMP-2 and TIMP-1, one study reported that MMP-2 levels were elevated only when cirrhosis had developed, while TIMP-1 had a diagnostic value in detecting earlier stage of fibrosis (50). Also, this study revealed that TIMP-1 levels had a strong correlation with histological inflammatory scores and that MMP-2 levels had no relationship to the stage of fibrosis in the noncirrhotic liver.

Other studies established that serum levels of MMP-1 had a declining tendency with the severity of liver fibrosis and inflammation and abnormal serum MMP-1 did not appear until the patients were in the advanced stages of fibrosis (51).

However, older studies which investigated the role of TIMP-1 in patients with various liver disease, comparing TIMP-1 with PIIINP, type IV collagen, laminin P1 and the histological aspect, suggested that the serum levels of TIMP1 may be useful to estimate hepatic fibrogenesis associated with active inflammatory activity (52).

Also, the serum levels of TIMP-1 were shown to correlate positively with the degree of fibrosis and a striking increase in serum TIMP-1 levels was observed in the late stage of fibrosis, but not in the mild stage (53).

The ratio of TIMP-1/MMP-1 could be useful in the diagnosis of hepatic fibrosis (51).

### Cytokines and chemokines associated with hepatic fibrosis

#### TGF- $\beta$ 1

Transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) is a homodimeric polypeptide that is secreted in an inactive form which requires activation. It has pleiotropic effects through membrane receptors on a wide variety of cells. In hepatic pathology, TGF- $\beta$ 1 is the most important stimulus for the production of ECM by HSC (54) and it is also an inhibitor of hepatocyte growth and proliferation (55).

In the liver biopsy from patients with chronic liver disease, TGF- $\beta$ 1, mRNA levels correlate with type I collagen mRNA (56).

The value of serum TGF- $\beta$ 1 levels has some limitations related to the contamination of the sample by TGF- $\beta$  from platelets, the interference with plasmin activity in the plasma that increases the amount of TGF- $\beta$ 1 through opening LAP-TGF- $\beta$  complex, the binding of TGF- $\beta$  at the sites of injury to ECM and to vascular endothelium, the sequestration by soluble proteins and the complicated clearance of TGF- $\beta$ 1. These factors explain why plasma levels of TGF- $\beta$ 1 are unlikely to be of diagnostic value (51,56).

However, some studies showed a good correlation between serum levels of total TGF- $\beta$ , and Knodell scores (57) and also a correlation with the rate of fibrosis progression (58).

Moreover, some authors established cut-off values with prognostic significance for patients with no progression of

fibrosis and those with progressive disease. A TGF- $\beta$ 1 level below 75 ng/ml was predictive for stable disease (58).

#### PDGF

Platelet derived growth factor (PDGF) is the main stimulus of HSC proliferation and migration and is upregulated following liver injury. PDGF-BB is the main subunit with the most important role for the signalling pathway in HSC (3,59).

The serum level of PDGF-BB was found to have the highest value for assessment of hepatic fibrosis, when compared to other eight markers (51).

The correlation of individual direct markers with fibrosis and inflammation is shown in Table V.

**Table V** The correlation of direct serum markers with the histological substrate (16)

Markers	Disease	Fibrosis	Inflammation
Procollagen type I	CVH*	+	+
PIIINP	CVH	+++	+++
Type I collagen	Various liver disease	+	+
Type IV collagen	CVM	++	++
Laminin	CVM	+++	++
Hyaluronic acid	CVM	+++	+
YKL-40	CVH	+++	+

\*CVH = chronic viral hepatitis.

### Combination of direct markers

The combination of direct markers to generate an algorithm capable of evaluating the existence of fibrosis and its stage is an alternative approach. There are several studies which made use of this approach.

Oberti et al (60) studied four specific markers of fibrosis: hyaluronic acid, PIII NP, laminin and TGF- $\beta$ , together with other nonspecific markers: prothrombin index, GGT, apolipoprotein-A1 (PGA score) and  $\alpha$ 2-macroglobulin in a prospective study. The best diagnostic accuracy was found for HA (86%), followed by laminin (81%), P III NP (74%) and TGF- $\beta$ 1 (67%).

Taken together, the results of the Oberti's study did not show any diagnostic advantage of the specific over nonspecific markers of fibrosis.

In another investigation, the serum markers of fibrosis: C-terminal peptide of procollagen I, PIIINP, collagen IV and serum prolylhydroxylase were studied in cirrhotic and noncirrhotic patients (27). By stepwise logistic regression analysis and ROC curves the authors established that collagen IV and PIIINP were independently associated with cirrhosis.

One study investigated the diagnostic value of PIIINP, PIVNP, HA, MMP-1, MMP-2 and TIMP-1 in order to assess by ROC the usefulness of serum direct markers for fibrosis staging and necroinflammatory grading in chronic hepatitis C (61). The authors concluded that HA and MMP-2 were most useful in that order for diagnosing stages greater than F2, while serum HA and PIVNP for diagnosing moderate

grade. Because of the great overlap among stages and grades they did not consider that the above mentioned investigated markers can replace liver biopsy for the assessment of liver histology, but have a value for the global clinical status judgement and for prognosis.

The European Liver Fibrosis Study, conceived as an international, multicenter, cross-sectional cohort study, compared the diagnostic performance of three direct serum markers: HA, PIIINP and TIMP-1 with liver biopsy to generate a diagnostic algorithm for estimating the severity of liver fibrosis. By adopting as thresholds sensitivity greater than 90% and specificity greater than 90%, this algorithm was found to exclude significant fibrosis. Also, this algorithm can detect cirrhosis with a sensitivity greater than 90% (62).

Recently, another study investigated the diagnostic value of a combination of three markers: HA, TIMP-1 and  $\alpha 2$ -macroglobulin, with the aim of generating an algorithm able to discriminate between significant and non-significant fibrosis. Establishing cut-off values for these markers, they may reliably differentiate chronic hepatitis C patients with moderate / severe fibrosis (F2 to F4 Metavir) from those with no or mild fibrosis (F0 to F1 Metavir) (63).

In an attempt to find a better combination of markers, serum levels of PDGF-BB, TGF- $\beta$ 1, MMP-1, TIMP-1, HA, PC III, collagen IV, laminin and mRNA-TIMP-1 and mRNA-MMP-1 in peripheral blood mononuclear cells (PBMCs) were investigated in patients with chronic viral B infection. Serum levels of PDGF-BB, TIMP-1 mRNA, the ratio TIMP-1 mRNA/MMP-1 mRNA in PBMCs and serum levels of TIMP-1 and TIMP-1/MMP-1 ratio were valuable markers for fibrosis assessment. The combination of serum PDG-BB, TIMP-1 mRNA and MMP-1 mRNA in PBMCs was the best test in screening of liver fibrosis (51).

### The role of fibrosis markers in assessing treatment efficacy and predicting disease progression

The dynamic assessment of direct markers of liver fibrosis: HA, PIIINP, YKL-60 and TIMP-1 showed decreased levels in patients who achieved a sustained biochemical or virological response and a good correlation with histological findings (64-67).

The fall of the TGF- $\beta$  levels after antiviral therapy suggests that interferon has also a direct antifibrotic effect through a direct inhibition of TGF- $\beta$  expression (68).

Noninvasive markers have also a prognostic value, by predicting clinical evolution and fibrosis progression. Serum HA levels have a great predictive value and correlate with Child-Pugh's score in patients with viral C cirrhosis.

HA and PIIINP were independently predictive of disease progression in primary biliary cirrhosis (81), serum laminin levels correlate with Child-Pugh's score of liver cirrhosis irrespective of etiology (38) and elevated levels of PIIINP and YKL-40 are predictive of shorter survival in alcoholic cirrhosis (70).

High basal levels of TGF  $\beta$ 1 allow for the identification of a subset of patients with chronic hepatitis C who will have progressive liver fibrosis (58), a statement that has been documented by serial evaluations of serum TGF- $\beta$ 1.

Patients with progressive hepatic fibrosis had a parallel increase in TGF- $\beta$ , levels.

### Limitations of the serum direct markers of liver fibrosis

Using either a single marker or a combination of tests, direct markers have some limitations:

- they reflect the rate of matrix turnover, not only deposition, and have the tendency to be more elevated when there is an associated high inflammatory activity. As a consequence, extensive matrix deposition might not be detected in the presence of minimal inflammation;
- they are not liver-specific and their serum levels may be elevated in the presence of concomitant sites of inflammation;
- serum levels of markers depend on clearance rates, which are influenced by dysfunction of endothelial cells, impaired biliary excretion or renal function (14).

### Indirect markers of liver fibrosis

Liver fibrosis may be predicted by using a single routine laboratory test that reflects alteration in hepatic function, or a combination of such tests.

#### Individual serum indirect markers of fibrosis

##### Serum ALT levels

Although serum ALT levels generally reflect liver injury, the correlation between ALT levels and necroinflammatory and fibrosis score is poor, especially in chronic viral C infection. However, an extensive study established that ALT levels had a good sensitivity and specificity for the prediction of histologic substrate (Table VI) (71).

**Table VI** Sensitivity and specificity of ALT for the prediction of a histologic score greater than A1F1

	ALT values				
	< N	N-1.5N	1.5N-2N	2N-3N	>3N
Sensitivity (%)	100-98	98-93	93-80	80-54	94-0
Specificity (%)	0-34	34-48	48-65	65-87	87-100

ROC analysis showed that the best theoretical ALT threshold with the best histologic predictive value is 2.25 times the upper limit of normal, but it implies the overlooking of 28% of patients with a histologic score greater than A1F1 Metavir. At the same time, among patients with persistently normal ALT levels, about 26% have a histologic score greater than A1F1, and a liver biopsy must be taken into consideration (71).

##### AST / ALT ratio

Assay of AST levels had a stronger correlation than ALT with hepatic fibrosis (72). The increase in ALT levels is

related to mitochondrial dysfunction and to reduced clearance of AST by hepatic sinusoidal cells. Reversal of the AST / ALT ratio was reported in patients who progress from chronic hepatitis to liver cirrhosis and the AST/ALT ratio of more than 1 had a good predictive value for advanced fibrosis or cirrhosis (73). A good correlation with Child–Pugh’s score, MELD score and monoethylglycinexylidide (MEGX) was found.

The AST/ALT ratio had also a predictive value. An AST/ALT ratio greater than 1.16 had 81.3% sensitivity and 55.3% specificity in identifying cirrhotic patients who died within 1 year of follow-up (73).

*Platelet count (PLT)*

Trombocytopenia is a valuable marker of advanced liver disease, but it may be related to many mechanisms: hypersplenism, myelosuppression by HCV, decreased thrombopoietin production, autoimmune process (74).

Combined assessment of the AST/ALT ratio and PLT had a high diagnostic value for cirrhosis (Table VII) (73).

**Table VII** Sensitivity, specificity, PPV and NPV of an AST/ALT rate of 1 and platelet count < 130 X 10<sup>3</sup> in the diagnosis of cirrhosis

Variable	Sensitivity(%)	Specificity(%)	PPV	NPV
AST/ALT>1	77.8	96.9	99.3	88.7
PLT<130x10 <sup>3</sup>	91.1	88.3	81.2	94.7
AST/ALT >1 or PLT <130x10 <sup>3</sup> /mm <sup>3</sup>	96.7	86.4	79.8	97.9
AST/ALT>1 and PLT< 130x10 <sup>3</sup> /mm <sup>3</sup>	72.2	98.8	97.0	86.5

*Prothrombin index*

Prothrombin time as an index that reflects the synthesis capacity of the liver is one of the earliest indicators of liver cirrhosis (75). In the HALT-C study, a multivariate logistic regression model that comprised prothrombin time, PLT, AST/ALT ratio and alkaline phosphatase was predictive of cirrhosis. In another study, prolonged prothrombin time correlated with the presence and size of esophageal varices (76). Prothrombin time is also a part of different composite indexes.

**Multicomponent indirect fibrosis tests**

In order to improve the diagnostic value of different laboratory tests, several combinations of indirect tests have been developed (Table VIII).

Two other studies are reported. Fortunato et al (83) combined the determination of pseudocholinesterase, fibronectin, prothrombin, ALT, N-acetyl-b-glucosaminidase, manganese superoxide dismutase and obtained a correct classification of cirrhosis in 81% of cases.

Sud et al made use of another combination: age, AST, HOMA-IR, cholesterol levels and past alcohol use and revealed an AUC of 0.77 which can identify at least Shener stage 2 (84).

With the aim of limiting the need for liver biopsy in patients with chronic hepatitis C, the MULTIVIRC group developed a panel of biochemical markers that combines six

markers: α2-macroglobulin, haptoglobin, GGT, total bilirubin, apolipoprotein A1 and ALT with the patient’s age and gender to generate a measure of fibrosis stage (FibroTest) and of necroinflammatory grade (ActiTest) of the liver (82).

**Table VIII** Multicomponent indirect serologic markers for liver fibrosis (16,17)

Study	Serological teste	Sensitivity(%)	Specificity(%)
APRI (90)	AST /platelet count	89	75
PGA (91, 92)	Protrombin index	91	81
PGAA (93)	GGT, apolipoprotein A1	79	89
Forns (94)	Protrombin index, GGT, apolipoprotein A1 α2-macroglobulin	94	51
FibroTest	Age, platelet count, GGT, cholestered levels	94	51
ActiTest (95)	Age, sex, ALT, GGT, bilirubin, 75 apolipoprotein A1, α2-macroglobulin, haptoglobin	75	85

The choice of these markers was justified by their significance in liver disease. α2-macroglobulin is an acute phase protein, that is a feature of HSC activation and as a consequence is related to hepatic fibrosis (85). It is also a proteinase inhibitor that can inhibit catabolism of matrix proteins, enhancing fibrotic process. Haptoglobin is negatively associated with fibrosis (86). The complex role of hepatocyte growth factor and TGF-β1 on the synthesis of these two markers explains the different behaviour of these proteins (101). GGT is associated with fibrosis and early cholestasis and an increase of epidermal growth factor may be the cause of increased GGT levels, parallel with the stage of fibrosis (87). Apolipoprotein A1 is trapped in extracellular matrix and decreases in liver fibrosis (88).

The test has been adopted in many countries including the USA (as Fibrosure).

Extensive studies have established the reference ranges of variables in healthy blood donors (104) as well as the absence of significant intra-individual variation of biochemical markers (89). Also the inter-laboratory variability of biochemical markers used for fibrosis or activity assessment had acceptable limits, without clinical consequences for the prediction of the stage of fibrosis and grade of activity (90). The essential requirement in obtaining comparable results between different centers is to make use of the same reagent kits and analyzers and to apply the quality charter which is available at [www.biopredictive.com](http://www.biopredictive.com) (91).

By using logistic regression, neural connection and ROC curves, the authors generated a numerical index that combines the above mentioned markers that give the measure of fibrosis stage and necroinflammatory grade (82).

FT-AT is conceived as a continuous linear biochemical assessment that provides a numerical quantitative estimate of liver fibrosis ranging from 0.00 to 1.0, corresponding to the Metavir scoring system or other semiquantitative scoring system (Table IX).

**Table IX** Conversion between FibroTest and fibrosis stage (92)

FibroTest	Fibrosis stage estimate		Ishak
	METAVIR	Knodell	
0.75-1.0	F4	F4	F6
0.73-0.74	F3-F4	F3-F4	F5
0.59-0.72	F3	F3	F4
0.49-0.58	F2	F1-F3	F3
0.32-0.48	F1-F2	F1-F3	F2-F3
0.28-0.31	F1	F1	F2
0.22-0.27	F0-F1	F0-F1	F1
0.00-0.21	F0	F0	F0

Selecting cut-off values for fibrosis score, the diagnostic value of FT was established (Table X).

**Table X** Integrated database with predictive values for significant hepatic fibrosis according to METAVIR conversion cut-offs (92)

Cut-off used for Metavir stages conversion	Sensitivity	Specificity	NPV	PPV
0.21	0.92	0.55	0.94	0.48
0.27	0.87	0.62	0.92	0.51
0.31	0.84	0.68	0.91	0.54
0.48	0.68	0.81	0.85	0.61
0.58	0.56	0.87	0.82	0.67
0.72	0.38	0.95	0.77	0.77
0.74	0.35	0.95	0.76	0.76
0.75	0.33	0.96	0.76	0.78

The FT had the highest value in discriminating insignificant fibrosis (F0 to F1 METAVIR) from significant fibrosis (F2 to F4 METAVIR). The overall sensitivity and specificity of FT for the correct identification of patients with METAVIR 2 were 75% and 85%, respectively.

According to preliminary results, by selecting cut-off values, the authors were able to individualize three categories of patients: a group with insignificant fibrosis (F0 to F1), a second group with significant fibrosis (F2 to F4) and a third group of patients who could not be adequately characterized and in whom biopsy would be necessary. In this way, the authors concluded that by the correct identification of the fibrosis stage, the number of biopsies could be reduced by up to 46% (82).

Further studies carried out by many other investigators showed that the diagnostic value of FT for consecutive stages of fibrosis was the same for both moderate and severe stages (65,92-94).

However, other reports gave less optimistic results: 18% of the patients with FT < 0.1 unlikely to have fibrosis had significant fibrosis on liver biopsy and 21% with score of FT > 0.6 who were likely to have significant fibrosis had mild fibrosis (95). But a prospective study demonstrated that 18% of discordances were due to biopsy failure, and only 2% to FT failure (93) and also the diagnostic value of FT-AT was established by several independent groups of investigators (86-97).

In cases with discordant results, the investigators must take into consideration several situations which could modify the value of components of FT:

- haptoglobin < 0.30 g/l: hemolysis (malarial attack, medication causing hemolysis, cardiac prosthesis, genetic hemoglobin disease);
- haptoglobin > 2,0 g/l or  $\alpha$ 2-macroglobulin > 4,0 g/l: acute inflammatory processes;
- bilirubin > 17 mmol / l, mainly unconjugated: Gilbert syndrome (the FT score should be calculated with conjugated bilirubin instead of total bilirubin) ;
- increased total bilirubin and GGT: fibrosing cholestatic hepatitis, other causes of cholestasis (93).

New studies confirm the value of FT to assess fibrosis in other liver diseases: viral B hepatitis, alcoholic liver disease, nonalcoholic fatty liver disease, as a marker of portal hypertension, and as a prognostic factor for survival.

## Conclusions

Despite the development of many biochemical markers, liver biopsy remains the gold standard for fibrosis assessment. However, serum markers are particularly useful in patients at risk for liver biopsy, in situations in which access to an expert pathologist is limited, for fibrosis staging with the purpose of deciding therapy or exclude cirrhosis, in assessing fibrosis in chronic liver diseases that can be diagnosed without liver biopsy (hereditary hemochromatosis, primary biliary cirrhosis, primary sclerosing cholangitis) and in monitoring disease progression or regression (15,16,62).

Synthesizing the available data, a ponderate conclusion can be formulated: "we may be approaching a time when serum biomarkers may become an integrated part of the assessment of patients with chronic liver disease, but published evidence suggests that these markers are not yet ready for prime time" (98).

## References

1. Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem* 2000; 275: 2247-2250.
2. Rojkind M, Dunn MA. Hepatic fibrosis. *Gastroenterology* 1979; 76: 849-863.
3. Gressner AM. The cell biology of liver fibrogenesis-an imbalance of proliferation, growth arrest and apoptosis of myofibroblast. *Cell Tissue Res* 1998; 292: 447-452.
4. Schuppan D, Ruehl M, Somasundaram R, Hahn EG. Matrix as a modulator of hepatic fibrogenesis. *Semin Liver Dis* 2001; 21: 351-372.
5. Friedman SL. Liver fibrosis-from bench to bedside. *J Hepatol* 2003; 38: S38- S53.
6. Arthur MJP. Fibrosis and matrix degradation. *Digestion* 1998; 59: 376-380.
7. Bonis PAL, Friedman SL, Kaplan MM. Is liver fibrosis reversible? *N Engl J Med* 2001; 344: 452-454.
8. Arthur MJP. Reversibility of liver fibrosis and cirrhosis following treatment for hepatitis. *Gastroenterology* 2002; 122: 1525-1528.

9. Maharaj B, Maharaj RL, Leary WP et al. Sampling variability and its influence on the diagnostic yield of percutaneous needle biopsy of the liver. *Lancet* 1986; 1: 523-525.
10. Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; 38: 1449-1457.
11. Desmet VJ. Scoring chronic hepatitis. *J Hepatol* 2003; 38: 382-386.
12. Guido M, Rugge M. Liver biopsy sampling in chronic viral hepatitis. *Semin Liver Dis* 2004; 24: 89-97.
13. Koukoulis GK, Shen J, Virtanen I et al. Vitronectin in the cirrhotic liver: An immuno-marker of mature fibrosis. *Hum Pathol* 2001; 32: 1356-1362.
14. Current assesment of fibrosis. Clinical care options (hepatitis). <http://clinicaloptions.com>.
15. Fontana RJ, Lok SFA. Noninvasive monitoring of patients with chronic hepatitis. *Hepatology* 2002; 36: S57-S-64.
16. Afdhal NH, Nunes D. Evaluation of liver fibrosis: A concise review. *Am J Gastroenterol* 2004; 127:1704-1713.
17. Pinzani M, Rombouts K, Colagrande S. Fibrosis in chronic liver disease: diagnostic and management. *J Hepatol* 2005; 42: S12-S36.
18. Afdhal NH. Staging fibrosis: time to abandon liver biopsy. *Expert viewpoint* June 2005. <http://clinicaloptions.com>.
19. Leroy V, Hilleret MN. Évaluation de la fibrose hépatique. *Hepato-Gastro* 2005; 12: 251-259.
20. Ramadori G, Zohrens G, Manns M et al Serum hyaluronate and type III procollagen aminoterminal peptide concentration in chronic liver disease. Relationship to cirrhosis and disease activity. *Eur J Clin Invest* 1991; 21:323-330.
21. Pares A, Deulofeu R, Gimenez A et al. Serum hyaluronate reflects hepatic fibrogenesis in alcoholic liver disease and is useful as a marker of fibrosis. *Hepatology* 1996; 24: 1399-1403.
22. Camps I, Garcia-Granero M, Riezu-Boj JI et al. Prediction of sustained remission of chronic hepatitis C after 12-months course of alfa-interferon. *J Hepatol* 1994; 21: 4-11.
23. Guechot J, Laria A, Serfaty L et al. Serum hyaluronon as a markers of liver fibrosis in chronic hepatitis C: Effect of alpha-interferon therapy. *J Hepatol* 1995; 22:22-26.
24. McHutchinson JG, Blau LM, DeMedina M et al. Measurement of serum hyaluronic acid in patients with chronic hepatitis C and its relationship to liver histology. Consensus Interferon Study Group. *J Gastroenterol Hepatol* 2000; 15: 945-951.
25. Schupan D. Connective tissue polypeptide in serum as parameters to monitor antifibrotic treatment in hepatic fibrogenesis. *J Hepatol* 1991; 13 (suppl 3): S17-525.
26. Schupan D, Stölzel U, Oesterling C, Somasundaram R. Serum assay for liver fibrosis. *J Hepatol* 1995; 22 (suppl 2) : 82-88.
27. Fabris C, Falletti E, Federico E et al. A comparison of four markers of fibrosis in diagnosis of cirrhosis. *Ann Clin Biochem* 1997; 34: 151-155.
28. Bensen KD, Horslev-Petersen K, Junker P et al. Serum aminoterminal procollagen type III in acute viral hepatitis. A long term follow-up study. *Liver* 1987; 7: 96-105.
29. Babbs C, Hunt LP, Haboubi NY et al. Type III procollagen peptide: a marker of disease activity and prognosis in primary biliary cirrhosis. *Lancet* 1988; I: 1021-1024.
30. Walsh KM, Fletcher A, MacSween RN, Marris AJ. Comparison of assays for N-terminal propeptide of type III procollagen in chronic hepatitis C by using receiver operating characteristic analysis. *Eur J Gastroenterol Hepatol* 1999; 11:827-831.
31. Murawaki Y, Ikuta Y, Nishimura Y et al. Serum markers for connective tissue turnover in patients with chronic hepatitis B and chronic hepatitis C: a comparative analysis. *J Hepatol* 1995; 23: 145-152.
32. Shahin M, Schupan D, Walherr R et al. Serum procollagen peptides and collagen type VI for the assessment of activity and degree of hepatic fibrosis in schistosomiasis and alcoholic liver disease. *Hepatology* 1992; 15: 637-644.
33. Hirayama C, Suzuki H, Takada A et al. Serum type IV collagen in various liver disease in comparison with serum 7S collagen, laminin and type III procollagen peptide. *J Gastroenterol* 1996; 31: 242-248.
34. Hayasaka A, Schupan D, Ohnishi K et al. Serum concentrations of the carboxyterminal cross-linking domain of procollagen type IV (NC1) and the aminoterminal propeptide of procollagen type III (PIIIP) in chronic liver disease. *J Hepatol* 1990; 10:17-22.
35. Murawaki Y, Koda M, Okamoto K et al. Diagnostic value of serum type IV collagen test in comparison with platelet count for predicting the fibrotic stage in patients with chronic hepatitis. *J Gastroenterol Hepatol* 2001; 16: 777-781.
36. Kropf J, Gressner AM, Negwer A. Efficacy of serum laminin measurement for diagnosis of fibrotic liver disease. *Clin Chem* 1988; 34: 2026-2030.
37. Walsh KM, Fletcher A, MacSween RNM, Morris AJ. Basement membrane peptides as markers of liver disease in chronic hepatitis C. *J Hepatol* 2000; 32: 325-330.
38. Körner T, Kropf J, Gressner AM. Serum laminin and hyaluronan in liver cirrhosis: markers of progression with high prognostic value. *J Hepatol* 1996; 25: 684-688.
39. Eriksson S, Fraser JRE, Laurent TC et al. Endothelial cells are a site of uptake and degradation of hyaluronic acid in the liver. *Exp Cell Res* 1983; 144: 223-228.
40. Engström – Laurent A, Lööf L, Nyberg A, Schröder T. Increased serum levels of hyaluronan in liver disease. *Hepatology* 1985; 5: 638-642.
41. Guéchet J, Poupon RE, Poupon R. Serum hyaluronan as a marker of liver fibrosis. *J Hepatol* 1995; 22 (suppl 2) : 103-106.
42. Guéchet J, Laudat A, Loria A et al. 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: 558-563.
43. Johansen JS, Christoffersen P, Moller S et al. Serum YKL-40 is increased in patients with hepatic fibrosis. *J Hepatol* 2000; 32:911-920.
44. Malinda KM, Ponce L, Kleinman HK et al. Gp 38 K a protein synthesized by vascular smooth muscle cells, stimulate directional migration of human umbilical vein endothelial cells. *Exp Cell Res* 1999; 250: 168-173.
45. Saitou Y, Shiraki K, Yamanaka Y et al. Noninvasive estimation of liver fibrosis and response to interferon therapy by a serum fibrogenesis marker, YKL-40, in patients with HCV-associated liver disease *World J Gastroenterol* 2005; 11: 476-481.
46. Tran A, Benzaken S, Saint-Paul MC et al. Chondrex (YKL-40), a potential new serum fibrosis marker in patients with alcoholic liver disease. *Eur J Gastroenterol Hepatol* 2000; 12: 989-993.
47. Friedman SL. Cytokines and fibrogenesis. *Semin Liver Dis* 1999; 19: 129-140.
48. Aparicio T, Lehy T. Metalloprotéinases matricielles en pathologie digestive. *Gastroentérol Clin Biol* 1999; 23: 330-341.
49. Iredale JP, Murhy G, Hembry RM et al. Human hepatic lipocytes synthesize tissue inhibitor of metalloproteinase 1. Implications

- for regulation of matrix degradation in liver. *J Clin Invest* 1992; 90: 282-287.
50. Boeker RH, Haberkorn CI, Michels D et al. Diagnostic potential of circulating TIMP-1 and MMP-2 as markers of liver fibrosis in patients with chronic hepatitis C. *Clin Chim Acta* 2002; 316: 71-81.
  51. Zhang BB, Min Cai W, Weng HL et al. Diagnostic value of platelet derived growth factor-BB, transforming growth factor- $\alpha$ 1, matrix metalloproteinase-1, and tissue inhibitor of metalloproteinase-1 in serum and peripheral blood mononuclear cells for hepatic fibrosis. *World J Gastroenterol* 2003; 2490-2496.
  52. Ueno T, Tamaki S, Sugawara H et al. Significance of serum tissue inhibitor of metalloproteinase -1 in various liver disease. *J Hepatol* 1996; 24: 177-184.
  53. Walsh KM, Timms P, Campbell S et al. Plasma levels of matrix metalloproteinase-2 (MMP-2) and tissue inhibitors of metalloproteinase-1 and-2 (TIMP-1 and TIMP-2) as noninvasive markers of liver disease in chronic hepatitis C. Comparison using ROC analysis. *Dig Dis Sci* 1999; 44: 624-630.
  54. Sasaki H, Pollard RB, Schmitt D, Suzuki F. Transforming growth factor- $\beta$  in the regulation of immune response. *Clin Immunol Immunopathol* 1992; 65:1-9.
  55. Nakamura T, Tomita Y, Hirai R et al. Inhibitory effect of transforming growth factor- $\beta$  on DNA synthesis of adult rat hepatocytes in primary culture. *Biochem Biophys Res Commun* 1985; 133:1042-1050.
  56. Breitkopf K, Lahme B, Tag CG, Gressner AM. Expression and matrix deposition of latent transforming growth factor beta binding proteins in normal and fibrotic rat liver and transdifferentiating hepatic stellate cells in culture. *Hepatology* 2001; 33: 387-396.
  57. Nellson DR, Gonzales-Peralta RP, Qian K et al. Transforming growth factor- $\beta$ 1 in chronic hepatitis. *J Viral Hepat* 1997; 4: 29-35.
  58. Kanzler S, Beumann M, Schirmacher P et al. Prediction of progressive liver fibrosis in hepatitis C infection by serum and tissue levels of transforming growth factor  $\beta$ . *J Viral Hepat* 2001; 8: 430-438.
  59. Pinzani M, Marra F. Cytokine receptors and signaling in hepatic stellate cells. *Semin Liver Dis* 2001; 21: 397-416.
  60. Oberti F, Valsesia E, Pilette C et al. Noninvasive diagnosis of hepatic fibrosis or cirrhosis. *Gastroenterology* 1997; 113: 1609-1916.
  61. Murawaki Y, Ikuta Y, Okamoto K et al. Diagnostic value of serum markers of connective tissue turnover for predicting histological staging and grading in patients with chronic hepatitis C. *J Gastroenterol* 2001; 36: 399-406.
  62. Rosenberg WMC, Voelker M, Thiel R et al. Serum markers detect the presence of liver fibrosis: a cohort study. *Gastroenterology* 2004; 127: 1704-1713.
  63. Patel K, Gordon SC, Jacobson I et al. Evaluation of a panel of non-invasive serum markers to differentiate mild from moderate-to-advanced liver fibrosis in chronic hepatitis C patients. *J Hepatol* 2004; 41: 935-942.
  64. Fabris P, Marranconi F, Bozzola L et al. Fibrogenesis markers in patients with chronic hepatitis C treated with  $\alpha$ -IFN. *J Gastroenterol* 1999; 34: 345-350.
  65. Yagura M, Murai S, Kojima H et al. Changes of liver fibrosis in chronic hepatitis C patients with no response to interferon- $\alpha$  therapy: including quantitative assesment by a morphometric method. *J Gastroenterol* 2000; 35: 105-111.
  66. Leroy V, DeTraversay C, Barnoud R et al. Changes in histological lesions and serum fibrogenesis markers in chronic hepatitis C patients non-responders to interferon alpha. *J Hepatol* 2001; 35: 120-126.
  67. Nojgaard C, Johansen JS, Kramp HB et al. Effect of antiviral therapy on markers of fibrogenesis in patients with chronic hepatitis C. *Scand J Gastroenterol* 2003; 38: 659-665.
  68. Castilla A, Poriato J, Fausto N. Transforming growth factor beta 1 and alpha in chronic liver disease. Effects of interferon alpha therapy. *N Engl J Med* 1991; 324: 933-940.
  69. Poupon RE, Balkan B, Guechot J et al. Predictive factors in ursodeoxycholic acid-treated patients with primary biliary cirrhosis. Role of serum markers of connective tissue. *Hepatology* 1994, 19: 635-640.
  70. Nojgaard C, Johansen JS, Christensen E et al. Serum levels of YKL-40 and PIINP as prognostic markers in patients with alcoholic liver disease. *J Hepatol* 2003; 39: 179-186.
  71. Pradat P, Alberti A, Poynard T et al. Predictive value of ALT levels for histologic findings in chronic hepatitis C: a European Collaborative Study. *Hepatology* 2002; 36:973-977.
  72. Gordon SC, Fang J W, Silverman AL. et al. The significance of baseline serum alanine aminotransferase on pretreatment disease characteristics and response to therapy in chronic hepatitis C. *Hepatology* 2000; 32: 400-404.
  73. Giannini E, Risso D, Botta F et al. Validity and clinical utility of the aspartate aminotransferase-alanine aminotransferase ratio in assessing disease severity and prognosis in patients with hepatitis C virus-related chronic liver disease. *Arch Intern Med* 2003; 163: 218-224.
  74. Peck-Radoslavljevic M. Hypersplenism. *Eur J Gastroenterol Hepatol* 2001; 13: 317-323.
  75. Croquet V, Vuillemin E, Ternisien C et al. Prothrombin index is an indirect marker of severe liver fibrosis. *Eur J Gastroenterol Hepatol* 2002; 14: 1133-1141.
  76. Pilette C, Oberti F, Aube C et al. Non invasive diagnosis of esophageal varices in chronic liver disease. *J Hepatol* 1999; 31: 867-873.
  77. Wai CT, Greenon JK, Fontana RJ et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; 38: 518-526.
  78. Poynard T, Aubert A, Bedossa P et al. A simple biological index for detection of alcoholic liver disease in drinkers. *Gastroenterology* 1991; 100: 1397-1402.
  79. Teare JP, Sherman D, Greenfield S M et al. Comparison of serum procollagen III peptide concentration and PGA index for assesment of hepatic fibrosis. *Lancet* 1993; 342: 895-898.
  80. Naveau S, Poynard T, Benattar C et al. Alpha-2 macroglobulin and hepatic fibrosis. Diagnostic interest. *Dig Dis Sci* 1994; 39: 2426-2432.
  81. Forn X, Ampurdanes S, Llovet J M et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; 36: 986-992.
  82. Imbert-Bismut, Ratzu V, Pieroni L et al. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: A prospective study. *Lancet* 2001; 357: 1069-1075.
  83. Fortunato G, Castaldo G, Oriani G et al. Multivariate discriminant function based on six biochemical markers in blood can predict the cirrhotic evolution of chronic hepatitis. *Clin Chem* 2001; 47: 1696-1700.
  84. Sud A, Hui JM, Farrell GC et al. Improved prediction of fibrosis

- in chronic hepatitis C using measures of insulin resistance in a probability index. *Hepatology* 2004; 39: 1239-1247.
85. Tiggelman AM, Linthorst C, Boers W et al. Transforming growth factor – beta induced collagen synthesis by human liver myofibroblasts is inhibited by alpha 2- macroglobulin. *J Hepatol* 1997; 26: 1220-1228.
  86. Bacq Y, Schillio Y, Brechot JF et al. Decrease of haptoglobin levels in patients with chronic viral hepatitis C. *Gastroenterol Clin Biol* 1993; 17:364-369.
  87. Edwards AM, Lucas CM, Boddams 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; 8:1837-1842.
  88. 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* 1996; 42: 525-534.
  89. Munteanu M, Messous D, Thabut D et al. Intra-individual fasting versus postprandial variation of biochemical markers of liver fibrosis (FibroTest) and activity (ActiTest). *Comparative Hepatol* 2004 ([www.comparative-hepatology.com/content3/1/3](http://www.comparative-hepatology.com/content3/1/3)).
  90. Halfan P, Imbert- Bismut F, Messous D et al. A prospective assessment of the inter-laboratory variability of biochemical markers of fibrosis (FibroTest) and activity (AntiTest) in patients with chronic liver disease. *Comparative Hepatol* 2002; 2: 3-7.
  91. Poynard T, Imbert–Bismut F, Ratzu V et al. FibroTest even better than liver biopsy? *Clin Chem* 2003. Electronic letter ([http:// www.clinchem.org/cgi/eletters/49/3/450](http://www.clinchem.org/cgi/eletters/49/3/450). Response: (21 March 2003).
  92. Poynard T, Imbert–Bismut F, Munteanu M 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. *Comparative Hepatol* 2004. [http:// www.comparative – hepatology. com /content 3/1/8](http://www.comparative – hepatology. com /content 3/1/8).
  93. Poynard T, Munteanu M, Imbert-Bismut F et al. Prospective analysis of discordant results between biochemical markers and biopsy in patients with chronic hepatitis C. *Clin Chem* 2004; 50:1344-1355.
  94. Poynard T, Imbert–Bismut F, Ratzin V. Serum markers of liver fibrosis. *Hepatology* 2004; 1: 25-33.
  95. Rossi E, Adams L, Prins A et al. Validation of the FibroTest biochemical markers score in assessing liver fibrosis in hepatitis C patients. *Clin Chem* 2003; 49: 450-454.
  96. Halfan P, Bourliere M, Deydier R 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. *Hepatology* 2003; 38: 188A.
  97. Le Calvez S, Thabut D, Messons D et al. FibroTest has higher predictive values than APRI for fibrosis diagnosis in patients with chronic hepatitis C. *Hepatology* 2004; 39: 862-863.
  98. Thuluvath PJ, Krok KL. Noninvasive markers of fibrosis for longitudinal assessment of fibrosis in chronic liver disease: are they ready for prime time? *Am J Gastroenterol* 2005; 100: 1981-1983.