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Non-invasive markers of fibrosis in chronic hepatitis C patients

Utilizarea markerilor non-invazivi pentru fibroză la pacienții cu hepatită cronică de tip C

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Abstract

Chronic hepatitis C has the potential to slowly progress towards the development of cirrhosis in an important number of patients infected with hepatitis C virus. Diagnosis of the stage of liver fibrosis in chronic hepatitis C is essential for making a prognosis and deciding on antiviral therapy. The most commonly used method of assessing the stage of liver fibrosis is biopsy. Although the technique is well documented and generally safe, it still suffers from important drawbacks, such as invasiveness, sampling error, interpretation variability. During the latest years, extensive research has been conducted in the development of non-invasive markers that can predict the severity of liver fibrosis. Either simple scores, like APRI or FIB-4, or more complex ones, like FibroTest, have been developed. These are calculated on indirect (aspartate aminotransferase, alanin aminotransferase, platelets, prothrombin time) or specific (hyaluronic acid, metalloproteinases) markers that are usually used in combination. The development of algorithms using several scores further improves their diagnostic performance. Although there is still need for refinement, non-invasive models for liver fibrosis show promising results.

Keywords: *liver fibrosis, non-invasive markers, chronic hepatitis C*

Introduction

Liver fibrosis in hepatitis C

Progressive fibrosis of the hepatic parenchyma leads to cirrhosis, nodule formation, altered hepatic function and risk of liver-related morbidity and mortality. Chronic viral hepatitis is among the most frequent conditions causing liver fibrosis.

Hepatitis C is a disease with various rates of progression. In general, it progresses slowly, with 30-40% of the infected patients recovering or having a benign outcome while 60-70% of the patients develop a chronic hepatitis. In about 20% of the cases of hepatitis C, the liver disease progresses to cirrhosis in 10-20 years and may be fatal in the absence of liver transplantation (1). Liver fibrosis does not progress with a constant speed, but follows an exponential evolution, with a markedly accelerated progression once it reaches F2 stage (2). There is little evidence that virologic factors, including viral load, viral genotype, and quasi-species diversity significantly affect the risk of progres-

sion of liver disease (3). It seems that host factors rather than viral factors correlate with fibrosis progression. The main risk factors for more rapid progression include: older age at time of infection, male gender, co-infection with HIV or with hepatitis B virus (1; 3). All studies show that alcohol is a very important co-factor in the progression of chronic hepatitis to cirrhosis (1). Other factors, including hepatic steatosis, schistosomal co-infection, iron overload, potentially hepatotoxic medications, and environmental contaminants, also may have important effects (1; 3).

An estimate of the current degree of fibrosis in a patient is important for several reasons. Although high grade histological activity may be associated with accelerated disease progression, the severity of chronic hepatitis C is mainly defined by the stage of fibrosis (4). Patients with stage F2 or F3 fibrosis (METAVIR scoring system) are thus the best candidates for antiviral treatment, with the highest chances of obtaining a sustained response, while advanced stages generally have an inferior response rate (4). If the degree of fibrosis is low, antiviral therapy may be less urgent, while patients with extensive fibrosis or cirrhosis at diagnosis, even if they have less chances to cure the infection, may need a „maintenance treatment" aimed at limiting disease progression and the risk of hepatocellular carcinoma (4).

Liver biopsy

Liver biopsy is an important tool in the evaluation of patients with chronic hepatitis C virus (HCV) infection as it provides a unique source of information on fibrosis and assessment of histology. The liver biopsy provides an opportunity to grade the severity of necro-inflammation and to stage the progression of fibrosis, which may then be considered in relation to the supposed duration of the disease, clinical status and biochemical abnormalities to make therapeutic decisions (1).

In the absence of counter-indications (e.g. coagulation disorders, emphysema), liver biopsy is usually performed percutaneously. In the presence of coagulation anomalies, liver tissue sample can be obtained through the jugular vein. The degree of fibrosis is described semi-quantitatively using validated scores. The most widely used system in Europe is METAVIR, which progressively replaced Knodell score, less reproducible, while in the USA, Ishak score is preferred (2). In addition to fibrosis staging scores, there is also a system for the semi-quantitative assessment of inflammation present in liver parenchima. Because of the wealth of information it provides, liver biopsy is considered the golden standard in the evaluation of liver status in HCV-infected patients.

Accuracy and reproducibility are essential in the histological assessment of disease severity in HCV infected patients; yet, needle liver biopsy has been shown to be associated with a high rate of sampling error in patients with diffuse parenchymal liver diseases (5). Sampling error may easily occur, as only a small fraction of about 15 mg is analyzed from an organ weighing 1500 g. It was found that differences of at least one stage of fibrosis between left and right lobes appear in 33% of the patients (5). In the same study, a sampling error may have led to underdiagnosis of cirrhosis in 14.5% of the patients.

In a study performed on surgical samples of livers from patients with chronic hepatitis C, Bedossa et al. (6) estimated that a correct evaluation of the extent of fibrosis using 15 mm length biopsies is achievable in only 65% of cases, while a maximum of 75% of cases can be correctly categorized by further increasing the length of the biopsy specimen up to 25 mm.

Recent standards suggest that optimal staging in chronic viral hepatitis should be performed with liver biopsy samples of 20-25 mm length and/or containing at least 11 complete portal tracts (7).

A single pass of percutaneous or transjugular liver biopsy usually provides an inadequate biopsy specimen according to these standards. However, multiple cores of transjugular liver biopsy can be obtained, in contrast to percutaneous liver biopsy, where more than one pass gives rise to increased complications (7).

Other limits of liver biopsy comprise inter-observer variability in categorizing the degree of fibrosis (8), high costs of the procedure, and its inability to assess the dynamics of the fibrosis process or the importance of individual mechanisms of fibrogenesis (2).

Non-invasive tests for liver fibrosis

Due to the limitations of liver biopsy, an intensive research effort has been recently conducted towards the development of alternative means for the evaluation of liver fibrosis. Research in this field was encouraged by the finding that fibrosis was a reversible process and by the expectation that antifibrotic therapies would be developed. There is an increased need to assess the level and evolution of fibrosis more frequently than the biopsy allows (8). This has permitted the description and validation of several non-invasive markers of fibrosis, mainly in chronic hepatitis C patients.

Single serum markers have been identified as possible indicators of fibrosis. For example, hyaluronic acid has been used alone to exclude significant fibrosis. However, most of the markers are not liver specific and can be affected by other clinical conditions, such as inflammation. Because they are not sufficiently predictive on their own, markers are used in combination in practice, in order to generate a score according to an algorithm. The score obtained is then used to give a fibrosis prediction. This approach has been shown to have a greater chance of success in discriminating minimal from significant fibrosis.

Most of the non-invasive models combining several individual markers have been developed on two groups of patients: a training group and a validation group. All candidate individual markers are measured on patients from the training group, then, after the final model is generated, its performance is assessed on the validation group.

In the development of almost all of the models, liver biopsy is used as a standard for staging of fibrosis. Usually, pre-treatment biopsy specimens achieving certain quality standards, such as a minimum length and/or a minimum of portal tracts, are required. Serum samples for the measurement of biochemical markers are obtained at the same time as or shortly before the liver biopsy.

In order to perform the statistical analysis, the desired fibrosis stage endpoints (diagnostic target) have to be chosen. A commonly used endpoint is significant fibrosis. In this case, the non-invasive model should be able to discriminate between stages F2, F3, F4 and stages F0, F1 (METAVIR system). The choice of this endpoint in the case of chronic hepatitis C patients is mainly based on the consensus recommendations to start the antiviral treatment when fibrosis becomes significant (≥F2, METAVIR) (4).

Statistically significant predictor markers are chosen by performing univariate analysis on all candidate markers tested in patients with and without the desired endpoint. Then, in order to identify independent factors associated with the presence or absence of the endpoint, a multivariate analysis is performed on significant predictor markers. A regression model is designed using the independent variables found and the diagnostic value of the equations is assessed by comparing the area under the receiver operating characteristic curves (AUROC). An ideal equation has an AUROC equal to 1, while 0.5 indicates an equation of no diagnostic value. The best cut-offs are selected from the receiver operating characteristic curves (ROC) by calculating sensitivity, specificity, positive and negative predictive values. The overall accuracy

(diagnostic accuracy) is calculated as the sum of true positives and negatives as a proportion of the total. An important characteristic of the positive and negative predictive values of a model is that they are dependent on the prevalence of the diagnostic target in the population to which the patient belongs.

There are two categories of non-invasive tests for liver fibrosis. First, methodologies related to liver imaging techniques, like ultrasound, computed tomography and magnetic resonance imaging are currently performed, especially if a diagnosis of cirrhosis is suspected. They can detect cirrhosis, but are unable to distinguish accurately between other stages of fibrosis. The second category comprises the non-invasive tests based on serum markers. These markers are usually classified as direct or indirect, according to their relationship to the process of fibrosis development (9). Direct markers are molecules that are directly involved in fibrosis physiopathology or are present in the extracellular matrix. Indirect markers are not directly involved in fibrogenesis or fibrolysis, but their serum concentration is influenced by the development of liver fibrosis.

Most **indirect markers** used in the design of non-invasive scores are simple parameters, readily available in current clinical practice: AST, ALT, cholesterol, γ-GT, bilirubin, gamma globulin, platelet count, INR. Other more sophisticated and expensive scores also include more specialized tests, such as: α2-macroglobulin, haptoglobin, apolipoprotein A1.

Platelet count decreases as liver fibrosis extends because they are sequestrated in the spleen and, on the other hand, because the low level of thrombopoietin produced by the liver.

Alpha 2-macroglobulin is a broad-spectrum inhibitor of endoproteases synthesized in the liver. Its serum levels increase with the degree of liver fibrosis (8). It can be assayed by immunonephelometry and immunoturbidimetry.

Haptoglobin is an acute phase protein whose concentration increases in inflammatory conditions. Its level decreases with increasing stages of fibrosis. It is usually assayed by immunonephelometry.

Apolipoprotein A1 is the major proteic component of high-density lipoproteins. Its levels decrease as the fibrosis progresses. It can be analyzed by immunonephelometry.

Direct biochemical markers include cytokines involved in the fibrogenetic process (TNFα, TGF-β1), components of the extracellular matrix, such as collagen, glycoproteins, proteoglycans and glycosaminoglycans, and molecules involved in the wound-healing process of the liver: metalloproteinases and tissue inhibitors of metalloproteinases.

Collagen markers include pro-collagen peptides, type I, III and IV collagen and laminin. The most extensively studied collagen marker is PIIINP, the N-terminal peptide of procollagen type III, cleaved from procollagen III during its excretion from fibroblasts. It can be analysed using immunoassays.

Hyaluronic acid is a structural glycosaminoglican present in the extracellular matrix. It has been used on its own as a single fibrosis marker or, more recently, in combination with other markers. Liver fibrosis causes the elevation of hyaluronic acid levels in serum. It can be assayed by ELISA technique.

Metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are proteins involved in the regulation of fibrogenesis and fibrolysis. The excess collagen deposition in the hepatic tissue, a characteristic of fibrosis, results from an increased collagen synthesis and a decreased collagen degradation mediated by increased TIMPs. MMPs and TIMPs are not currently assessed in routine clinical laboratories. They can be tested in an ELISA format.

The main limit of the use of cytokines and proteins of the extracellular matrix as indicators of liver fibrosis is the complexity and re-

producibility of the analytical methods implied. Further studies are needed in order to assess the utility of direct markers in the evaluation of liver fibrosis, as several studies have suggested that indirect markers, which are less expensive, have similar diagnostic accuracy to them (7).

Combinations of several markers in the calculation of scores

Since the first report (10) published in 2001 on a combination of several biochemical serum markers used in the calculation of a score which could predict the degree of fibrosis in patients with chronic hepatitis C (8), there has been a great interest in this field and an important number of other scores have been described (*Table 1*).

FibroTest was first described in 2001 for hepatitis C patients (10) and is probably the most validated non-invasive score for liver fibrosis (7). Its calculation algorithm is licensed to BioPredictive (www.biopredictive.com) and uses five serum parameters (apolipoprotein A1, γ-GT, bilirubin, haptoglobin and α2-macroglobulin), with adjustments according to age and gender. It was first tested and validated on 339 HCV monoinfected patients (205 patients in the training group and 134 in the validation group). Its calculation returns results between 0 and 1. In the original report, the AUROC for the prediction of significant fibrosis (≥F2 on Metavir) was between 0.83 and 0.87. FibroTest scores between 0 and 0.1 provided a negative predictive value of 100% for the detection of significant liver fibrosis, whereas scores from 0.6 to 1 gave a positive predictive value of more than 90% for significant fibrosis.

In a systematic review (11) published in 2007 on the diagnostic accuracy of FibroTest in chronic hepatitis C, which included 9 studies (1679 patients), FibroTest was found to perform well in the prediction of significant fibrosis, with an area under the summary ROC curves of 0.81, and had excellent utility for the identification of HCV-related cirrhosis, with an area under the curve of 0.9. The authors also found a potential publication bias for this test, but also acknowledged that those findings should be interpreted cautiously, since tests for publication bias had not been fully validated in meta-analyses of diagnostic test accuracy.

FibroTest cannot be performed in the presence of sepsis (because of high levels of haptoglobin and α2-macroglobulin), causes of elevated bilirubin, either hereditary, such as Gilbert's syndrome, or acquired, such as acute hepatitis and cholestasis. Haemolysis is also a limiting condition for the use of Fibrotest, as it lowers the levels of circulating haptoglobin.

In a cohort of 476 consecutive untreated patients (estimation group, 351 patients; validation group, 125 patients) with chronic hepatitis C, Forns et al. (12) tested and validated a score based on age, γ-GT, cholesterol and platelet count that could predict the absence of significant fibrosis in more than one third of patients with chronic HCV infection. The lower threshold (<4.2) had a negative predictive value of 96%, while the use of the higher threshold had a positive predictive value of 66%, making it less suitable for the prediction of higher stages of fibrosis.

The use of cholesterol levels in the calculation of Forns score makes the results susceptible to interferences from the presence of dyslipidemias or from cholesterol-lowering drugs.

Wai et al. tested and validated one simple model consisting of routine laboratory data to predict both significant fibrosis and cirrhosis among patients with CHC on a group of 270 patients (192 in the trending set and 78 in the validation set) (13). The new score, AST to platelet ratio index (APRI), was designed to amplify the opposing effects of liver fibrosis on AST and platelet count. Its AUROCs for the detection of significant fibrosis (Ishak ≥ F3) and cirrhosis (Ishak ≥ F5) were 0.88 and 0.94, respectively. Two cut-off points were chosen to

Table 1. Several non-invasive scores used in the diagnosis of liver fibrosis in patients with chronic hepatitis C

Score	Author, year	Age	Gender	AST	ALT	γ-GT	Total bilirubin	Urea	apoA1	Cholesterol	Haptoglobin	α_2-macroglobulin	Gamma globulin	Hyaluronic acid	TIMP	Prothrombin	Platelets	Formula
Fibrotest	Imbert-Bismut et al., 2001	X	X			X	X		X		X	X						
Forns	Forns et al., 2002	X				X				X							X	**Forns score** = 7.811 – 3.131 x ln platelets (g/L) + 0.781 x x ln γ-GT (IU/L) + 3.647 x ln age (years) – 0.014 x cholesterol
APRI	Wai et al., 2003			X													X	**APRI** = (AST (IU/L)/upper limit of normal) x 100/PLT (10^9/L)
FIBROSpect	Patel et al., 2004											X		X	X			
Fibrometer	Cales et al., 2005			X				X				X		X		X	X	**Fibrometer** = -0.007 PLT (10^9/L) - 0.049 PI (%) + 0.012 AST (UI/L) + + 0.005 α_2-macroglobulin (mg/dL) + 0.021 HA (μg/L) - 0.270 urea (mmol/L) + 0.027 age (years) + 3.718.
Hepascore	Adams et al., 2005	X	X			X	X					X		X				**Hepascore** = y/(1 + y), where y = exp (-4.185818 – 0.0249 x age + 0.7464 x gender (male = 1, female = 0) + 1.0039 x x α_2-macroglobulin + 0.0302 x HA + 0.0691 x bilirubin – 0.0012 x γ–GT
FIB-4	Sterling et al., 2006	X		X	X												X	**FIB-4** = age (year) x AST (IU/L)/[platelets (10^9/L) x ALT (IU/L)]$^{1/2}$
FibroIndex	Koda et al., 2007			X									X				X	**FibroIndex** = 1.738 – 0.064 x platelets (10^4/mm^3) + 0.005 x x AST (IU/L) + 0.463 x gamma globulin (g/dL)

AST = aspartate amino transferase, ALT = alanine amino transferase, γ-GT = gamma glutamil transpeptidase, HA = hyaluronic acid, PI = prothrombin index, PLT = platelet count, TIMP = tissue inhibitor of metalloproteinases; IU = international units

predict the absence (APRI ≤ 0.50) or presence (APRI ≥ 1.50) of significant fibrosis and two different cut-off points were chosen to predict the absence (APRI ≤ 1.00) or presence (APRI ≥ 2.00) of cirrhosis. Patients with significant fibrosis or cirrhosis could be identified with negative predictive values of 90% and 100% and positive predictive values of 91% and 65% respectively.

In a systematic review (14) on the diagnostic accuracy of the APRI for the prediction of hepatitis C-related fibrosis, which included 22 studies (4266 patients), the APRI performance was found slightly lower than initially reported. APRI accuracy was not affected by the prevalence of advanced fibrosis, or study and biopsy quality. The authors of the review concluded that the major strength of the APRI was the exclusion of significant HCV-related fibrosis. Because the APRI is based on routinely performed, inexpensive laboratory parameters, it was considered to potentially be the ideal tool because most HCV-infected patients reside in regions with limited healthcare resources (14). Different thresholds than those initially chosen for APRI were also used with promising results in HIV/HCV coinfected patients (15). Alcohol intake is a major limitation on the use of APRI in daily clinical practice as alcohol may have direct effects on AST levels and platelets (9).

FibroIndex was recently proposed by Koda et al. and combines AST levels, gamma globulin and platelet count (16). It was constructed on a test cohort of 240 patients and validated on 120 patients. The two thresholds chosen had low sensitivity and good specificity for the diagnosis of significant fibrosis.

Hepascore requires the measurement of bilirubin, γ-GT, α2-macroglobulin and hyaluronic acid levels. Its calculation formula also considers the age and gender of the patient. It only implies one cutt-off value: 0.5. In the original study (17) performed on 221 HCV infected patients, a score ≥ 0.5 had a positive predictive value of 88% for significant fibrosis (≥ F2 on Metavir), while a score < 0.5 had a negative predictive value of 95% for the absence of advanced fibrosis (≥ F3 on Metavir).

FIBROSpect combines hyaluronic acid, TIMP-1 and α2-macroglobulin. It is licensed and commercially available in the USA. The test proved to be excellent for excluding either significant fibrosis or cirrhosis (9).

Fibrometer, developed by Calès et al. (18), requires prothrombin index, platelet count, AST, urea, α2-macroglobulin and hyaluronic acid.

Other scores combining direct and indirect markers have also been described, but they have rarely been externally validated by other teams (9).

In the recent years, studies performing direct comparisons among the wide array of existing scores are more and more frequent. A prospective, independent validation of six non-invasive scores for liver fibrosis in chronic hepatitis C (MP3, FibroTest, Fibrometer, Hepascore, Forns' score and APRI) performed on 180 patients, found overall diagnostic performances very similar to those originally reported (19). When used alone, most non-invasive scores do not yield more than 75-85% accuracy in patients with chronic hepatitis C. The authors found that Fibrometer, Fibrotest, MP3, APRI, Forns' score and Hepascore had overall diagnostic performances, as determined by AUROCs for the diagnosis of ≥F2, close to each other and these were not altered by separating patients according to genotype (1 *versus* non 1), length of biopsies, and presence or absence of sinusoidal fibrosis, steatosis or intrahepatic iron load (19). For a diagnosis of ≥ F3, Fibrometer had the best performance, but its superiority was statistically significant only compared with the Forns index. However, the Forns score had not been designed to distinguish between patients with significant and advanced fibrosis (F2 *vs.* F3) (12).

Algorithms combining several scores

It has been shown that the diagnostic performance of non-invasive markers of liver fibrosis can be greatly improved by combining them in stepwise algorithms (20). Sebastiani's algorithm (20) highlights the concept of combining non-invasive scores of fibrosis to increase their diagnostic accuracy. The authors evaluated the diagnostic performance of Fibrotest, APRI and the Forns index in 190 CHC patients at the same time as LB. Then, they developed three different algorithms for the diagnosis of significant fibrosis (≥ F2 by METAVIR) in patients with elevated or normal ALT, and for the diagnosis of cirrhosis. Significant fibrosis in patients with elevated ALT was identified with high diagnostic performance (>94% accuracy), using APRI as the screening test, followed by Fibrotest in APRI non-classified cases and restricting LB to patients classified as F0-F1 by non-invasive tests. This algorithm avoided biopsy in around 50% of cases with no instances of underestimations and a 5% rate of overestimations. It was the first study in which these non-invasive markers of liver fibrosis had been combined in the attempt to improve diagnostic accuracy.

Sebastiani's sequential algorithms were developed on the basis mainly of the PPV or NPV of each marker (APRI and Fibrotest). Later, Leroy et al. (19) were the first ones to test the statistical independence of several scores, in order to propose a logical algorithm. Interestingly, some combinations including MP3 + APRI, Fibrotest + APRI and MP3 + Fibrotest gave greater results than single scores. The statistical independence can be explained by the fact that these scores do not share the same biological parameters (19). Finally, the authors proposed an algorithm using a combination of Fibrotest and APRI, which allowed the detection of significant fibrosis in chronic hepatitis C patients with elevated transaminases with more than 90% accuracy.

Leroy's and Sebastiani's algorithms have been compared on a series of 188 monoinfected HCV patients (21). Leroy's algorithm had a 98% PPV for the prediction of significant fibrosis, while Sebastiani's had a 100% NPV for the exclusion of significant fibrosis. Though the overall accuracy of both algorithms was excellent, with a slightly better performance of Leroy's, the application of Sebastiani's algorithm resulted in a greater reduction of liver biopsies (54% *vs.* 19%).

Recently, Bourlière et al. tested five different sequential algorithms on more than 450 patients. In this group of patients, the performance of Sebastiani's algorithm was similar to that reported in the original study, with an accuracy rate of 90% and no biopsy needed in 44% of cases (22). Replacing Fibrotest with HepaScore in Sebastiani's algorithm only slightly increased the number of avoided biopsies (45% *vs.* 44%).

Use of scores in clinical practice

The existence of such a great number of markers and scores considered for the prediction of the extent of liver fibrosis in chronic hepatitis C patients can be confusing for the clinician. It also suggests that none of them is free of limitations.

Some of these methods, such as APRI and Forns' index, leave many patients unclassified. Most of them are not able to identify individual stages of fibrosis. Although the detection of significant fibrosis is an important endpoint, highlighted by current recommendations for the management of chronic hepatitis C, identification of other stages may also be useful. For example, detection of mild fibrosis can help identify patients who would benefit from early changes in diet and lifestyle. Conversely, identification of severe fibrosis and cirrhosis can help identify patients who need closer surveillance of the onset of complications (23).

Leroy et al. (19) suggested that discordances between the results of non-invasive

scores and biopsy were attributable to scores' failure rather than biopsy failure, because patient classification by the serum models was not influenced by the length of biopsy specimen and diagnostic performance was improved when using scores in combination.

An important limitation of serum markers is the standardization of the analytical methods used to measure protein concentrations. Variability due to the use of several equipments, reagents and calibrators for the dosage of serum analytes, especially by immunonephelometry and immunoturbidimetry is an important limitation for biochemical non-invasive markers of fibrosis. The existence of a quality control program in the laboratory which performs the tests is crucial (8).

Another cause for the limited diagnostic accuracy of serum marker models found when they are compared to liver biopsy is that the latter is used to describe the amount of fibrosis as a categorical variable, while serum marker based scores are continuous variables. As the statute of "gold standard" attributed to liver biopsy is more and more often questioned during the recent years, the real value of non-invasive markers is still to be defined.

A less studied feature of non-invasive scores is their potential prognostic value for the evolution of the HCV-related liver disease. The 5-year prognostic value of the FibroTest for predicting cirrhosis decompensation and survival in patients with chronic HCV infection was assessed by Ngo et al. in a prospective cohort of 537 patients (24). The authors found that FibroTest was a better predictor than biopsy staging for HCV complications and for HCV-related deaths. The prognostic value of FibroTest was still significant in multivariate analyses after taking into account histology, treatment, alcohol consumption, and HIV coinfection.

It is commonly expected from non-invasive diagnostic tests for liver fibrosis to be able to fully replace liver biopsy. This expectation is simply unrealistic. Biopsy offers a great deal of information not only about the stage of fibrosis, but also about tissue architecture, presence and severity of necro-inflammation or presence of other diseases. Although it has been stated that not all that detailed information is always necessary in a given patient (23), this is only partially true. Upon the examination of a biopsy specimen, a trained specialist makes several connections and observations which are far more important that the simple return of a numerical value corresponding to the extent of inflamation and fibrosis. The result given by a pathologist is a conclusion of everything he observes in a particular specimen.

Scores based on indirect markers, such as APRI and FIB-4, which are virtually cost-free, readily available and easy to calculate, can give, if not an exact evaluation, at least some interesting information about the status of liver fibrosis. This means that simple scores may have a role in first-line assessment of liver-disease patients, although a more reliable evaluation of liver fibrosis is often necessary in the majority of patients for making therapeutic and management decisions (9).

The development of algorithms combining several scores overcomes some of their limitations and gives promising results. The example of Leroy's (19) and Sebastiani's (20) algorithms, both using the same scores (FibroTest and APRI), with different levels of reduction of the need for liver biopsy, shows that there is still room for improvement.

So, can we trust non-invasive markers for liver fibrosis? This is still a highly debated question. Since the description of the first serum score for liver fibrosis by Imbert-Bismut et al. (10), there have been many attempts to develop non-invasive tests, such that it is rather unusual to read an issue of any specialist hepatology journal, which does not describe a new serum model (8), but there is still a lot to improve. Until the discovery of more reliable, organ-specific, biochemical markers, the use of several actual models in combination is prob-

ably the way to go. Even so, the clinician has to be aware of their limitations. Nevertheless, close contact with the laboratorian has to be maintained, in order to be able to identify all the analytical interferences that can arise in a given patient.

Probably in the near future, the ability to measure disease progression, regression, and response to treatment by serial measurement of serum markers will give clinicians even more valuable information to aid management decisions.

Abbreviations

ALT = alanin amino transferase
APRI = AST to platelet ratio index
AST = aspartate amino transferase
AUROC = area under the receiver operating characteristic curves
CHC = chronic hepatitis C
HCV = hepatitis C virus
INR = International normalized ratio
MMPs = metalloproteinases
NPV = negative predictive value
PIIINP = the N-terminal peptide of procollagen type III
PPV = positive predictive value
ROC = receiver operating characteristic curve
TGF-β1 = transforming growth factor beta 1
TIMPs = tissue inhibitors of metalloproteinases
TNFα = tumor necrosis factor alpha
γ-GT = gamma glutamil transpeptidase

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