Non-invasive evaluation of hepatic fibrosis: don't count your chickens before they're hatched

M Pinzani

There is an increasing desire for non-invasive tests to assess both the stage of liver fibrosis and the rate of progression of fibrogenic chronic liver diseases and so reduce the need for repeated liver biopsies. However, higher quality non-invasive diagnostic procedures are still needed

Cost pressures and recognition of the limitations of liver biopsy have led to an increasing desire for non-invasive tests to assess both the stage of liver fibrosis and, more importantly, the rate of progression of fibrogenic chronic liver diseases (CLD). In addition, the increase in knowledge of the mechanisms regulating the fibrogenic process in CLD and the consequent knowledge of its dynamic features (that is, scarring as the net result of extracellular matrix deposition and degradation) have led to the need for diagnostic tools with higher prognostic value and flexibility for longitudinal follow up.

There are two main groups of non-invasive methodologies for the evaluation of hepatic fibrosis and its progression. The first group, defined "serum markers", is aimed at predicting fibrosis stage and, possibly, other prognostic information, using parameters measurable in serum. The second group includes methodologies derived from elaboration of parameters obtainable with the current liver imaging techniques (ultrasound, computed tomography (CT) scan, magnetic resonance) or to the innovative use of principles of physics (that is, transient elastography).

Among serum markers we can distinguish indirect and direct markers.¹ Indirect markers are based on single or algorithmic elaboration of commonly observed alterations in liver function that do not necessarily reflect extracellular matrix metabolism (that is, platelet count, aspartate aminotransferase (AST), and total cholesterol) (table 1). Assessment through indirect markers is in general easy to obtain as it is based on routine laboratory tests or tests easily available in a hospital general laboratory. The best known tests based on indirect markers include: the PGA (prothrombin index, γ -glutamyl-transpeptidase (GGT),

apolipoprotein)² and the PGAA (same as PGA plus α_2 macroglobulin)³; Bonacini index (platelet count, alanine aminotransferase (ALT)/AST ratio, international normalised ratio),⁴ Fibrotest (α_2 macroglobulin, haptoglobin, gamma globulin, apolipoprotein, bilirubin),⁵ Forns fibrosis index (age, platelet count, GGT, cholesterol),⁶ and APRI index (AST/platelet ratio).⁷

Direct markers reflect actual extracellular matrix turnover within the liver: total amount of matrix, matrix deposition, matrix removal. They are based on knowledge of the fine cellular and molecular mechanisms of fibrogenesis and they have evolved accordingly in the past decade. This approach, which is an attempt to translate the basic mechanisms into clinically useful applications, is however not simple. Indeed, the requirements for the ideal direct serum markers of liver fibrosis (that is, liver specificity, high sensitivity for fibrogenesis/fibrololysis, known half life and excretion route, easily measurable and inexpensive) are rather difficult to match and an extensive evaluation of their potential is still under investigation. Indeed, the diagnostic performance of these markers and their ability to assess the severity and progression of liver fibrosis has been disappointing, although some individual assays have been shown to be promising in detecting cirrhosis⁸⁻⁹ in alcoholic liver disease (hyaluronic acid),¹⁰ or milder fibrosis in non-alcoholic fatty liver disease.¹¹ Other markers have been reported to reflect changes in liver histology attributable to antiviral therapy.¹²⁻¹³

An alternative approach has recently been provided by the European Liver Fibrosis study, an international multicentre cohort study including more than 1000 patients with a wide range of CLD, in which the relationship between serum levels of nine surrogate markers

of liver fibrosis, fibrosis/fibrololysis, and fibrosis stage in liver biopsies scored by three expert pathologists, working together or independently, was evaluated.¹⁴ Different algorithms, obtained by discriminant analysis of a test set of samples, were then evaluated for their ability to distinguish between individual histology scores. Similar performance characteristics were found with algorithms that incorporated hyaluronic acid, collagen IV, collagen VI, laminin, aminoterminal peptide of procollagen III, tissue inhibitor of metalloproteinase 1 (TIMP-1), and matrix metalloproteinase 2 in varying combinations. Overall, analysing the fibrosis score versus the algorithm discriminant score allowed clear differentiation between "mild" fibrosis (F1-F2) and "significant" fibrosis (F3-F4). The results of this promising approach are now the subject of internal and external validation in larger cohorts of patients with different CLD before they can be proposed as validated tools for extensive clinical use. Finally, attempts have been made to verify the diagnostic efficacy of combinations of direct and indirect serum markers. In a recent study, an algorithm including hyaluronic acid, TIMP-1, and α_2 macroglobulin was shown to be effective in differentiating moderate/severe fibrosis (F2-F4) from those with no/mild fibrosis (F0-F1), although accurate delineation between stages was not possible.¹⁵

It is predictable that technological advancements in liver imaging will allow reliable and non-invasive assessment of hepatic tissue structure in the future. This possibility would allow elimination of the actual limitations related to sampling error and the need to rely on serum markers. At present, CT and MR can indicate the presence of cirrhosis with high specificity but with very low sensitivity. In addition, these approaches do not allow any delineation of fibrotic stage.¹⁶⁻¹⁸ The value of Doppler-ultrasonography (US) is still unclear. Some authors report that compensated cirrhosis could be diagnosed in 90% of cases using some Doppler-US parameters and, above all, the hepatic vein spectrum.¹⁹ Other studies report a weak correlation between Doppler-US parameters and portal hypertension.²⁰ Recently published data indicate that US allows monitoring with a sufficient degree of accuracy the transition from advanced fibrosis to cirrhosis by employing three parameters: liver surface nodularity, caudate lobe hypertrophy, and pattern of hepatic venous blood flow.²¹

In addition to imaging techniques, a non-invasive medical device based on transient elastography (FibroScan) has

Table 1 "Indirect" serological markers for evaluation of liver fibrosis with relative sensitivity and specificity, as reported by the studies

Name	Parameters	CLD	Sensitivity (%)	Specificity (%)	Study (ref)
PGA index	Prothrombin index, GGT, apolipoprotein A1	Mixed	91	81	2
PGAA index	Prothrombin index, GGT, apolipoprotein A1, α_2 macroglobulin	Alcohol	79	89	3
Bonacini index	Platelet count, ALT/AST ratio, INR	HCV	46	98	4
Fibrotest	α_2 macroglobulin, haptoglobin, γ -globulin, apolipoprotein A1, bilirubin	HCV	75	85	5
Forns fibrosis index	Age, platelet count, GGT, cholesterol	HCV	94	51	6
APRI index	AST/platelet ratio	HCV	89	75	7

CLD, chronic liver diseases; HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; APRI, AST platelet ratio index; INR, international normalised ratio; GGT, γ -glutamyl-transpeptidase.

been proposed for measurement of liver stiffness, considered as a direct consequence of the fibrotic evolution of CLD. The results of two recent studies performed in cohorts of patients with chronic hepatitis C suggest that this system has potential in detecting significant fibrosis (that is, $>F2$) or cirrhosis.^{22, 23}

Several considerations emerge from an overall critical analysis of the proposed methodologies. Firstly, the diagnostic efficacy of non-invasive methods is evaluated in terms of sensitivity (that is, the proportion of truly diseased persons, as measured by the gold standard, who are identified as diseased by the test under study), specificity (that is, the proportion of truly non-diseased persons, as measured by the gold standard, who are so identified by the diagnostic test under study), and negative and positive predictive values (that is, the probability that a person with a positive test is a true positive, or that a person with a negative test truly does not have the disease). Predictive values of a diagnostic test are determined by the sensitivity and specificity of the test, and by the prevalence of the condition for which the test is used. In addition, receiver operating characteristic and area under the receiver operating characteristic curves, commonly employed for graphic illustration of the diagnostic efficacy of a test, basically show the trade off between sensitivity and specificity (any increase in sensitivity will be accompanied by a decrease in specificity). It is therefore clear that the reliability of the test depends heavily on the gold standard and hence on the correct interpretation of liver histology in terms of grading and staging. As a paradox, the trustworthiness of non-invasive markers relies on the diagnostic procedure (that is, liver biopsy) they are supposed to replace because it is

imperfect apart from being invasive. Furthermore, even assuming that the stage of fibrosis was obtained by analysing liver biopsies matching the current guidelines²⁴ and that intra- and inter-observer variability was maximally reduced (that is not the case in the large majority of studies), qualitative descriptors of stage of fibrosis (that is, for METAVIR $F0 > F4$) are generally employed in the test as quantitative measures. In other words, a score $F4$ is used as double $F2$. This is, however, incorrect as fibrosis progression is not a linear process and the rate of progression may be subject to acceleration and deceleration that cannot be standardised. There are in addition several other possible problems related to interpretation of these results.^{1, 25}

Finally, it should be stressed that all of the proposed non-invasive methodologies perform much better in detecting the presence of advanced fibrosis and cirrhosis (that is, METAVIR $F3$ – $F4$) while they are insufficient in detecting non-significant or low-moderate fibrosis (that is, $F0$ – $F2$). Therefore, their clinical utility in the decision to treat process is limited. For example, in the case of a patient with chronic hepatitis C virus (HCV) infection, normal liver tests, and exclusion of cirrhosis obtained by one of the non-invasive methods, liver biopsy may still be necessary for treatment decisions.

The development of non-invasive methodologies implies that these tools should complement and enrich the information derived from liver histopathology, particularly in the long term dynamic assessment of patients with CLD. However, since the beginning of their history, most if not all non-invasive methods have been referred to as "surrogate" markers, thus implying that they could be cheap and/or fast substitutes for liver biopsy. New systems

are continuously proposed and, to date, studies detailing new methodologies have found easy access to top specialised journals. Consequently, it is not rare to find that they are directly used by clinicians for routine use without an appropriate phase of internal and, more importantly, external validation. In the clinical context in which execution of liver biopsy has traditionally been a key requirement for diagnosis and for deciding whether or not to start a treatment, the use of a non-invasive system rapidly becomes a dogma, sometimes with definite ethical and commercial biases. This has led to a scenario where the feelings of hepatologists towards non-invasive evaluation of hepatic fibrosis ranges from confused to very critical.

Luckily, as in the example provided by the paper by Macías and colleagues²⁶ published in the current issue of *Gut* (see page 409), clinical investigators start wondering how these systems could fit into their everyday routine and how efficient they are in particular subsets of patients. In this paper, the aim was set at a very simple level—that is, detect advanced fibrosis in HCV-human immunodeficiency virus (HIV) coinfection by employing two methods with internal and external validation (Forns index, APRI, and Bonacini index). The results of the study suggest that only a very limited percentage of these patients would benefit from exclusion of liver biopsy. The authors offer a number of possible explanations to justify the low performance of these validated methodologies in coinfecting patients: faster fibrosis progression, younger age of patients, concomitant antiretroviral treatment, etc. However, the conclusion is that application of these methods in groups of patients with special features should be even more cautious and further evaluation is needed. This is also strongly suggested by another recent and extensive evaluation of the performance of "simple" non-invasive tests for the detection of advanced fibrosis/cirrhosis in HCV related CLD performed by other authors.²⁷

In another paper published in this issue of *Gut*, Foucher and colleagues²⁸ report on the diagnostic efficacy of liver stiffness measurement for the detection of cirrhosis in patients with different types of CLD (see page 403). The results of the study indicate that detection of different cut off values of liver stiffness by transient elastography allows allocation of cirrhotic patients into different groups based on clinical manifestations. Although this method requires further evaluation, its clinical utility in the initial clinical workup of cirrhotic patients appears relevant. In this group

of patients, transient elastography, together with US, could represent a fast screening tool for prioritising further diagnostic evaluation and, if this is confirmed in longitudinal follow up studies, for allocating cirrhotic patients to a specific class of risk. However, it would be inadequate to suggest that this method could replace a complete diagnostic evaluation of this group of patients.

In conclusion, we are in indeed in a period of transition that hopefully will lead to non-invasive diagnostic procedures of higher quality. These procedures will reduce the need for repeated liver biopsies and will be employed in the dynamic longitudinal follow up of patients with CLD. In any case, before we count our chicken we should know them better.

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Correspondence to: Professor M Pinzani, Dipartimento di Medicina Interna, Università degli Studi di Firenze, Viale G B Morgagni, 85, 50134 Firenze, Italy; m.pinzani@dmf.unifi.it

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