POINT OF VIEW

Noninvasive assessment of liver fibrosis. Serum markers and transient elastography (FibroScan)

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ABSTRACT

Both the prognosis and potential treatment of chronic liver disease greatly depend on the progression of liver fibrosis, which is the ultimate outcome of chronic liver damage. Historically, liver biopsy has been instrumental in adequately assessing patients with chronic liver disease. Histological assessment allows clinicians both to obtain diagnostic information and initiate adequate therapy. However, the technique is not exempt of deleterious effects. Multiple diagnostic tests have been developed for the staging of fibrosis using noninvasive methods, most of them in the setting of chronic hepatitis C. The goal of this paper is to review available data on the staging and assessment of liver fibrosis with two methods: serum markers and transient elastography (FibroScan®).

Key words: Liver fibrosis. Liver biopsy. Transient elastography. FibroScan. Serum markers.

INTRODUCTION

Both the prognosis and potential treatment of chronic liver disease greatly depend on the progression of liver fibrosis, which represents the ultimate consequence of chronic liver damage. This is a dynamic situation where two extreme processes collide: fibrogenesis and fibrolysis (1). This entails an accumulation of collagen, as well as other proteins in the extracellular matrix, in the tissue. Progressive deposition of these substances eventually re-

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sults in disrupted liver morphology, parenchymal function impairment, and ultimately portal hypertension and its related sequels.

In the last few years progress has been made in understanding the cellular and molecular mechanisms leading to fibrogenesis, particularly regarding the relevance of inflammatory mediators, apoptosis, and the role of hepatic stellate cells. These studies have led to attempts at translating this evidence into clinical practice, and to the consequent development of biological markers allowing to properly stage liver disease.

LIVER BIOPSY

Historically, liver biopsy (LB) has been the key test for an appropriate assessment of patients with chronic liver disease. Histological assessment allows the clinician both prognostic information and adequate treatment (2). Traditionally, this procedure provided further understanding on the pathological basis of liver conditions and their progression, as well as objective data on which to ground our diagnoses (3). However, the technique is not exempt of deleterious effects. Hospital stay becomes longer for up to 1-5% of patients undergoing LB, mainly due to bleeding complications. Similarly, the procedure-related death rate has been reported as 1/1,000-1/10,000 (4,5). The risk for complications is on the other hand proportional to the number of needle passes as well as the presence of certain conditions, including sepsis and coagulation disorders (6).

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LB also has limitations such as sampling errors and inter-observer variability. In a normal-build adult the mass of liver tissue is around 1500 g. An LB sample represents a parenchymal fraction of 1/25,000-1/50,000. In the last few years several studies were reported showing that diagnostic reliability depends on sample size. Thus, Colloredo et al. (7), in a study including 161 biopsies, found that inflammation extent and fibrosis stage were considered more benign when shorter or thinner samples were assessed. These authors believed that correctly assessing histological lesions required at least 11 portal spaces, which was only the case for biopsies at least 2 cm long and 1.5 mm wide.

Histological diagnosis uncertainty is also apparent when results obtained from two biopsies from the same liver are compared. In a recent study where laparoscopic biopsies were taken from both liver lobes, a diagnosis of cirrhosis was arrived at for only one of them (the other biopsy was interpreted as grade 3) in 14.5% of patients. In addition, staging differences reached at least one grade between both lobes in approximately 33% of patients (8). Another study using the METAVIR classification (9) showed that only 65% of 15-mm biopsies (the theoretically recommended size) and 75% of 25-mm samples allow correct staging. Even with two samples at least 15 mm in size from only one site, differences in fibrosis of at least 1 stage were described for 45% of patients (10).

Issues derived from assessment subjectivity add to those related to representativity. Various studies showed extensive variability in the interpretation of lesions by different pathologists (inter-observer variability) and even one same pathologist when assessing the same sample at two separate times (intra-observer variability). In the METAVIR study 10 expert pathologists specialized in liver disease examined 30 LB samples from patients with HCV infection; while some lesions were similarly assessed by all of them, broad differences were reported for others. The latter included erosive necrosis, activity, lobular necrosis, and Knodell's index (11).

On the other hand, one would often want to be aware of the progression of liver disease in order to assess therapy response. Limitations entailed by repeat LB as regards potential patient risks demands the development of new techniques for liver fibrosis assessment. On all these grounds, non-invasive diagnostic tests (serum markers, imaging modalities) have been developed of late mainly to assess liver fibrosis severity.

The following pages attempt to review available data on the better-known serum markers as well as transient elastography (FibroScan®).

SERUM FIBROSIS MARKERS

Under ideal conditions a serum fibrosis marker should be organ-specific, easy to measure, reproducible, and inexpensive. In addition, it should be useful not only for fi-

brosis staging but also for monitoring disease progression and therapy effectiveness.

During the last few days multiple studies have been reported on various biological markers measured in blood or serum samples for fibrosis staging with no need for LB. Most are cross-sectional diagnostic-test studies where measured markers are compared to histology findings, which represent the gold standard. However, improved follow-up designs allow for repeat measurements to predict clinical outcome or complications for liver disease.

Serum markers used for non-invasive diagnosis of liver fibrosis mainly fall into two categories: a) indirect markers: those that do not directly reflect extracellular matrix metabolism. More often than not they are also used as routine measurements, and include transaminases, cholesterol, or platelet count; and b) direct markers: usually comprised of extracellular matrix degradation or synthesis by-products, including procollagen III aminoterminal peptide (PIIINP), laminin, etc. (Table I).

Whatever the case, the goal of most studies is not to provide an accurate diagnosis of fibrosis stage with no need for LB, but rather to tell patients with minimal fibrosis apart from individuals with clinically significant fibrosis (equal to or higher than F2 according to the METAVIR classification, or greater than F3 according to Ishak's classification). This cutoff was established because it represents the fibrosis stage from which therapy is recommended to patients with HCV-related liver disease (12,13).

Table I. A classification of fibrosis serum markers

<i>Classification</i>	<i>Biomarker</i>
Routine lab tests	AST / ALT ratio Platelet count GGT Prothrombin activity Total bilirubin Albumin
Acute-phase reagents	A2MG Haptoglobin APOA1
ECM remodeling markers	Procollagen IV carboxyterminal peptide Procollagen IV aminoterminal peptide (collagen 7S) Collagen IV MMP YKL-40 HA
ECM deposition markers	Procollagen I carboxyterminal peptide PIIINP TIMP TGF-β

ECM: extracellular matrix; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma glutamyltransferase; A2MG: alpha2-macroglobulin; APOA1: apolipoprotein A1; MMP: matrix metalloprotease; HA: hyaluronic acid; PIIINP: procollagen III aminoterminal peptide; TIMP-1: tissue inhibitor of metalloprotease-1; TGF- β: transforming growth factor β.

As previously mentioned, the development of liver fibrosis does not follow a steady linear slope over time, but there is rather an unstable balance with periods of greater and lesser fibrogenic activity or of fibrosis regression. A major limitation of biological liver fibrosis markers is their continual quantitative nature, which does not necessarily reflect the complexity of the fibrotic process. As will be discussed below, this entails, among other things, that they all discriminate the presence of extreme stages (F0 and F4), but greater overlapping for intermediate stages makes their discrimination difficult.

There follows a review of the serum markers most commonly described in the literature, with a particular focus on their behavior as diagnostic tests using validity parameters.

APRI (AST to Platelet Ratio Index)

The AST-to-platelet ratio index, better known as APRI, resulted from an analysis of treatment-naïve patients with chronic hepatitis C. In this model by Wai et al. (14) a statistically significant positive correlation was found between fibrosis severity and AST levels on the one hand, and a negative correlation with platelet count on the other hand. APRI is estimated using the formula: $\text{AST}/\text{upper limit of normal AST}$, divided by platelet count (10^9 cells/L), and multiplied by 100. AUC-ROC values to predict significant fibrosis (fibrosis stage > 3 according to Ishak) and cirrhosis were 0.80 and 0.89, respectively. An $\text{APRI} < 0.5$ managed to exclude clinically significant fibrosis (CSF) with a sensitivity (St) of 91% and a specificity (Sp) of 47%, with a positive predictive value (PPV) and a negative predictive value (NPV) of 61 and 86%, respectively. For $\text{APRI} > 1.5$ CSF was found with an St of 41%, an Sp of 95%, a PPV of 88%, and a NPV of 64%. When APRI was lower than 0.5 or higher than 1.5, 51% of patients were correctly classified, that is, with absent or present CSF. In the diagnosis of cirrhosis using cutoffs < 1 and > 2 the absence or presence of cirrhosis was correctly established in 81% of cases. An APRI below 1 ruled out cirrhosis with a NPV of 98%, and an APRI above 2 entailed a PPV of 57%. However, only a small number of patients was to do without LB since a diagnosis with CSF is reliable for an APRI higher than or equal to 1.5 in only 24% of patients. Certainty is higher when cirrhosis is excluded, which is achieved for 85% of cases when APRI is lower than 2 (15).

As previously discussed, because of the established cutoffs a relevant percentage of patients, approximately 40%, will have values in an indeterminate area for fibrosis stage prediction. This fact was also observed in a group of 131 patients with CHC where diagnostic discrimination was compared for three models: APRI, Forns' index, and Sydney index. The measurement of insulin resistance, included in the latter model, allowed to moderately increase the predictive power for both CSF

and advanced fibrosis. However, values resulted in an indeterminate range in 43, 44 and 36% of patients, respectively (16).

To get around this shortcoming Snyder et al. (17) suggested the use of a second test for indeterminate cases - FIBROSpect II in subjects with chronic hepatitis C (CHC), thus reducing the percentage of subjects with indeterminate results from 43% to 26%, as well as the subsequent need for LB.

Regarding the use of this scoring system in transplant recipients, Pissia et al. (18) recently reported a study assessing APRI, Forns' index, and FIB-4 for fibrosis staging in liver diseases of diverse etiology. AUC-ROC values for CSF prediction were 0.87 for APRI and 0.78 for FIB-4.

Forns' index

In an attempt to differentiate patients with HCV hepatitis with CSF ($F \geq 2$ according to Scheuer/METAVIR) from patients without other stages of fibrosis, Forns et al. (19) developed a multivariate analysis-based model in a cohort of 476 subjects. Their index uses four variables: age, GGT, cholesterol, and platelet count. The NPV to exclude fibrosis equal to or higher than F2 reached 96% for scores below 4.2. However, on applying the higher cutoff (> 6.9) PPV was only 66%. The usefulness of this index is therefore restricted to patients with early-stage fibrosis.

When the usefulness of Forns' index and APRI are compared for CSF detection both are seen to closely correlate with fibrosis, and their discriminatory power to predict mild fibrosis (F0-F1) is similar. In a comparative study of both methods by Gómez et al. (20) Forns' index NPV to exclude CSF was 71%, and increased to 73% when APRI was used. By combining both methods this NPV increased up to 83% (for patients with a Forns' index < 4.2 and $\text{APRI} < 0.5$). At the other end of the spectrum Forns' index showed a PPV in the diagnosis of advanced fibrosis (F3-F4 in Scheuer's classification) of 79%, while APRI's was only 54%. By combining both indices PPV increased up to 86% in this situation. In addition, in the subgroup of patients with genotype 1 the predictive power of both models increased, particularly when used in combination (NPV of 95% for mild fibrosis and PPV of 92% for stages F3-F4).

In special populations, as is the case with subjects coinfecting with HCV and HIV, this test's overall discriminatory power was superimposable to results obtained with APRI or FIB-4, especially in the prediction of advanced fibrosis stages ($F \geq 3$), according to Tural et al. (21). However, also in the setting of coinfecting subjects, on trying to predict the presence of CSF ($F \geq 2$) the percentage of correctly classified cases using Forns' index is only 25%, and improves using APRI and FIB-4 to 39 and 70%, respectively (22).

On the other hand the usefulness of this and other markers is questioned for patients with CHC and normal transaminases, where a test's overall diagnostic power, as measured with AUC-ROC, decreases in a statistically significant manner when compared to subjects with hypertransaminasemia; this is particularly the case with Fibroindex and Forns' test (23).

Forns' index weaknesses include the inclusion of cholesterol in the formula. This parameter varies according to HCV genotype, and values are lower for genotype 3 and become normal or increased following antiviral therapy (24). Furthermore, the inclusion of platelet count, as in APRI, is not adequately standardized among laboratories (25), which may induce some heterogeneous results.

Fibrotest

In 2001 Imbert-Bismut et al. suggested an index based on a mathematical formula combining five variables: total bilirubin, GGT, haptoglobin, alpha-2-macroglobulin (A2MG) and apolipoprotein A1 (APOA1).

A2MG is a protease inhibitor synthesized by activated stellate cells, hence its levels increase with fibrosis stage. Conversely, *haptoglobin* levels decrease when fibrosis increases because of its direct association with profibrotic cytokine TGF- β 1. The latter decreases in serum when fibrosis extent increases because of elevated hepatocyte growth factor. The inclusion of APOA1 is justified because of its affinity in binding ECM when fibrosis increases, which renders its levels lower (26).

The resulting model was designated FibroTest (Bio-Predictive; Paris, France), with a scoring range of 0 to 1. The original report showed that, in patients with HCV-related liver disease, its NPV was 100% for values up to 0.1 regarding accuracy to exclude CSF (F2 to F4 in METAVIR), and that, for values between 0.6 and 1, its PPV is higher than 90% to confirm CSF. According to reported data, the number of LBs avoided would have been 46% (26). This same team uses this model for validation in subsequent studies. An analysis with retrospective data collection in patients with HCV infection who were being followed up in the setting of an interferon (IFN) clinical trial found a statistically significant decrease in fibrosis stage, as measured with Fibrotest (FT), in subjects with sustained viral response versus non-responders and relapsing responders. Such differences were not detected by measuring hyaluronic acid (HA), which was used as a comparator fibrosis marker (27). Later on, in another clinical trial to assess the effectiveness of pegylated interferon plus ribavirin, they found similar results regarding lower FT results when histologically improved fibrosis was detected, and also reported that, for indices above 0.3, sensitivity was 90% and PPV reached 88% for CSF detection (28).

FT results were also compared to patient history (designated "historical index" in their report) including gen-

der, alcohol use, and age at LB, for CSF prediction. Thus, PPV was 70% for an FT score > 0.8 , and NPV was 87% for an FT score ≤ 0.2 . Their "historical index" provided a PPV of 65% for scores above 0.6, and a NPV of 84% for scores equal to or lower than 0.2 (29).

In a review by this same group of 16 published papers, the NPV to exclude CSF using FT, with a prevalence of 31%, was 91%. When FT results were directly compared to other fibrosis markers such as HA, APRI (30) or Forns' index in the same patients, the former had greater diagnostic power (on comparison of AUC-ROCs), was more sensitive to differentiate stage F1 from F2, and showed a more linear correlation with fibrosis stages (31).

On the other hand, Castera et al. (32) described that, when FT results were combined with transient elastography (FibroScan [FS], Echosens, Paris, France) and results were consistent, which occurred in 70 – 80% of patients, said results were also consistent with those of LB for 84% of patients with F2 stage, for 95% with F3, and for 94% for F4. By combining these methods, LB could have been avoided for 77% of patients with CSF. However, in 10% of patients with CSF fibrosis extent would have been underassessed, and overestimated in 1.5%.

An independent paper from Australia found poorer results than reported by European groups on assessing FT usefulness. Rossi et al. (33) found that NPV was 85% for scores < 0.1 , and PPV boiled down to 78% for scores above 0.6. The proportion of false negative results in diagnosing CSF in this study was 18%, and that of false positive results was 21%. Furthermore, only in 46% of cases could LB have been avoided, and conflicting results would have amounted to 19% of cases.

While most reports analyzing FT results referred to patients with CHC and hypertransaminasemia, results in other subgroups have been published of late, including patients followed up for HBV hepatitis on treatment with lamivudine (34) or adefovir (35), patients with HIV/HCV co-infection (36), subjects infected with HCV with normal transaminase levels (37), and individuals undergoing renal transplantation with associated chronic liver disease by HBV or HCV (38). In a recently reported meta-analysis FT showed a diagnostic discriminatory power, as measured by AUC-ROC, similar in patients with either HCV or HBV infection, in non-alcoholic fatty liver disease, and in alcoholic liver disease (39).

On the other hand, in cirrhotic patients it has shown a close, statistically significant correlation with the presence of severe portal hypertension (≥ 12 mmHg) (40).

Anyway, FT results could be altered by variations in any formula component (available at: <http://www.freepatentsonline.com/7225080.html>). False positive results would be detected with decreased haptoglobin from hemolysis, which is common in patients on treatment with ribavirin, or in Gilbert's syndrome, because of increased bilirubin. On the other hand, false negatives result from inflammatory processes with increased acute phase

reagents, haptoglobin and A2MG (41). These hypotheses were confirmed by Fontanges et al. (42) in a series of patients with CHC, where increased serum GGT and A2MG levels were seen to contribute to fibrosis over-staging.

FIB-4

In patients with HCV/HIV coinfection, Sterling's team, based on a multivariate analysis of 832 cases with retrospective data collection regarding liver histology, developed a fibrosis predictive model including age, AST, platelets, and ALT for variables. In the original study the AUC-ROC value to differentiate fibrosis stages ≥ 4 according to Ishak's classification was 0.765. In the validation group, using a cutoff < 1.45 , 90% of patients would have no advanced fibrosis (NPV), with a sensitivity of 70%. Alternatively, with a cutoff > 3.25 PPV was 65% with Sp = 97%. The authors report that by using these cutoffs 71% of LBs could be avoided in the validation group (43). In a subsequent study this model's results were compared to FT versus LB-derived histology (44). Here the METAVIR scale was used to assess fibrosis extent. AUC-ROC for FIB-4 was 0.85 to detect advanced fibrosis ($F \geq 3$). FIB-4 values to exclude advanced fibrosis reached a NPV of 94.7% with St = 74.3%. At the other end, values > 3.25 showed a PPV of 82.1% with Sp = 98.2%. Of all 847 LBs analyzed, 72.8% were correctly classified with these cutoff points. Agreement with FT results for values < 1.45 or > 3.25 was 92.1 and 76%, respectively. Recently, its use has been extrapolated to a sample of subjects with chronic hepatitis B. Its power to distinguish moderate from advanced fibrosis was higher than APRI's, and consistent with FT results in 89% of patients for advanced fibrosis exclusion (45).

Other models using combined markers

In a prospective clinical study of patients on antiviral therapy for CHC, Lok et al. (46) developed a model to predict cirrhosis based on routine laboratory testing, including platelet count, AST/ALT ratio, and INR. Values lower than 0.2 for cirrhosis exclusion resulted in only 8% of false positive results (NPV: 86%), whereas these reached 15% for indices above 0.5 (PPV: 75%). By using this model LB could be avoided for 50% of patients.

In order to distinguish patients with CHC and CSF, the Australian group of Sud et al. developed an index designated FPI (fibrosis probability index) including age, history of alcohol intake, insulin resistance, and AST and cholesterol levels. NPV was 69% for scores ≤ 0.2 , and PPV reached 97% for scores ≥ 0.8 . With this index, LB could be avoided for 48% of patients with mild fibrosis (47).

Calès et al., in turn, used different marker combinations related to liver disease etiology, either alcoholic or viral. For the latter case platelet count, prothrombin index, AST, A2MG, HA, urea, and age were combined into an index they designated Fibrometer; the authors concluded that its AUC-derived diagnostic accuracy was higher than that of Fibrotest, Forns' index, and APRI (48).

On the other hand, Adams et al. (49) similarly tried to establish an algorithm to predict fibrosis stage in treatment-naïve subjects with chronic hepatitis C. This gave rise to the Hepascore predictive model, which includes bilirubin, GGT, HA, A2MG, age and gender for variables. In the validation group AUC-ROC values were 0.82, 0.90 and 0.89 to discriminate CSF, advanced fibrosis and cirrhosis, respectively. Furthermore, Hepascore results are consistent with those of FT within one same sample of subjects in 82% of cases, and with liver biopsy in 88% of patients (50).

AST, gamma-globulin, and platelet count values allowed Koda et al. (51) to develop the so-called Fibroindex model that, in the original study, showed better AUC-ROC values versus APRI and Forns' index in the sample of studied subjects. Furthermore, in a cohort of patients with two liver biopsies, before and after interferon therapy, the model correlated adequately to histologically established changes in fibrosis stage.

Finally, while the population of subjects with HCV-induced liver disease is the key target in these studies, fibrosis prediction is also useful for other causes of liver disease. Thus, in a sample of 32 patients with hereditary hemochromatosis, Castiella et al. (52) described that a fibrosis index higher than 480,000, resulting from multiplying age by liver iron levels, and a platelet count lower than 200,000 allowed to rule out advanced fibrosis in 94% of patients.

Models based on direct fibrosis markers

In liver fibrosis both qualitative and quantitative changes may be detected in various extracellular matrix (ECM) components. Some are fibrogenesis markers, and some are fibrolysis markers. Potential biomarkers include collagen synthesis or degradation by-products, enzymes involved in ECM synthesis or degradation, glycoproteins, and proteoglycans. None of the criteria defined thus far meets the requirements of an ideal marker, since all of them respond to variations in their metabolism or excretion.

As with studies based on routine lab tests, combined markers are more useful than single markers. HA has been shown to be the marker that best correlates with fibrosis extent in patients with CHC; its NPV to exclude cirrhosis is 99%, but a PPV of only 30% makes it unsuitable to predict its presence (53). Regarding collagen metabolism by-products, procollagen III amino-terminal peptide (PIIINP) has been most studied; it is a poorer fibrosis marker when com-

pared to HA, but reflect inflammation extent better (54). Collagen IV and laminin, a basal membrane constituent, may predict the presence of severe acute alcoholic hepatitis, helping to decide on the use of corticosteroids in patients with this condition and already in a cirrhotic stage (55).

A glycoprotein that seems to be a fibroblast growth factor, YKL-40, has also been correlated with the presence of fibrosis, and may seemingly predict the survival of patients with chronic alcoholic liver disease (56).

On the other hand, ECM degradation is known to occur via collagen-degrading endopeptidases designated matrix metalloproteinases (MMPs), whose activity is regulated by tissue inhibitors (TIMPs). Disbalance here seems to be a factor involved in the development of liver fibrosis through decreased MMP activity and increased TIMP effects (57). The results of these markers as fibrosis detectors vary among studies, and comparisons are difficult because of the many enzyme subtypes encountered (58,59).

As with studies using routine lab tests, several authors have reported their experience with combinations of more specific fibrosis markers.

Using PIIINP and matrix metalloproteinase 1 (MMP-1), Leroy et al. (60) described that NPV was 75% in CSF exclusion for scores lower than 0.3, and PPV was 76% in the diagnosis of fibrosis in stages F2-F4 for scores > 0.3.

In patients with various types of chronic liver disease the European Liver Fibrosis (ELF) group developed a model including age, HA, PIIINP, and tissue inhibitor of metalloproteinase 1 (TIMP-1) that detected with high probability the absence of CSF (NPV: 92%), and was particularly useful in patients with alcoholic liver disease or non-alcoholic fatty liver disease (61).

Patel et al. (62) developed another algorithm they designated FibroSpectIISM for the detection of CSF that included 3 markers: TIMP-1, HA, and A2MG. With a CSF prevalence of 52% and scores higher than 0.36 this index was predictive with an accuracy of 75% and PPV of 74%.

In patients with HIV/HCV coinfection a combination of HA, albumin and AST (SHASTA index) allows to discriminate patients with mild fibrosis (F0-F2 in modified Ishak's classification) from those with advanced fibrosis ($F \geq 3$). Using a cutoff for this index below 0.3 advanced fibrosis was detected with a NPV above 94%. At the other end, for scores > 0.8 PPV was 100%. However, with this model a significant percentage of patients (58%) could not be classified in either end (63).

Table II summarizes the internal validity parameters as well as variables included in the models above.

TRANSIENT ELASTOGRAPHY (FIBROSCAN®)

Physics and technique

FS (Echosens, Paris, France) includes a probe, a specifically-designed electronic analysis system, and a control unit installed on a personal computer. The probe

has a piston-shaped low-frequency vibrator (50 Hz). The wave generated by the piston's pulse travels through the liver. A 5-MHz ultrasound transducer is fitted to the piston's axis. The device breaks up wave propagation into time and space components, which allows to estimate its speed across the tissue.

The physical principle on which it is based considers the liver a zero-viscosity (no resistance to tangential deformity), isotropic (same physical properties in all directions) medium where measured elasticity would be expressed according to Young's module as $E = 3\rho V_s^2$, where V_s is the velocity of the wave and ρ is the medium's density. Since in soft tissues density is nearly constant and approaches that of water, a ρ value of 1000 kg/m³ is used. Elastic wave propagation velocity is the slope of the space-time straight line, which is generated in an area located at 24 to 45 mm under the skin surface. Measurements here avoid wave distortions as induced by the subcutaneous tissue and liver capsule in most patients.

From the above equation elasticity, measured in kPa, is directly proportional to wave propagation velocity. Since stiffer tissues allow a faster elastic wave passage, elasticity values will be higher here. Therefore, progressive liver stiffening associated with advancing fibrosis results in higher elasticity values.

As per the original description (64), measurements are performed in the liver's right lobe, with the patient in the supine position and a maximally abducted right arm over the head. The hepatic area of interest should be at least 6 cm thick, and interference by vascular structures should be avoided. The piston should be placed in an intercostal space, on the mid-axillary line, perpendicular to the skin on which it rests. Ten measurements are usually performed in each patient. FS-derived data include median elasticity, a measure of dispersion (interquartile range), procedure duration, and percentage of success (estimated as the number of valid acquisitions-to-total acquisitions ratio). FS results are deemed valid provided that: a) the interquartile range remains inferior to 30% of median value; and b) the percentage of success is at least 60% (65).

Regarding the volume of analyzed tissue, the virtual cylinder on which the measurement is performed is 20 mm long and 20 mm in diameter, which approximately represents 1% of total liver mass, a percentage far more relevant than usually provided by a LB specimen, which is around 1/50,000 (66).

Reproducibility

In the original description Sandrin et al. (64) described little inter- and intra-observer variability in 15 patients with HCV infection, with a standardized coefficient of variation of 3.2 (range 2-18%) and 3.3%, respectively.

Such data have been subsequently verified in a wide series of 800 patients with liver disease of diverse etiologies,

Table II. Serum markers predictive of liver fibrosis. Model variables and validity parameters

Name (reference)	Components	Fibrosis stage	Cutoff	St (%)	Sp (%)	PPV (%)	NPV (%)
APRI (14)	AST Platelets	Ishak > 3	< 0.5	91	47	61	86
			< 1.5	41	95	88	64
Forns' index (19)	Age GGT Cholesterol Platelets	Scheuer > F2	< 4.2	94	51	40	96
			> 6.9	30	95	66	80
Fibrotest (26)	A2MG Alpha2-globulin Gammaglobulin APOA1 GGT Total bilirubin	METAVIR > F2	0.3	87	59	63	85
FIB-4 (43)	Age AST Platelets ALT	Ishak > 3	≤ 1.45	70	74	42	90
			≥ 3.25	22	97	65	82
Lok's model (46)	Platelets AST / ALT INR	METAVIR F4	< 0.5	40	99	84	90
FPI (47)	Age AST Cholesterol HOMA-IR Alcohol ingestion	Scheuer > F2	0.2	85	48	70	69
			0.8	42	98	97	54
ELF (61)	Age PIIINP TIMP-1 HA	Scheuer > F2	0.063	95	29	28	95
			0.564	30	99	89,5	83
FibroSpect II (62)	HA TIMP-1 A2MG	METAVIR > F2	0.36	77	73	76	75
SHASTA index (63)	HA AST Albumin	Ishak > 3	0.1	100	52	44	100
			0.8	15	100	100	76

AST: aspartate aminotransferase; GGT: gamma glutamyltransferase; A2MG: alpha2-macroglobulin; APOA1: apolipoprotein A1; HOMA-IR: homeostasis model assessment of insulin resistance; PIIINP: procollagen III aminoterminal peptide; HA: hyaluronic acid; TIMP-1: tissue inhibitor of metalloprotease-1.

where intraobserver and interobserver agreement was assessed using the interclass correlation coefficient (ICC), which was 0.98 for both. However, ICC was seen to decrease for subjects with BMI > 25 kg/m², in the presence of steatosis, and with early fibrosis (F < 2). Elasticity values as assessed by two technicians were found on the same fibrosis cutoff in 88% of patients with F ≥ 2, in 92% of those with F ≥ 3, and 91% of those with F4 (67).

Diagnostic capability

Viral liver disease

In the study by Sandrin et al. (64) transient elastography was performed in 91 patients with HCV-related liver

disease, and a definitive analysis was performed for 67. In 93% of cases with F0-F1 fibrosis according to METAVIR elasticity was ≤ 5.1 kPa. On the other hand, 94% of cases in F ≥ 2 stage this value was equal to or higher than 7.6 kPa.

Subsequently, another report with prospective, multi-center data collection by Ziolk et al. in 251 patients with HCV infection, either alone or associated with other liver conditions concluded that using a cutoff for F ≥ 2 at 8.47 kPa, and at < 3 kPa to exclude F2, would avoid LB for 42% of subjects. Optimal cutoffs included 8.74, 9.56, and 14.52 kPa for F ≥ 2, F ≥ 3, and F4, respectively (68).

Later on, Castera et al. compared in patients with CHC the diagnostic accuracy of FS versus two serum markers (FT and APRI). This analysis in 193 patients, with a highly homogeneous distribution of fibrosis stages, de-

scribed a cutoff that was slightly different from the above one for the diagnosis of $F \geq 2$ (7.1 kPa). The remaining optimal cutoffs were 9.5 and 12.5 kPa for $F \geq 3$ and F4, respectively. In addition, an algorithm was suggested where, should FS and FT results be consistent, no LB would be necessary, with treatment strategy being based solely on values provided by both techniques (32). However, in 10% of cases, based exclusively on these techniques, treatment would have been incorrectly discouraged, and 1.5% of patients would have received an unnecessary therapy.

Colletta's team (37), using the cutoffs previously reported by Ziol et al. (68), compared the diagnostic accuracy of FS and FT for the staging of fibrosis in patients with HCV-related liver disease and normal transaminase levels. While in this report the prevalence of $F \geq 2$ stages was 35%, only 12.5% of cases has F3, and no cirrhosis was seen, it was concluded that elastography was superior to FT in the identification of fibrosis stages in a noninvasive manner, with a diagnostic accuracy for FS, measured using Youden's index, of 100 *versus* 43% for FT. Moreno-Otero et al., in a series of 28 patients with CHC and normal transaminase levels, also described a mean FS value of 6.35 kPa, which suggested that fibrosis was minimal or absent. No comparison with the reference test was performed in this study (69).

The ability of FS to predict fibrosis stage was also assessed in patients coinfecting with HIV and HCV. Elastography's AUC-ROC for diagnosing cirrhosis in this group of patients, when compared to serum markers such as APRI, FIB-4, AST/ALT ratio and platelet count, was higher in a statistically significant way (70). A recent systematic review by Shaheen et al. (71) of studies by Castera (32), Colletta (37), Ziol (68), and Ledinghen (70) suggests that diagnostic accuracy is excellent for the detection of cirrhosis associated with HCV, but usefulness is lower for earlier stages.

The usefulness of FS has also been recently reported in patients with HBV hepatitis. The study by Marcellin et al. (72) included 202 patients, and a statistical analysis could be performed for 173. In the remaining 14% elasticity could not be measured. AUC-ROC values FS were 0.81, 0.93 and 0.93, respectively, for predicting $F \geq 2$, $F \geq 3$ and F4. Cutoffs for which the highest St + Sp values were obtained were 7.2 kPa for $F \geq 2$, 8.1 kPa for $F \geq 3$ and 11 kPa for F4.

Other causes of liver disease

In 101 patients with cholestatic disease (PBC and PSC), Corpechot et al. (73) reported there is adequate correlation between elasticity and fibrosis stage, even when each of these conditions is analyzed separately. In the multivariate analysis only HA levels ($> 50 \mu\text{g/L}$) and FS value ($\geq 9.8 \text{ kPa}$) were variables independently associated with extensive fibrosis. Optimal cutoffs for FS

were 7.3, 9.8 and 17.3 kPa for $F \geq 2$, $F \geq 3$ and F4, respectively. This correlation in patients with PBC between elasticity and presence of fibrosis was later confirmed in a study by Gómez-Domínguez et al., where its role in the follow-up of these patients is suggested (74).

In a range of liver conditions including two thirds of HCV infection cases, Gómez-Domínguez et al. described a St of 94% with a NPV of 50% using a cutoff for $F \geq 2$ at 4 kPa, whereas for a diagnosis with cirrhosis an elasticity value above 16 kPa has a St of 96% with a PPV of 80% (75).

As regards the impact of steatosis on FS-measured elasticity, the possibility was initially suggested that, since fat tissue is softer than normal parenchyma, the presence of fatty liver would decrease measurements (64). However, a letter published by Yoneda et al. provided evidence that the correlation between elasticity and fibrosis severity is not modified by steatosis extent (76). In fact, our own group published an abstract stating that the diagnostic accuracy of FS for the detection of CSF ($F \geq 2$), advanced fibrosis ($F \geq 3$) and cirrhosis, as measured with AUC-ROC, is higher when compared to APRI and Forns' test in subjects with histologically-proven non-alcoholic fatty liver disease (77).

Diagnosis of cirrhosis

Regarding cirrhosis, FS has been reported to exhibit a higher power for its exclusion as compared to its prediction. According to Ganne-Carrié et al., a value higher than 14.6 kPa has a St of 95% and NPV of 96%. False negative results resulted in 29% of cases from the presence of macronodular cirrhosis, and in the remaining 71% from absence of mild inflammatory activity (78).

Foucher et al., in turn, in a study with prospective case collection in 711 patients with various liver conditions, established a number of cutoffs from which several complications secondary to liver cirrhosis would develop, including esophageal varices at 27.5 kPa and ascites at 49.1 kPa (79).

Portal hypertension screening

Of special interest is the possibility of detecting portal hypertension with this technique. The study by Vizzutti et al. (80) assessed the ability of FS to detect this complication *versus* the hepatic venous pressure gradient (HVPG) and varices at endoscopy. A positive correlation was seen between FS and HVPG. FS sensitivity and specificity for the detection of clinically significant portal hypertension (CSPH) were 97 and 92%, respectively, for a cutoff $\geq 13.6 \text{ kPa}$; to predict severe portal hypertension (HVPG $\geq 12 \text{ mmHg}$), NPV and Sp were 91 and 94%, respectively. However, as with HVPG, FS could not differentiate between esophageal varices grades. In another cohort of

165 patients with cirrhosis of diverse etiologies Kazemi et al. found that elasticity < 19 kPa was highly predictive of absent esophageal varices with grade > 2, with a NPV of 93% and Sp of 84% (81). This may suggest a potential role of FS in the selection of patients who should undergo endoscopic screening for esophageal varices (82). However, other authors claim that elastography lacks adequate diagnostic accuracy for delaying endoscopic screening for cirrhotic patients based exclusively on results obtained (83).

Relapsing infection with HCV post-transplant

The measurement of elasticity with FS may prove useful for assessing the severity of relapsing infection with HCV during the post-transplant period. Thus, Carrión et al. not only confirm the finding of a marked direct correlation between FS results and HVPG measurements also in this group of patients, but note that values smaller than 8.5 kPa have a specificity of 90% for CSF detection, and a PPV of 92%. None of the patients in this study with higher elasticity figures had advanced fibrosis or CSPH as measured by HVPG (84). A team led by Harada agrees on the usefulness of FS for fibrosis staging in patients with relapsing HCV infection post-transplant, with a higher diagnostic power –as measured with AUC-ROC– as compared to HA, collagen IV, ALT levels, and APRI. In this series, where the prevalence of $F \geq 2$ is 37.5%, optimal cutoffs are 9.9, 15.4 and 26.5 kPa for $F \geq 2$, $F \geq 3$ and F4, respectively (85). Another interesting contribution in this setting is that by Rigamonti et al. (86), who followed up for 6 to 21 months 40 transplanted patients undergoing 2 liver biopsies. This study noted a statistically significant correlation between increased elastography values and fibrosis stage, with a sensitivity of 86% and a specificity of 92% for predicting an increase in fibrosis stage.

Elastography for special groups

Regarding children with chronic liver disease, FS was better than serum markers, FT and APRI (87), as was in subjects with post-transfusional iron overload. In the latter group a particularly low cutoff is reported, namely 6.25 kPa, for the diagnosis of advanced fibrosis, $F \geq 3$, which the authors attribute to the sample's younger age when compared to other previously reported studies (88). More recently transient elastography and various serum markers have been compared in C282Y homozygotes *versus* a control group. In no subject in the latter group did FS values suggest the presence of fibrosis in $F \geq 2$ stages. While there was correlation between elastography values and the study serum markers, none was found for ferritin levels (89).

In patients with Crohn's disease on methotrexate FS showed no good correlation with cumulative drug doses,

and median values were similar in a drug-naïve group and a group with a cumulative dose higher than 1500 mg. Other non-invasive markers that were assessed (APRI, FT, HA) also showed no statistically significant differences between both groups. This study had a serious methodological fault: LB was only carried out in patients with persistent hypertransaminasemia or elasticity figures > 8.7 kPa (90).

Also in subjects receiving methotrexate, but now for psoriasis, Berends et al. (91) proposed FT to detect the presence of significant fibrosis, and FS to rule it out. Of note, 60% of all initially eligible subjects failed to eventually participate in the study. An FT > 0.31 was able to adequately detect 83% of patients with CSF, whereas an FS value above 7.1 kPa represented a likelihood of 88% in identifying patients with no CSF.

Table III summarizes the reported cutoff, internal validity parameters, and AUC-ROC values according to the origin of liver disease.

Limitations

The influence of inflammatory activity data on the diagnostic capability of FS is being studied of late. Thus, Coco et al. (92), in a study including patients with HBV- and HCV-related liver disease, described that, in patients with identical fibrosis stages, those with normal serum ALT levels had a lower elasticity value as compared to those with biochemical activity. In addition, during follow-up, FS measurements increased with transaminase levels in subjects with viral disease exacerbation. The study reported for $F \geq 2$ a cutoff of 8.3 kPa with a sensitivity of 85% and NPV of 79%, and for F4 a cutoff of 14 kPa with a specificity and PPV of 98%.

These findings were confirmed by Sagir et al., who performed FS measurements in patients with acute liver failure of various origins (HBV, drug toxicity, autoimmune). In 75% of patients elasticity values were consistent with a diagnosis of cirrhosis using a cutoff at 12.5 kPa, which neither LB nor ultrasounds supported. Furthermore, in 6 cases follow-up identified a vast improvement in elasticity after recovery from the acute event and once transaminase levels had returned to normal. On the other hand, statistically significant differences were found in bilirubin levels and age when patients above and below said cutoff were compared, these being higher in the group with elasticity > 12.5 kPa (93). In a similar study by Arena et al. in patients with acute viral hepatitis a positive correlation was reported between FS values and AST and ALT levels, and the authors concluded that the presence and extent of necroinflammatory activity should be considered particularly in patients with little or no fibrosis, $F \leq 2$, on interpreting FS-derived information (94).

Another complication that makes FS results difficult to interpret is a finding by Millonig et al. (95). In their study

Table III. Different cutoffs for the diagnosis of liver fibrosis with transient elastography

Reference	Cause of liver disease	Stage (METAVIR)	Cutoff	St (%)	Sp (%)	PPV (%)	NPV (%)	AUC-ROC
Castera (32)	HCV	F $\geq$ 2	7.1	67	89	95	48	0.83
		F $\geq$ 3	9.5	73	91	87	81	0.90
		F4	12.5	87	91	77	95	0.95
Colletta (37)	HCV	F $\geq$ 2	8.74	100	100	100	100	
Ziol (68)	Various etiologies	F $\geq$ 2	8.74	55	84	87	51	0.79
		F $\geq$ 3	9.6	84	85	71	93	0.91
		F4	14.5	84	94	76	96	0.97
de Ledinghen (70)	Coinfection HCV/HIV	F $\geq$ 2	4.5	93	11	62	50	0.72
		F4	11.8	100	93	81	100	0.97
			14.5	88	96	88	96	
Marcellin (72)	HBV	F $\geq$ 2	7.2	70	83	80	73	0.81
		F $\geq$ 3	8.1	86	85	65	95	0.93
		F4	11	93	87	38	99	0.93
Corpechot (73)	PBC / PSC	F $\geq$ 2	7.3	82	79	85	75	0.92
		F $\geq$ 3	9.8	89	90	84	93	0.95
		F4	17.3	87	95	76	97	0.96
Gómez-Domínguez (75)	Various etiologies	F $\geq$ 2	4	94	33	88	50	0.74
		F $\geq$ 3	11	58	89	78	76	0.72
		F4	16	89	96	80	98	0.94
Ganne-Carrié (78)	Various etiologies	F4	14.6	79	95	74	96	0.95
Carrión (84)	Relapsing HCV in graft	F $\geq$ 2	8.5	90	81	79	92	0.9
		F4	12.5	100	87	50	100	0.98
Harada (85)	Relapsing HCV in graft	F $\geq$ 2	9.9	90	91	86	44	0.92
		F $\geq$ 3	15.4	75	95	82	93	0.96
		F4	26.5	100	98	83	100	0.99
Mirault (88)	Iron overload	F $\geq$ 3	6.25	80	70	57	88	0.82
Coco (92)	HBV and HCV	F $\geq$ 2	8.3	85	91	94	79	0.93
		F4	14	78	98	98	82	0.96

AUC-ROC: area under the ROC curve; PBC: primary biliary cirrhosis; PSC: primary sclerosing cholangitis.

15 patients with bile flow obstruction underwent elastography before cholestasis resolution with ERCP. Elasticity values increased at baseline decreased following the procedure. In fact, the mean reduction of elasticity values was 1.2 $\pm$ 0.56 kPa per 1 g/dL of bilirubin reduction. Only 2 subjects showed no decrease in elasticity – one had cirrhosis, and one suffered from multiple liver metastases. These findings are consistent with those reported by our team in an abstract, where FS was performed for 58 subjects with non-HCV liver disease. Median elasticity in the presence of intrahepatic cholestasis was higher versus patients with no cholestasis in a statistically significant manner. Furthermore, in the multiple regression analysis only fibrosis stage and the presence of cholestasis had a statistically significant correlation with FS results (96). Even a stasis liver seems capable of increasing liver elasticity values, which may eventually return to normal following the resolution of heart failure (97).

Despite such limitations, two meta-analyses found FS useful for cirrhosis confirmation or exclusion, with a mean diagnostic discrimination power of 94-96% as

measured using AUC-ROC. This value markedly decreases to 84-87% when attempting to differentiate between stages F $\geq$ 2 (98;99). However, some authors state that, when used concomitantly with other clinical data and noninvasive diagnostic tests, FS may prove a highly useful tool in guiding difficult decision-making concerning treatment (100).

CONCLUSION

In chronic liver disease patient prognosis and treatment options depend on fibrosis stage, including the potential development of cirrhosis and its complications. Liver biopsy is the most valuable test for liver fibrosis staging; however, given its invasive nature, noninvasive tests have been recently tried to document liver disease stage.

Serum markers for liver fibrosis range from APRI or Forns' index, non-patent models based on measurements readily available to clinicians, to "pay" models such as

Fibrotest, which require less commonly used laboratory studies. Thus far, the latter seems to demonstrate a higher diagnostic discrimination power in the prediction of fibrosis stage. However, the number of currently available predictive models makes data analysis difficult to the extent that none has been usually implemented to this day in our setting.

Transient elastography, despite limitations and given its relative availability and simplicity, seems to find a place in clinical practice. However, as with serum fibrosis markers, its ability to discriminate between adjacent fibrosis stages is scarce. Its clinical role will likely be greater when specific cutoff points are used for each disease.

In any case, maybe we should not consider noninvasive tests to replace liver biopsy. Both techniques are supplementary and allow a more thorough assessment of the complex fibrosis process in our patients. The future may bring initial combined assessments (biopsy plus noninvasive methods) to inform on baseline fibrosis, with only noninvasive tests used during follow-up in subjects with clearly correlated results.

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