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Noninvasive markers of fibrosis: key concepts for improving accuracy in daily clinical practice

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ABSTRACT

Noninvasive markers of fibrosis have emerged as an alternative to the staging of fibrosis by means of liver biopsy. Apart from being noninvasive and thus lacking the adverse effects of liver biopsy, they offer some advantages such as reduced risk of sampling error, objectiveness in the interpretation of the result, appropriateness for repeated measurements and lower cost. Many studies have validated different panels of blood markers and imaging/transient elastography for the estimation of fibrosis with acceptable accuracy. Clinical scenarios leading to inacurate or failed estimation must be acknowledged, as well as the fact that performance of blood markers and transient elastography, and their diagnostic cut-off values vary among specific liver diseases. The combination of two blood markers or of a blood marker and transient elastography has been shown to increase accuracy of the estimation. Further, unlike liver biopsy the noninvasive markers of fibrosis are not associated with a ceiling effect after cirrhosis is identified, but can discriminate early from advanced stages of cirrhosis. Longitudinal studies have shown their utility as predictors of complications from portal hypertension and mortality, outperforming liver biopsy. In conclusion, noninvasive markers of fibrosis provide major advantages over liver biopsy. The reported performance of some of the available tests -particularly when used in combination- make them a reliable tool, very attractive for daily clinical practice.

Key words. APRI. Fibrotest. Transient elastography. Mortality.

INTRODUCTION

Although liver biopsy continues to be an invaluable diagnostic method in hepatology as well as the gold standard to evaluate fibrosis, it is an invasive procedure with the potential for side effects: pain in 84% of cases, bleeding in ≈ 1/500, and death in ≈ 1/10,000.[1] Furthermore, liver biopsy is an expensive procedure when one considers physician, nursing, pathology and facility costs. Because fibrogenesis is a dynamic process, under specific clinical situations the ongoing surveillance of fibrosis progression is necessary, hence mandating repeated evaluations. This multiplies anxiety and the economic burden, making liver biopsy a less than ideal method to evaluate fibrosis. For these reasons, noninvasive markers for the estimation of liver fibrosis have been developed, most of them based on combinations of blood parameters or using transient elastography (TE). This review will describe the fundamentals behind noninvasive markers of fibrosis, as well as some of the relevant validation studies with their reported diagnostic accuracies. The aim is to reveal useful information that can help the clinician in daily practice, including the variations in tests according to the liver disease and the known limitations under specific clinical situations. Other noninvasive markers addressing hepatic inflammatory activity or steatosis will not be discussed.

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Liver biopsy

Whether via a percutaneos or transjugular procedure, using an aspiration or cut needle, liver biopsy

Table 1. Semiquantitative methods for staging fibrosis in liver biopsy.

Chronic hepatitis B and C		NAFLD
METAVIR	Ishak	Brunt
F0-No fibrosis.	F0-No fibrosis.	F0-No fibrosis.
F1-Fibrous portal expansion.	F1-Fibrous expansion of some portal areas with or without short fibrous septa.	F1A-Mild perisinusoidal fibrosis.
F2-Periportal fibrosis with rare septa formation.	F2-Fibrous expansion of most portal areas with or without short fibrous septa.	F1B-Moderate perisinusoidal fibrosis.
F3-Abundant bridging fibrosis.	F3-Fibrous expansion of most portal areas with occasional portal to portal bridging.	F1C-Only portal/periportal fibrosis.
F4-Cirrhosis.	F4-Fibrous expansion of portal areas with marked portal-portal and portal-central bridging.	F2-Both perisinusoidal and portal/periportal fibrosis.
	F5-Marked bridging with occasional nodules (incomplete cirrhosis).	F3-Bridging fibrosis.
	F6-Cirrhosis.	F4-Cirrhosis.

yields a sample of ≈1/50,000 times the size of the liver used for the semiquantitative assessment of fibrosis. Diverse scales have been developed for the staging of fibrosis, with METAVIR and Ishak as the most popular systems for viral hepatitis, and the Brunt score for nonalcoholic fatty liver disease (NAFLD)[2] (Table 1). In spite of the standardization of fibrosis staging, the main limiting factor continues to be sampling variability, which can lead to an inaccurate assessment of fibrosis in up to 33% of cases, and disagreement between pathologists regarding staging in about 30% of biopsies.[3] Furthermore, it has been shown that the smaller the liver biopsy, the lower the stage of fibrosis observed,[4] suggesting that by increasing the size of the liver biopsy sample, the accuracy of fibrosis assessment is improved. It is thus recommended that the optimal liver biopsy specimen should exceed 20-25 mm long, and/or include at least 11 portal tracts.[3] However this goal is a very difficult to achieve and less than half of liver biopsies fulfill this requirement. In their systematic review Cholongitas, *et al.* reviewed 32 studies reporting the length of liver biopsy (12 reporting the number of portal tracts) and could determine that the mean ± SD length was 17.7 ± 5.8 mm, whereas the mean ± SD number of portal tracts was 7.5 ± 3.4.[3]

Another important issue is the use of semiquantitative scales to stage fibrosis. The distance between adjacent stages, as well as the clinical implications of progressing one stage (*i.e.* F0 to F1 ≠ F2 to F3), is certainly not equal because despite the use of the numbers, the scales are not linear. There is also the issue of a ceiling effect to all of the fibrosis scoring systems. Once the diagnosis of cirrhosis (F4) is established, it is not possible to determine more advanced stages (stages 2 to 4 of cirrhosis) with higher risk of complications on the basis of liver biopsy alone, and other diagnostic methods have to be considered (*e.g.* hepatic venous pressure gradient, HVPG).[5] These issues highlight the fact that liver biopsy is a very imperfect gold standard for assessing fibrosis, greatly limiting its utility in validation studies of noninvasive markers. Ideally, validation studies for noninvasive markers of fibrosis should include liver biopsies with a length ≥ 2 cm (or ≥ 11 portal tracts) and perhaps base the analysis in more objectively quantitative methods with a continuous scale such as the collagen proportionate area.[6] Unfortunately most of the validation studies have based their conclusions on biopies ≥ 1.0-1.5 cm long with METAVIR as the gold standard.

NONINVASIVE MARKERS: GENERAL CONCEPTS

Noninvasive markers of fibrosis offer several advantages over liver biopsy: near absence of adverse effects (only those associated with phleobotomy, when applicable) and reduced risk of sampling error, objectiveness in the interpretation of the result, lack of a ceiling effect, appropriateness for repeated assessment, and lower cost. Nevertheless, as with any diagnostic test, studies show that there is a variable degree of overlap among different stages of fibrosis score (0-4) and most of the tests are not applicable

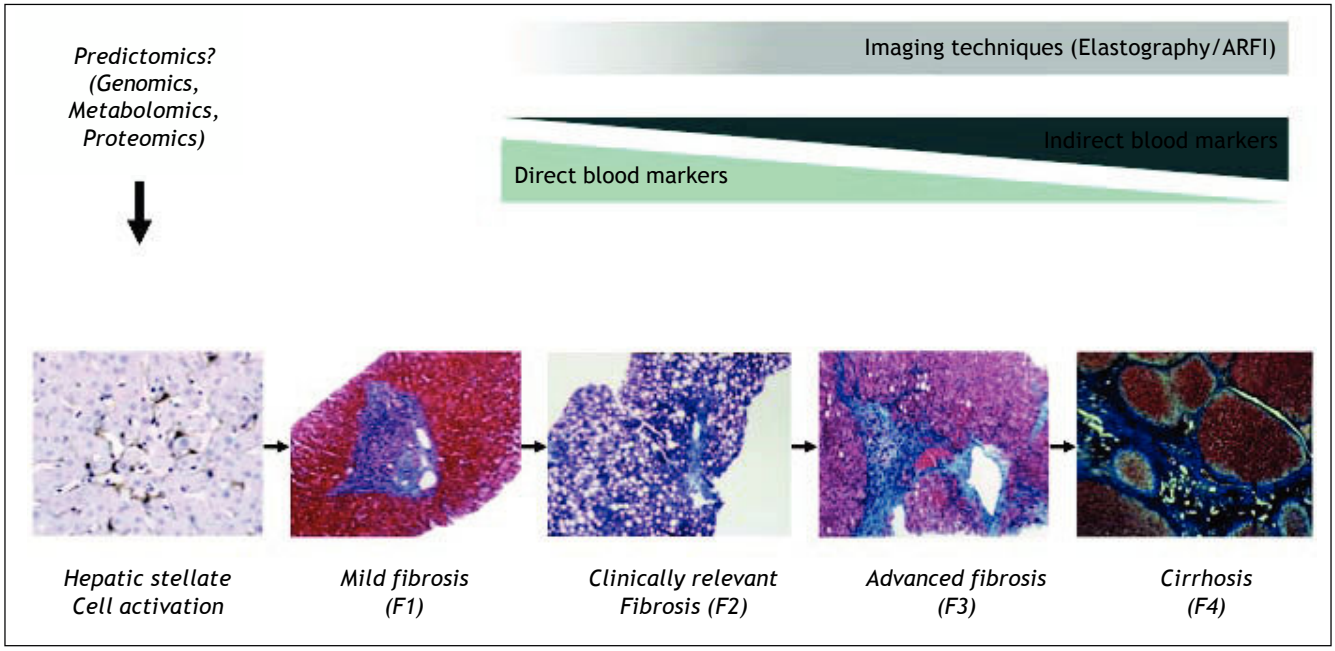

Figure 1. *Noninvasive assessment of liver fibrosis. Most of the non-invasive methods currently used have a good accuracy to identify clinically relevant/advanced fibrosis (F ≥ 2/3). It is possible that in the near future the incorporation of genetic, metabolic and proteomic profiles could be a feasible strategy to allow the identification of liver fibrosis at its early stages. ARFI, acoustic radiation force impulse (photographs courtesy of Rosa Miquel and Cristina Millán, Hospital Clinic, Barcelona, Spain).*

to all liver diseases or clinical scenarios. It it likely that future advances in the 'omics' (genomics, metabolomics and proteomics) may be useful in the future yielding an accurate prediction of fibrosis progression even at the very early stages of fibrogenesis (Figure 1).

Remarkably, the gold standard in the validation studies for noninvasive markers of fibrosis has been the liver biopsy, despite the fact that the gold standard will yield equivocal results in about a third of cases. Consequently, noninvasive markers will appear to be particularly inferior to liver biopsy whenever the latter misclassifies the stages of fibrosis, even when the former are more accurate. Thus, with the current validation methodology, noninvasive markers by definition will never outperform liver biopsy even if they are in truth better assessors of liver fibrosis. Interestingly, it has been shown that the larger the size of the biopsy specimen, the better the accuracy of noninvasive markers of fibrosis suggesting that it is the accuracy of biopsy that limits the correlation with the new systems.[7,8] Clearly, cross-sectional comparisons with liver biopsy are not enough to determine the real usefulness of noninvasive markers, but rather longitudinal studies assessing their predictive value for clinical outcomes must be analyzed. These should include not only death and complications from portal hypertension, but regression of fibrosis with resolution of the underlying liver disease (*eg.* sustained virological response in viral hepatitis, alcohol abstinence in alcoholic liver disease, and improvement in metabolic conditions in non-alcoholic steatohepatitis [NASH]).

Because of their dynamic nature, chronic viral hepatitis C and B (CHC, CHB) are perhaps the two liver diseases that benefit the most from noninvasive assessment of fibrosis. Determining disease progression to clinically relevant fibrosis (CRF or F ≥ 2) or early stages of cirrhosis (F4) cannot be performed with routine tests and they are of great importance in daily clinical practice: CRF determines disease progression and the need for antiviral treatment, whereas cirrhosis has prognostic implications both for antiviral response and survival, and dictates the need to start screening tests for esophageal varices and hepatocellular carcinoma. Thus, both positive and negative predictive values (PPV, NPV) for F ≥ 2 and F4 are the most important parameters to consider. Because of the variation in the PPV and NPV according to prevalence of these stages among studies, a direct application of results from published studies is limited. In the ideal world, each liver center would know their F ≥ 2 and F4 prevalences

Table 2. Formulas and performance of blood markers for the estimation of clinically relevant fibrosis and cirrhosis.

Blood marker	Formula	Etiology, N	AUROC (95%CI) ≥ F2	CUV, PPV / NPV	AUROC (95%CI) = F4	CUV, PPV/NPV
APRI[10]	([AST/ULN]/platelets) x 100	CHC, > 4500	0.77 (SE = 0.012)	> 0.5, 55/69% > 1.5, 82/63%	0.83 (SE = 0.013)	> 1, 55%/69% > 2, 82%/63%
FIB-4[13]	(Age x AST)/(platelets x ALT$^{1/2}$)	CHC, 847	NE	NE	0.85 (0.82-0.89) ††	< 1.45, 39%/95% > 3.25, 82%/88%
FibroIndex[15]	1.738 - (0.064 x platelets¶) + (0.005 x AST) + (0.463 x γglobulin)	CHC, 240	0.86 (0.81-0.92)	≤ 1.25, 87/62% ≥ 2.25, 94/59%	NE	NE
Forns[16]	7.811 - [3.131 x ln (platelets)] + [0.781 x ln(GGT)] + [3.467 x ln(age)] - [0.014 x cholesterol]	CHC, 125	0.81	< 4.2, 40/96% > 6.9, 66/80%	NE	NE
ASPRI[18]	Age* + [(spleen diameter [cm]/platelets) - 100]	CHB, 346	NE	NE	0.91 (0.87-0.95) > 12, 96%/83%	< 5, 32%/100%
ELFG[19]	(-0.014 x ln[age])+(0.616 x ln[HA])+ (0.586 x ln [PIIINP]) + (0.472 x ln [TIMP1]) - 6.38	Varied, 921	0.78 (0.74-0.82)	NR	0.89 (0.84-0.94)	0.102, 35%/92%†† 0.826, 90%/77%
FibroSpect II[22]	Algorithm including HA, TIMP1, A2M	CHC, 294	0.83 (0.79-0.88)	> 0.36, 76/75%	NE	NE
Hepascore[25]	-4.185818 - (0.0249 x age) + (0.7464 x gender**) + (1.0039 x A2M‡) + (0.0302 x HA§) + (0.0691 x TB‖) - (0.0012 x GGT)]	Viral, 1,238	0.78 (0.75-0.80)	≥ 0.5, 84/58%	0.86 (0.83-0.88)	≥ 0.84, 43%/93%
Fibrometer[25]	- (0.007 x platelets†) - (0.049 x PI) + (0.012 x AST) + (0.005 x A2M‡) + (0.021 x HA§) - (0.27 x urea) + (0.027 x age) + 3.718	Viral, 1,204	0.79 (0.76-0.81)	0.411, 72/72%	0.86 (0.83-0.89)	0.442, 58%/92%
FibroTest[25]	(4.467 x $\log_{10}$A2M‡) - (1.357 x $\log_{10}$HGB‡) + (1.017 x $\log_{10}$GGT) + (0.0281 x age) + (1.737 x $\log_{10}$TB‖) - (1.184 x LPA1‡) + (0.301 x gender**) - 5.540	Viral, 1,197	0.78 (0.75-0.81)	0.48, 0.79/63%	0.82 (0.79-0.85)	0.74, 40%/93%
PGAA[34]	Sum of TP, GGT, LPA1 y A2M (0-4 points each one)	ALD, 624	NE	NE	ND	≥ 12, 92%/89%
NAFLD fibrosis index[39]	- 1.675 + (0.037 x age) + (0.094 x BMI) + (1.13 x Glu‡‡) + (0.99 x AST/ALT) - (0.013 x platelets) - (0.66 x albumin)	NAFLD, 253	NE	NE	0.84 (0.81-0.88)††	< -1.455, 52%/88% > 0.676, 82%/80%

Age is expressed in years, albumin in g/dL, ALT, AST and GGT in UI/L, cholesterol in mg/dL, γglobulin in g/dL, BMI in kg/m^2, platelets in 10^9/L, PI protrombin index in percentage, urea in mmol/L; unless otherwise stated. * Sum 1 point for every each 10 years starting at 30 years, until reaching 5 points in patients ≥ 70 years (< 30 years = 0). † In g/L. ‡In mg/dL. § In mcg/L. ‖ In mcmol/L. ¶ Expressed in 10^4/mm^3. **Gender: male = 1, female = 0. ††In this study comparison was between F0-2 and F3-4. ‡‡Glucose > 110 mg/dL or diabetes gives 1 point, whereas their absence equals 0. **A2M:** alpha-2-macroglobulin. **HA:** hyaluronic acid. **APRI:** AST/plaquetas ratio index. **ELFG:** European Liver Fibrosis Group. **HGB:** haptoglobin. **LPA1:** apolipoprotein-A1. **NE:** not evaluated. **NR:** not reported. **PIIINP:** amino-terminal propeptide of type III collagen. **SPRI:** spleen/platelets ratio index. **TB:** total bilirubin. **TIMP:** tissue inhibitor of matrix metalloproteinase 1. **ULN:** upper limit of normal.

Table 3. Possible caveats of noninvasive markers for the estimation of fibrosis and their effects on interpretation.

Limiting factor	Blood markers Effect
Hemolysis	Reduces haptoglobin → Fibrotest ↑
Gilbert syndrome	Increases bilirubin → Fibrotest ↑
Inflammatory condition	Increases α2–macroglobulin → Fibrotest ↑ Increases γglobulin → Fibroindex ↑
Postprandium, gastrectomy[9]	Increases hyaluronic acid → Fibrometer ↑
Active alcoholism	Increases GGT → Fibrotest ↑
Statin use, HCV genotype 3	Reduces cholesterol → Forns ↑
Limiting factor	Transient elastography Effect – Possible solution
BMI ≥ 28 - 30 kg/m^{2*}	Failure - Use XL - probe
Narrow intercostal space	Failure or TE ↑ - Patient reposition
Ascites	Failure - Assess need for TE, diuretics
Elevated ALT	TE ↑- Repeat after acute event improves
Hepatic infiltration	TE ↑- Assess need for TE, alternative method
Retrogade vascular congestion	TE ↑- Consider alternative method
Extrahepatic cholestasis	TE ↑- Consider alternative method

*Likely the main reason for failure in these patients is an increased thoracic adiposity, which can better be defined as a thoracic girth > 110 cm.

(pre-test probability), which would allow for more accurate use of the noninvasive markers of fibrosis (higher prevalence increases PPV but decreases NPV, and viceversa).

BLOOD MARKERS

Blood markers used in the estimation of liver fibrosis can be classified as direct, when they measure components derived from the extracellular matrix or hepatic stellate cell (*e.g.* hyaluronic acid, α-2-macroglobulin), or indirect, when they are molecules released by hepatic parenchyma after fibrosis injury (*e.g.* cellular damage [GGT, AST], are indicative of compromised hepatic synthesis [bilirubin, INR], or are markers of portal hypertension [platelets, gamma globulin]). Even though direct markers allow an earlier assessment of liver fibrosis, they lack specificity as non-hepatic scarring processes can yield positive results. It is generally considered that the combination of direct and indirect markers can provide a better estimation of fibrosis, as they combine the need for both sensitive and specific parameters. Given that direct markers sometimes require sophisticated laboratory techniques, it is advisable to request them at reference laboratories with good quality control and standardization processes; otherwise, proposed cut-off points may inadvertently be modified.

None of the existing blood markers fulfills criteria as an ideal noninvasive marker of fibrosis: simple, accesible, low cost, accurate and reliable. In spite of this, available methods offer many advantages. Many panels have been described to date but only those considered most relevant due to either popularity or good performance characteristics, will be described (Table 2). Also relevant are the clinical situations for which noninvasive markers are less valid, or possible pitfalls with suggestions to improve accuracy.[9] Some of these are shown in table 3.

Viral hepatitis

The APRI (AST to platelet ratio index) combines two biological phenomena that occur during progression to cirrhosis and portal hypertension: the increase in AST and decrease in platelet count, respectively. Given its simplicity it continues to be very useful in clinical practice, even though a recent meta-analysis including 40 studies showed some limitations according to the reported PPV and NPV.[10] APRI has been validated for CHC and CHB, as well as for coinfection with HCV/HIV.[10,11] FIB-4 is another simple method which makes use of the biological phenomena of APRI, considering the concomitant decrease in ALT, while AST increases. This was originally designed to be used in HCV/HIV coinfection and was validated against the Ishak fibrosis scale,[12]

but has now been validated for CHC and CHB with METAVIR as well.[13,14] The limitation of APRI or FIB-4 in CHC is the lack of ability to differentiate early stages of fibrosis (F0-2), however they are useful to detect advanced fibrosis (F3-4) with high values in the area under the receiver operator characteristic curve (AUROC) reported.[13] For CHB, a PPV of 91% and NPV of 93% for the diagnosis of cirrhosis was reported.[14] Fibroindex adds to AST and platelet count the determination of gamma globulin under the assumption that this increases in the presence of portosystemic shunting. One might expect that the test is not appropriate to evaluate early stages of fibrosis, however it showed a high AUROC with a PPV of 94% for the detection of CRF in CHC.[15] Forns' index is based on patient age, which is associated with more advanced fibrosis particularly in CHC; GGT as a marker of biliary damage, and thus commonly elevated in advanced fibrosis; and cholesterol, which decreases due to impaired synthetic capacity or as a direct cytopathic viral effect (more evident with genotype 3).[16,17] The major utility of the Forns index is to rule out CRF with an NPV of 96%. It was been validated for both CHC and CHB. ASPRI (Age-Spleen-Platelet Radio Index), validated in CHB, uses patient's age and an ultrasonographic parameter –spleen diameter– in combination with platelet count (from its predecesor SPRI).[18] This system is based on clear pathophysiologic principles of portal hypertension and may be very useful in ruling in/out cirrhosis in CHC and CHB. However, two possible caveats are the operator-dependent reliability of ultrasonographic spleen measurement, as well as variations in body composition that may affect measurement or the normal relation with platelet count.

Unlike the previously described estimators that were based only on indirect markers, there are two that exclusively combine direct markers. The ELFG (European Liver Fibrosis Group) measures hyaluronic acid (HA), amino-terminal propeptide of type III collagen (PIIINP), the tissue inhibitor of matrix metalloproteinase 1 (TIMP1), as well as age. The first two biomarkers are components of the extracellular matrix and the third one is a fibrogenesis regulator that inhibits collagen degradation and hepatic stellate cell apoptosis. ELFG has shown a good AUROC for the identification of cirrhosis in CHC, presumably with a high NPV given the reported sensitivity of 91%.[19] A more recent study demonstrated that eliminating age from the formula does not change the usefulness of the test, and now this is known as ELF (Enhanced Liver Fibrosis).[20] Fibrospect II (Prometheus Labs., San Diego, CA) uses an algorithm including HA, TIMP1 and α-2-macroglobulin (A2M). The latter is a protease inhibitor expressed by the hepatic stellate cell upon activation.[21] The validation study of Fibrospect II in CHC found a fair AUROC but reported PPV and NPV that are difficult to use in clinical practice.[22] Notably, a subsequent study using quantitive measurements of fibrosis did not show improved accuracy for this tool.[23] The major issue with either ELF or Fibrospect II is the relatively limited accessibility of the tests required in general clinical practice.

Among the tests combining direct and indirect markers of fibrosis, three tests are remarkable for their consistency in multiple studies and some meta-analyses: Hepascore, Fibrometer and Fibrotest. Hepascore uses demographic factors, makers of biliary damage and hepatic synthesis, as well as HA and A2M.[24] Fibrometer (BioLiveScale) combines age, indirect markers of portal hypertension, cellular damage and hepatic synthesis (including urea), with the same direct markers as Hepascore: HA and A2M.[7] Fibrotest (Biopredictive, Paris, Francia; Fibrosure-Labcorp, Burlington, VT) is probably the most studied noninvasive marker of fibrosis and it includes demographic factors, indirect markers for biliary damage and hepatic synthesis (bilirubin, apolipoprotein-A1 [LPA1], haptoglobin [HGB]), and A2M as a direct marker. FIBROSTIC was a multicentric (23 centers) study performed in France directly comparing these 3 methods in 1,307 patients with viral hepatitis (913 with CHC, 284 with CHB and 110 coinfected with HIV).[25] According to the AUROC reported in FIBROSTIC, the 3 tests have a similar clinical usefulness for both the diagnosis of CRF and cirrhosis. However, none of the tests was ideal. By taking a careful look at the PPV for the diagnosis of CRF, 16 to 28% of patients would erroneously be advised to receive antiviral treatment despite having only stage F0-1 disease (false positives); whereas based on the NPV, 25 to 42% of patients would be misclassified as having mild disease (F0-1) (false negatives) and may forego antiviral therapy. The results for cirrhosis are not much better. With the low PPV, about 50% of patients would be unnecessarily included in surveillance protocols for esophageal varices and hepatocellular carcinoma (false positives); importantly however, the NPV was good with only about 7 to 8% of patients with cirrhosis who would be missed and therefore not get appropriate surveillance (false negatives). However, it must be mentioned that the prevalence of cirrhosis in this study was rather low (14%), which likely

caused underestimation of the PPV and overestimation of the NPV.[25] Similar results have been reported in other studies and meta-analyses (including a recent one for CHB).[26-29] At least part of the discordance in AUROC results between studies can be explained by 'spectrum bias': variations in the representation of the different stages of fibrosis (*e.g.* prevalence of F4 oscilates between 10% and 25% among studies), and their transformation from an ordinal to a binary scale (F0-4) by clustering adjacent stages (F0-1 vs F2-4 or F0-3 *vs.* F4). When statistical methods adjusting for spectrum bias are used (DANA, Obuchowski),[30] a modest improvement in the 'real accuracy' of blood markers has been observed for the 3 tests with AUROC for the diagnosis of CRF increasing from 0.80-0.82 to 0.83-0.85 after adjustment.[31] Accumulation of future studies assessing blood markers for the noninvasive estimation of fibrosis with ajustment for spectrum bias may provide a better understanding on their accuracy.

Other liver disease

In both alcoholic liver disease (ALD) and NAFLD fibrosis deposition differs from viral hepatitis in that it starts with a perisinusoidal and perivenular distribution instead of the classical periportal fibrosis[32] (Table 1). This phenomenon may cause blood markers designed for viral hepatitis to have different performance characteristics when applied to ALD and NAFLD. Not only may the sensitivity, specificity, PPV and NPV change but the thresholds used to rule in or out various degrees of fibrosis may also differ between diseases. These differences may be important clinically to ensure that the decisions are made with the specific test characteristics for a given disease in mind. Arguably, there may be some advantage to developing specific markers for diseases with different patterns of fibrogenesis.

Several panels have been specifically developed or validated for alcoholic liver disease (ALD). The PGA index, named after its components: protrombin, GGT and LPA1, was initially developed for ALD. The diagnostic accuracy was improved with the addition of A2M, hence becoming PGAA. Because of the methodology employed in these studies it is difficult to ascertain the accuracy for the diagnosis of CRF, but PGAA is a good discriminator for cirrhosis with PPV and NPV close to 90%.[33,34] Remarkably, Fibrometer, ELFG and Fibrotest have all been validated in ALD, showing better performance than what has been observed in CHC. The reported AUROC for ALD and CHC, respectively were: Fibrometer 0.92 (SE = 0.03) and 0.83 (SE = 0.02); ELFG 0.94 (SE = 0.06) and 0.77 (SE = 0.04); and Fibrotest (meta-analysis) 0.88 (0.81-0.84) and 0.79 (0.76-0.82).[7,19,27] Recently, one study compared the accuracy of Fibrotest, Fibrometer and Hepascore and did not find differences among them for the diagnosis of alcoholic cirrhosis: Fibrotest and Fibrometer AUROC = 0.94 ± 0.02, Hepascore AUROC = 0.92 ± 0.02, and were significantly greater than those of nonpatented biomarkers (APRI, Forns, FIB4; $P < 0.01$).[35] Another recent study showed relatively limited usefulness of the Forns index in ALD.[36]

HA by itself has also shown adequate diagnostic usefulness and it outperformed Fibrotest for the diagnosis of CRF (AUROC 0.94 and 0.83, respectively) but not for cirrhosis (AUROC 0.93 and 0.95, respectively), according to one study.[37] Finally, serum markers of hepatocyte death and apoptosis have recently been evaluated in fibrosis progression among alcoholic patients. Lavallard, *et al.* found that citokeratin 18 (CK18) and its fragments showed good diagnostic accuracy (AUROC 0.84, 95% CI 0.76-0.90 and 0.76, 95% CI 0.66-0.83; respectively) for predicting advanced fibrosis among heavy drinkers.[38]

The case of NAFLD is similar to ALD as it has been studied both with panels developed specifically based on its pathophysiology, as well as with the other existing panels that have been validated for this disease. Among the specific tests, the NAFLD fibrosis index stands out as it considers age, BMI, glucose and parameters of cellular damage, synthetic function, and portal hypertension. It allows for the correct classification of advanced fibrosis (F3-4) with a good AUROC and a high NPV of 88%.[39] The BARD index is very similar to the previous one, also considering anthropometry and glucose, and has yielded similar results for the discrimination of advanced fibrosis.[40] Fibrotest has been validated for NAFLD with an AUROC resembling CHC, but different cut-off points are used to classify patients: a result < 0.3 excludes $F \geq 2$ in 90% of cases, whereas > 0.7 confirms it in 73%.[41] The validation of Fibrometer and ELFG in NAFLD yielded improved accuracy when compared to CHC (Fibrometer AUROC 0.94, ELFG AUROC 0.87),[19,42] similarly to what was described with ALD; and ELF has proved to be equally useful to its parent panel ELFG in predicting fibrosis in NAFLD.[43] Although these nonspecific panels seem to outperform NAFLD-specific indexes, direct comparisons have not been reported in the same study. In a study comparing FIB-4 with both NAFLD fibrosis and BARD indices, the former yiel-

Table 4. Performance of studies using elastography for the estimation of clinically relevant fibrosis and cirrhosis.

Elastography	Etiology, N	AUROC (95%CI) ≥ F2	CUV, PPV/NPV	AUROC (95%CI) = F4	CUV, PPV/NPV
ET–FS[54]	CHC, 183	0.83 (76–0.88)	7.1, 95%/48%	0.95 (0.91–0.98)	12.5, 77%/95%
ET–FS[55]	CHC, 251	0.79 (0.73–0.84)	8.8, 88%/56%	0.97 (0.93–1.0)	14.6, 78%/97%
ET–FS[56]	CHB, 173	0.81 (0.73–0.86)	7.2, 80%/73%	0.93 (0.82–0.98)	11.0, 38%/99%
ET–FS[25]	Viral, 1307	0.76 (0.74–0.79)	5.2, 65%/72%	0.90 (0.87–0.92)	12.9, 53%/95%
ET–FS[63]	ALD, 103	0.91 (0.85–0.98)	7.8, 93%/70%	0.92 (0.87–0.98)	19.5, 69%/88%
ET–FS[62]	NAFLD, 246	0.84 (0.79–0.90)	7.0, 70%/84%	0.95 (0.91–0.99)	10.3, 46%/99%
ERM[69]	Varied, 141	0.99 (0.98–1)	2.5, 93%/100%	0.99 (0.99–1)	4.1, 86%/100%

ded better results;[44] and another study showed Hepascore, Fibrotest and FIB-4 to have better accuracy than both BARD index and APRI.[45]

In autoimmune and other liver diseases there is a paucity of studies of noninvasive estimation of liver fibrosis through blood tests. APRI, FIB-4, Fibroindex and Fibrotest have shown to have limited usefulness in autoimmune hepatitis and primary biliary cirrhosis, although the Forns index looked to be promising for the identification of cirrhosis.[46-48]

TRANSIENT ELASTOGRAPHY OF THE LIVER

TE measures the stiffnes or ability of a tissue not to undergo deformation when mechanical stress is applied to it, by measuring the propagation velocity of a shear wave within the tissue: the stiffer the tissue the faster the shear wave propagates. The principle is similar to percusion during clinical exam in that we can identify the content of a tissue (*i.e.* solid, liquid, gas) according to the audible feedback we generate. With the Fibroscan (FS) (Echosens, Paris, France), a vibrating probe with low frequency and amplitude (50 MHz) is mounted to an ultrasound transducer (3.5 MHz). The probe generates a shear wave or elastic wave that propagates through the hepatic tissue and the ultrasound transducer captures the wave. The data are processed to express the elastic wave as a function of time represented in an elastogram, and stiffness is provided as a numeric output expressed in kilopascals (kPa). The results range from 2.5 to 75 kPa. The probe assesses a total hepatic volume of 3 cm^3, greater than 100 times the size of a liver biopsy, thus theoretically reducing sampling error. With the standard probe, measurement starts at a depth of 2.5 cm from the skin (up to 6.5 cm in depth) and hence the evaluation in patients with an excess amount of fat over the right hypochondrium may be limited.[49]

TE offers some advantages over other noninvasive markers of fibrosis: the procedure is easy to perform, is relatively operator-independent, is easy to learn (> 100 cases), and provides immediate results.[49] Moreover, it is a reliable method with an intra-class correlation coefficient of 0.98, particularly in stages F ≥ 2.[50] However, caveats remain, some of which are shown in table 3.

TE determinations should be considered valid when there are 10 succesful measurements with a success rate > 60%, and when the interquartile range (IQR) of all measurements is ≤ 30% of the value of the median (IQR/M < 30%); although more recent data suggested that an IQR/M < 21% would increase the accuracy of the test.[51] According to a study including 7,261 patients, it was not possible to obtain at least one measurement in 4% of individuals, and in 17% measurements were unreliable, mostly in relation to an IQR/M > 30%. The main limiting factors leading to a failed study were the lack of experience of the operator and a BMI > 28 kg/m^2.[52] For heavier patients a new probe (XL) allowing examinations at a depth from 3.5 to 7.5 cm below the skin has been designed, increasing the rate of succesful measurements. The validation study showed that the rate of failed measurements could be decreased from 16% with the standard probe to 1.1% with the XL probe, in patients with a BMI ≥ 28 kg/m^2.[53] However, even with the XL-probe BMI continues to be the main factor favoring discordance with biopsy results, particularly in patients with a BMI ≥ 40 kg/m^2.[54]

Table 4 summarizes some of the published studies regarding the noninvasive estimation of liver fibrosis with TE. As can be observed the cut-off points used for CRF and cirrosis vary among studies. A recent meta-analysis evaluated the diagnostic accuracy of TE showing that the sentitivity and specificity for the diagnosis of CRF (31 studies) were 79% (95%CI: 74-82%) and 78% (95%CI: 72-83%), respectively, and the median of reported cut-off values was 7.2 kPa (range of 4 to 10.1 kPa). For the diagnosis

of cirrhosis (30 studies) sensitivity was 83% (95%CI: 79-86%) and specificity 89% (95%CI: 87-91%), with a median cut-off of 14.5 kPa (range 9 to 26.5 kPa).[55]

Viral hepatitis

TE by means of FS has proved to be very useful for both CHC and CHB. As shown in table 4, the AUROC are particularly high for cirrhosis but less accurate for the diagnosis of CRF. This is related to some overlap among adjacent stages that is more evident in the low spectrum of fibrosis. Although the clinical application for CRF seems to be somewhat limited, in cirrhosis the NPV are all ≥ 95%.[56-58] Whether statistical methods adjusting for the spectrum bias (DANA, Obuchowski) will be useful for TE is unknown, but such an approach seems rational and a recent study also showed a modest improvement when standard AUC were compared to Obuchowski (0.82 *vs.* 0.84, respectively).[31] Although the aforementioned meta-analysis showed that the cut-off values to discriminate CRF or cirrhosis were slightly higher for CHC than for CHB, it must be considered that TE readings are elevated during ALT flares in CHB and they return to baseline after normalization of ALT.[59,60] Some authors have proposed to use a cut-off point of 10.1-12 kPa for F4 when ALT is normal, and another of 13.4-15.5 kPa when ALT is above the upper limit of normal.[61,62]

Other liver diseases

Despite some controversies about the effect of steatosis on TE results,[57,63,64] this technique has been used for fibrosis evaluation among patients with ALD and NAFLD showing good diagnostic accuracy, comparable to that of viral hepatitis[64,65] (Table 4). Other studies have shown similar results[36,53,66,67] and support the utility of TE in these patients. Different cut-off values for cirrhosis have been suggested for ALD/NAFLD than for viral hepatitis, which may reflect differences in the fibrogenic process between diseases. Similar to CHB flares, severe steatohepatitis, particularly in ALD, may also affect the TE results.

Recently, variation in the accuracy of TE as a function of AST levels was evaluated in patients with alcoholic steatohepatitis. By performing sequential TE before and after of alcohol detoxification, Mueller, *et al.* could observe a parallel decrease of both TE and AST, with the former decreasing more than 3.5 kPa in patients arriving with an AST ≥ 100 U/L, after a fall in AST. In a second cohort of patients it was confirmed that the lower the AST at the time of assessment the more accurate the TE results.[68] This study provides evidence that, in patients with ALD, a more accurate noninvasive assessment of fibrosis stage by TE can be achieved if the degree of steatohepatitis is considered. However, validation in large and independent series is still needed before making specific recommendations in routine clinical practice.

As with blood markers, there is a lack of validation studies for TE in autoimmune and other liver diseases. Nonetheless, and in spite of being diseases of heterogeneous involvement within the liver, TE has shown to be useful in a limited sample of patients with either primary biliary cirrhosis or primary sclerosing cholangitis.[69]

Magnetic resonance elastography and other imaging modalities

Magnetic resonance elastography (MRE) uses the same principles as TE, differences being that the low amplitude (60 MHz) vibrating signal is provided by a pneumatic transducer positioned over the right hypochondrium (or last ribs at the back) and the elastic waves are captured by the magnetic resonance machine. With this method the elastogram can involve the whole liver instead of only the 3 cm^3 of FS, and it is not limited by a narrow intercostal space, also allowing the evaluation of obese patients and those with ascites.[70] Nevertheless, the technique suffers from the same drawbacks of all magnetic resonance approaches, such as the longer-time for acquisition and interpretation of results, and elevated cost. In addition, it cannot be used to assess patients with significant iron overload because of signal-to-noise limitations. Table 4 shows the performance characteristics of a representative study using MRE. This study also reported on the superiority of MRE over FS-TE, particulary at early stages of fibrosis (AUROC for CRF with MRE was 0.99 *vs.* 0.84 with FS-TE; for cirrhosis with MRE 0.99 *vs.* 0.93 with FS-TE), due to better discrimination of adjacent stages; and it also showed improved reliability.[71] Given the very high predictive values reported by this study for both CRF and cirrhosis, MRE seems to be a very promising tool for noninvasive estimation of fibrosis and confirmatory large studies are awaited. However, availability and cost may be important limiting factors even in specialized centers.

There are other imaging techniques to assess fibrosis noninvasively: diffusion-weighted imaging

through magnetic resonance; and acoustic radiation force impulse (ARFI) and real-time elastography, which make use of ultrasound technology. A study with diffusion-weighted imaging showed it to be inferior to MRE,[72] and in the case of ARFI[73-75] most of the available data does not support that it outperforms TE, although it is more accurate than APRI.[76] However, a recent study used ARFI to evaluate liver stiffness in 20 different spots of the right lobe (segments V to VIII) in patients with CHC, providing a larger and more varied sample than TE. With this novel approach authors could demonstrate superiority of ARFI over TE, particularly for the diagnosis of CRF and advanced fibrosis.[77] This may be especially useful for liver diseases with a heterogenous distribution such as primary biliary cirrhosis and primary sclerosing cholangitis. It will be interesting to follow the performance of ARFI in future studies, as access to this technology may be favored over FS-TE and MRE in some centers.

COMBINING BLOOD MARKERS AND TRANSIENT ELASTOGRAPHY

Tables 2 and 3 clearly demonstrate improved performance of noninvasive tests for the diagnosis cirrhosis over CRF. The main issue for all tests is discrimination between adjacent stages of fibrosis. Fibrotest is more accurate in the lower range of fibrosis stages (F0 *vs.* F1), while TE performs better with more advanced fibrosis (F3 *vs.* F4).[26] Moreover, some of the predictive values seem to complement each other supporting the concept that the combination of a blood marker and TE would yield greater diagnostic accuracy. This has been investigated for CHC[78] and CHB.[61,62] In patients with CHC, a report showed that an algorithm combining APRI and Fibrotest correctly classified 97% of patients with CRF (PPV 96%, NPV 100%), and would have avoided 48% of biopsies; whereas an algorithm combining Fibrotest and FS correctly classified 96% of patients with cirrhosis (PPV 95%, NPV 96%) and would have avoided 79% of biopsies.[79] These results make the combination of noninvasive markers of fibrosis look very attractive for daily clinical practice. Moreover this has been confirmed by a more recent publication with a larger sample, also proposing a new noninvasive algorithm combining Fibrometer and FS-TE with very promising results.[80] It is worth mentioning that the new clinical practice guidelines from the European Association for the Study of Liver on CHC have endorsed the use of noninvasive markers of fibrosis, and recommend to combine them to increase diagnostic accuracy.[81]

Longitudinal studies with noninvasive markers of fibrosis

Sustained virological response (SVR) after treatment with interferon and ribavirin in CHC patients is associated with regression of fibrosis after long-term follow up. A few studies have evaluated the change in noninvasive markers of fibrosis after antiviral treatment, noting that in patients who achieve an SVR there is a significant decrease (APRI, FIB-4, Forns, ELF, Fibrotest, Fibrospect II) not observed in those without an SVR.[82-85] The improvement in Fibrospect II and ELF as early as 24 weeks after the end of treatment suggests reversion of the profibrogenic environment in the liver, as these panels include molecules involved in matrix remodelling and stellate cell activation.[83,85] Perhaps not surprisingly, it has been shown that low scores from blood markers before the start of antiviral treatment are predictive of treatment response, similar to what has been described for early stages of fibrosis on liver biopsy.[83,85,86]

With viral clearance, there is also evidence of improvement in TE 24 weeks after the end of treatment, although the effect seems to be low to moderate.[84,87] One study that performed TE at baseline and 2 years after antiviral treatment demonstrated a clear decrease in patients who achieved an SVR (10.3 and 5.4 kPa, $p < 0.001$, respectively), but not in patients who continued to be viremic (10 and 11.3 kPa, respectively).[88] Also, a long-term follow up study (14 years) of CHC patients with advanced fibrosis treated with antiviral treatment showed a significant difference in liver stiffness between patients with and without an SVR (7 kPa [5-11] *vs.* 17 Kpa [10-29 kPa], $p < 0.001$, respectively).[89] In the case of CHB there is one study showing improvement in TE reading after 12 months of treatment with entecavir (11.2 kPa baseline and 7.8 kPa at two years, $p = 0.009$).[90] Although a confirmatory liver biopsy was not required in these studies, the results shown strongly support the concept of regression of fibrosis as a consequence of viral eradication/control in patients with CHC or CHB. Whether the identification of regressed fibrosis with these methods translates into improved prognosis is still unknown but would influence how we follow patients. Prospective studies aiming to detect improved outcomes (*i.e.* decreased rate of decompensation, hepatocellular carcinoma or death) along with the

degree of change in noninvasive markers will allow identifying what the clinically important change is. This is particularly relevant as it is conceivable thinking that the initial improvement in noninvasive markers of fibrosis after viral eradication/control is to some extent related to resolved inflammatory activity, and not to regressed fibrosis. Long-term follow up studies may clarify things and set the stage for accurate prognostication with noninvasive markers after regressed fibrosis.

The association between noninvasive markers of fibrosis and portal hypertension or robust outcomes (*i.e.* death and complications from portal hypertension) has also been assessed. There is evidence showing that TE correlates with HVPG ($\rho = 0.858$, $p = 0.001$), and a TE measurement of > 21 kPa had a PPV of 93% and NPV of 91% for predicting an HVPG ≥ 10 mmHg, according to one study including 150 patients with varied etiologies of liver disease.[91] Follow up of these patients showed that TE (same cut-off value of > 21 kPa) was also useful to predict complications from portal hypertension such as development of ascites, hepatic encephalopathy, variceal bleeding, hepatocellular carcinoma and death.[92] Although indirectly assessing liver fibrosis, spleen stiffness measured by elastography better correlates with HVPG when compared to liver stiffness,[93] and it is another promising method for identifying clinically relevant portal hypertension in the form of gastroesophageal varices.[94-95] The association with hepatocellular carcinoma and death was also recently shown for TE readings > 10kPa in patients with CHB.[96]

The prognostic significance of the blood panels has also been evaluated. The correlation between Fibrotest and HVPG is moderate ($\rho = 0.58$, $p = 0.001$),[97] but a Fibrotest determination ≥ 0.58 is associated with the development of complications from portal hypertension and increased mortality in CHC patients.[98] Similar findings have been reported for Fibrotest in CHB,[99] and for ELF in primary biliary cirrhosis.[100] In ALD a Fibrotest value > 0.58 is also associated with higher mortality, and this effect is independent of liver biopsy stage, whereas Fibrometer A and Hepascore did not show any independent effect on mortality.[35]

A recent study evaluated liver biopsy, blood markers and TE as predictors of 5-year mortality in patients with CHC. As seen in table 5, both Fibrotest and TE were associated with mortality, outperforming the prognostic value of fibrosis stage by liver biopsy, and independently of antiviral treatment response.[101] Thus, noninvasive markers of fibrosis can predict both complications from portal hypertension and mortality even once cirrhosis is identified, a major benefit over liver biopsy. This can be explained by the fact that they can differentiate early from advanced cirrhosis (*i.e.* stage 1 *vs.* stages 2-4), due to the lack of a ceiling effect as observed with liver biopsy after reaching F4.

Table 5. Multivariate model for the association between noninvasive markers of fibrosis and 5–year mortality in chronic hepatitis C.

Pronostic marker	RR (95%CI)	P
TE	2.9 (2.0–4.3)	< 0.0001
Fibrotest	60 (14–255)	< 0.0001
Actitest	0.19 (0.07–0.53)	0.002
Antiviral treatment	0.28 (0.19–0.42)	< 0.0001
Age	1.03 (1.01–1.04)	0.002

N = 1457, including 89 deaths. RR: relative risk. CI: confidence interval.

CONCLUSIONS

Noninvasive markers of fibrosis are making their way into daily clinical practice, predominantly for the identification of CRF and/or cirrhosis. Their accuracy and reliability are good enough to be used in the clinical field and may be underestimated due to imprecision of the 'gold standard' to which they are compared (i.e. liver biopsy). Use of non-invasive tests provides conclusive enough results to avoid half of the liver biopsies performed for staging of fibrosis. It is critical to remember that non-invasive markers perform differently in distinct clinical scenarios and therefore it is necessary to consider different cut-off values with their associated PPV and NPV for each clinical scenario, and in some instances for each specific liver disease. Although most of the approaches have fairly good test characteristics, the greatest accumulated experience exists with APRI, Fibrotest, Fibrometer, TE and MRE. Results may be improved with all tests with knowledge of clinical situations that may lead to test failure as well as possible solutions. The combination of noninvasive markers increases their diagnostic performance and therefore it is useful to consider more than one test, either in sequential or parallel algorithms, according to the information they provide (complementary tests with either high sensitivity or high specificity, or strengthening of results when two tests with similar performance coincide). Noninvasive markers provide a major advance over liver biopsy in terms of the ability to do longitudinal

evalutions, particularly given their utility in predicting clinical events and even mortality. It has taken a remarkable international effort to develop the various noninvasive approaches available today and the future holds promise of improving current tools as well as the development of novel approaches. Liver biopsy still has a major diagnostic role for the hepatologist, but its days as the gold standard for fibrosis evaluation and prognostication may be numbered.

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