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TOPIC HIGHLIGHT

2015 Advances in Cirrhosis

Non-invasive diagnosis of liver fibrosis and cirrhosis

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Abstract

The evaluation and follow up of liver fibrosis and cirrhosis have been traditionally performed by liver biopsy. However, during the last 20 years, it has become evident that this "gold-standard" is imperfect; even according to its proponents, it is only "the best" among available methods. Attempts at uncovering non-invasive diagnostic tools have yielded multiple scores, formulae, and imaging modalities. All are better tolerated, safer, more acceptable to the patient, and can be repeated essentially as often as required. Most are much less expensive than liver biopsy. Consequently, their use is growing, and in some countries the number of biopsies performed, at least for routine evaluation of hepatitis B and C, has declined sharply. However, the accuracy and diagnostic value of most, if not all, of these methods remains controversial. In this review for the practicing physician, we analyze established and novel biomarkers and physical techniques. We may be witnessing in recent years the beginning of the end of the first phase for the development of non-invasive markers. Early evidence suggests that they might be at least as good as liver biopsy. Novel experimental markers and imaging techniques could produce a dramatic change in diagnosis in the near future.

Key words: Liver; Fibrosis; Cirrhosis; Non-invasive; Serum biomarkers; Ultrasonography; Computerized tomography; Magnetic resonance imaging

Core tip: Liver fibrosis (leading to liver cirrhosis), and not inflammation and cytolysis, is the main cause of liver disease-associated morbidity and mortality. During the last 20 years, it has become evident, even to its proponents that liver biopsy, is no longer the "gold-standard". At most, it is the old standard. Non-invasive diagnostic scores, formulae, and imaging modalities, all of which can be repeated as often as required, are cheaper, better tolerated, safer, and more acceptable

to the patient than liver biopsy. Although their accuracy is still controversial, early evidence indicates that they might be at least as good as liver biopsy.

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INTRODUCTION

Until the mid-20th century, chronic liver diseases could be diagnosed ante mortem with certainty only at a very advanced stage, usually after the onset of cirrhosis[1,2]. The first quantifiable noninvasive markers of liver disease were serum levels of liver enzymes. Alkaline phosphatase and transaminases became available in 1930 and 1955-1956, respectively. The spectrum of chronic liver disease expanded, and the submerged part of the chronic liver disease "iceberg" became known. Almost simultaneously (1958) Menghini introduced his "one second liver biopsy" technique and needle. Examination of liver tissue "Intra Vitam" became possible and contributed to the exposure of additional hidden parts of the iceberg, both qualitatively and quantitatively. Various imaging techniques came later and contributed their share.

The introduction of more and more efficient therapeutics in the 1980's transformed hepatology from a mainly descriptive discipline into an active one, able to cure many patients. More precise quantitation of the degree of liver damage became necessary for "To treat or not to treat" decisions, but neither liver biopsy (LB)[3] nor single parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), or platelet numbers were adequate. Standing on the shoulders of giants, Child and Turcotte[4] and Maddrey *et al*[5], investigators combined the power of single parameters by inserting them into various scores and formulae, thus greatly improving their predictive power.

The following review is a practical guide for the clinician.

OVERVIEW OF LIVER FIBROSIS

Liver fibrosis, leading to liver cirrhosis, is the result of several processes, which include the stimulation of fibrogenesis [extracellular matrix (ECM) synthesis] and regulation of fibrolysis (ECM degradation)[6,7]. It is initiated by a variety of insults leading to the death of hepatocytes, prominently viral infections [hepatitis B virus (HBV), hepatitis C virus (HCV)], alcohol, and diet [non-alcoholic fatty liver disease (NAFLD)]. This leads to activation of hepatic stellate cells (HSCs), which is the main mechanism leading to liver fibrosis[8]. HSCs are a main storage for retinol, a precursor of vitamin A, and control ECM turnover by secretion of matrix metalloproteinases (MMPs) and MMP-inhibitors (TIMPs). Three stages are involved in the fibrogenic process through HSC activation: a pre-inflammatory phase of HSC activation by dying hepatocytes, an inflammatory phase, when HSCs are further stimulated to transdifferentiate to myofibroblasts (MFB)[9], and a postinflammatory phase, when MFBs secrete stimulating cytokines and ECM components. These cytokines can stimulate MFBs and HSCs, creating a positive feedback loop that perpetuates the fibrogenic process. The main cytokine mediating this effect is transforming growth factor beta (TGF-β)[10]. TGF-β stimulates ECM gene expression and decreases ECM degradation by downregulation of MMPs and upregulation of TIMPs. HSCs can also be activated through oxidative stress in the form of reactive oxygen species, an important pathway in alcoholic liver injury, NSFLD, and iron overload. The oxidative species can also be produced by activated Kupffer cells. MFBs change the structure of the ECM by altering the types of deposited collagen, laminin, glycoproteins, and proteoglycans (for example heparan sulfate). Changes in the secretion and degradation of ECM components are used as biomarkers for some of the noninvasive screening techniques. The change in ECM structure, in turn, increases ECM stiffness, a change that is measured in some of the physical techniques for noninvasive diagnosis of liver fibrosis.

SERUM MARKERS

Many of the serum markers are enzymes that are measured in routine laboratory tests but are not specific to the liver and can be released upon inflammation of other tissues.

Others are secreted molecules, such as bilirubin, alpha-fetoprotein, alpha-2-macroglobulin, haptoglobin and apolipoprotein A1.

Albumin is specifically secreted from the liver, and its levels are reduced mainly in severe liver disease but also in other clinically relevant diseases (inflammatory diseases, renal diseases with significant proteinuria, malnutrition, protein losing enteropathy). Therefore, although albumin is a good indicator of ill health, it lacks specificity for liver disease.

None of these markers is of much use by itself but are useful when combined in marker panels[11,12].

Combinations of biomarkers or marker panels have been established in recent years for clinical use. The most common ones are summarized in Table 1. They are all based on indirect biomarkers (see below), except for hyaluronic acid or hyaluronan (HA) and panels that include it (Fibrometer and Hepascore).

Although some of these markers, or combinations of them, are now established in clinical use, their prognostic value is not clear-cut. They are increasingly useful in the exclusion of advanced fibrosis and

Table 1 Diagnostic accuracy of established serum markers

Test	Parameters	Prognosis	Sensitivity	Specificity	AUROC
APRI	AST, platelet count	Significant fibrosis	81	55	0.77
		Cirrhosis	77	75	0.84
FIB-4	Platelet count, AST, ALT, age	Significant fibrosis	64	68	0.74
		Cirrhosis	90	58	0.87
Fibrotest	Haptoglobin, α2-macroglobulin, apolipoprotein A1, γGT, bilirubin	Significant fibrosis	92	38	0.79
		Cirrhosis	83	76	0.86
Forns Index	Age, platelet count, γGT, cholesterol	Significant fibrosis	88	52	0.76
		Cirrhosis	98	27	0.87
HA	hyaluronan	Significant fibrosis	-	-	0.75
		Cirrhosis[1]	65	86	0.92
HepaScore	Bilirubin, γGT, hyaluronan, α2-macroglobulin, age, gender	Significant fibrosis	66	79	0.79
		Cirrhosis	72	86	0.89
Fibrometer	Platelet count, prothrombin index, AST, α2-macro-globulin, hyaluronan, urea, age	Significant fibrosis	69	81	0.82
		Cirrhosis[1]	62	87	0.90

[1]All values are medians. Except for these values, which were taken from Ref. [11], all other values are from Ref. [42]. APRI: AST to platelet ratio index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase, γGT: γ-glutamyltransferase.

cirrhosis but do not distinguish well early and intermediate stages of fibrosis, a problem shared with the past "gold standard" - LB[3,13].

However, some novel experimental markers hold promise of improving this noninvasive diagnostic ability in the near future.

The biomarkers can be divided into direct and indirect markers. Direct biomarkers reflect the changes in the ECM structure, including markers of ECM turnover, fibrogenesis, and fibrolysis. Indirect biomarkers are related to liver damage and/or decline in liver function, during the development of fibrosis and cirrhosis. They have also been called class I (direct) and class Ⅱ (indirect) biomarkers[14]. For the sake of clarity, we will discuss established and experimental markers separately.

Established serum markers

AST/ALT ratio: ALT and AST commonly misnamed "Liver function tests" are actually "Liver damage tests", as they are released from damaged cells. Taken together, they yield much more information than each one alone.

De Ritis *et al*[15] proposed the AST/ALT ratio in 1957, only 2 years after these tests were described. Williams and Hoofnagle from the National Institutes of Health (NIH) described very similar findings in 1988: "In the majority of cases of chronic viral hepatitis, the AST/ALT ratio was less than 1.0. However, there was a statistically significant correlation between the AST/ALT ratio and the presence of cirrhosis. Among 100 patients with chronic type B hepatitis, the mean AST/ALT ratio was 0.59 in those without cirrhosis and 1.02 in those with cirrhosis. Furthermore, the AST/ALT ratio often rose to greater than 1.0 when cirrhosis first became manifest. Thus, the finding of an AST/ALT ratio of greater than 1.0 in a patient with nonalcoholic liver disease should suggest the presence of cirrhosis. In addition, the use of the AST/ALT ratio as a means of separating alcoholic and nonalcoholic liver disease must be tempered with the knowledge that this ratio may be less helpful in the presence of cirrhosis".

Testa's group from Genoa showed in 1999 that an AST/ALT ratio of < 1 correctly classified 170 patients suffering from chronic hepatitis, and misclassified seven patients suffering from cirrhosis as suffering from chronic hepatitis. Thus, a ratio < 1 rules out cirrhosis with a great degree of certainty. The AST/ALT ratio performed less well among 171 cirrhotics; indeed, 130 had a ratio > 1, but 41 had a ratio of < 1. There was also a strong correlation between the De Ritis index and monoethylglycinexylidide (MEGX) formation, and indocyanine green (ICG) clearance[16].

It is fascinating to note that 16 years later, in the European Association for the Study of the Liver (EASL) 2015 postgraduate course, one of the take home messages was identical: "Simple and complex serum based tests have > 90% predictive value for excluding cirrhosis, though are poorly predictive of cirrhosis"[17].

McPherson *et al*[18], from Newcastle upon Tyne found that the De Ritis index could avoid LB in 69% of NAFLD patients and had a negative predictive value (NPV) to exclude advanced fibrosis of 95% at a cutoff of 0.8. The other scores, the Bard, the Fibrosis 4 (OFIB-4), and NAFLD fibrosis score, also performed very, well saving 38%-62% of biopsies.

APRI: stands for AST-Platelet Ratio Index. It is calculated in the following way: APRI = [AST level (/ULN)/Platelet counts (10^9/L)] × 100 and is one of the simplest marker panels that can diagnose significant fibrosis and cirrhosis with acceptable accuracy[19]. It has been extensively evaluated in HCV. A meta-analysis including 40 studies and a total of 8739 HCV patients showed that APRI had an area under the receiver operating characteristic (AUROC) of 0.77 for

the diagnosis of significant fibrosis (≥ F2), 0.80 for severe fibrosis (≥ F3), and 0.83 for cirrhosis[20]. Similar results for cirrhosis were found for a group of chronic HBV patients[21]. Recent studies indicate that APRI was comparable to other, more complex established panels in excluding advanced but not moderate fibrosis[22,23]. In a comparison of four tests (FibroTest, APRI, FIB-4, and Forns' Score) before and after telaprevir treatment of 1208 chronic HCV patients, APRI showed the most significant decrease[24], confirming the validity of this test found in previous studies[25,26]. A meta-analysis of 22 studies (n = 4266) showed that the summary AUROCs of APRI for significant fibrosis and cirrhosis were 0.76 [95% confidence interval (CI), 0.74-0.79] and 0.82 (95%CI: 0.79-0.86), respectively. For significant fibrosis, an APRI threshold of 0.5 was 81% sensitive and 50% specific. At a 40% prevalence of significant fibrosis, this threshold had a NPV of 80% and could reduce the necessity for liver biopsies by 35%. For cirrhosis, a threshold of 1.0 was 76% sensitive and 71% specific. At a 15% cirrhosis prevalence, the NPV of this threshold was 91%[27].

The World Health Organization(WHO) guidelines on the assessment of the degree of liver fibrosis and cirrhosis in hepatitis C patients suggested that "In resource-limited settings, the aminotransferase/platelet ratio index (APRI) or FIB-4 tests be used for the assessment of hepatic fibrosis rather than other noninvasive tests that require more resources such as elastography or Fibrotest". (of note, this was a conditional recommendation, based on low quality of evidence)[28].

NAFLD, firmly established as a clinical entity only in 1979[29], is rapidly becoming the most prevalent liver disease in affluent society. Because it is asymptomatic and lacks serological markers, its onset and course are even more insidious than viral and autoimmune liver diseases. Thus, an ultrasound (US) scan of the liver is the first diagnostic step. However, as shown by Tapper's group from Boston on 358 patients with biopsy proven NAFLD, 17.6% of patients diagnosed with steatosis also suffer from "biopsy proven" advanced fibrosis, and 16.7% (one in six) of the patients without US detected steatosis had advanced nonalcoholic steatohepatits (NASH), defined as an NFALD activity score (NAS) score > 4. Clearly, US alone does not suffice, and the authors recommended adding APRI. An APRI value > 1 was the most significant predictor of advanced fibrosis in the study population. The predictors of having advanced NASH are being female, having a body mass index (BMI) of > 30, and an AST > 40. In indeterminate cases, a LB should be seriously considered[30].

In 2015, Xiao *et al*[31] from Chengdu compared APRI and FIB-4, the two most validated noninvasive indices, in a meta-analysis of 39 articles with 9377 hepatitis B patients. For the diagnosis of cirrhosis, APRI had an AUROC of 0.726, and FIB-4 had an AUROC of 0.844. The authors concluded that APRI and FIB4 can identify HBV related fibrosis with moderate sensitivity and accuracy.

FIB-4: Is a combination of four simple variables: AST, ALT, age, and platelet count. It is calculated with the following formula:

The FIB-4 index = [age (years) × AST (IU/L)]/[platelet count (10^9/L) × ALT (IU/L)]$^{1/2}$.

It was initially evaluated in human immunodeficiency virus (HIV)/HCV coinfected patients[32]. FIB-4 performed similarly to FibroTest in the diagnosis of advanced fibrosis and cirrhosis in HCV patients[33], and also in a more recent study of 89 HBV and HCV patients[34]. It was also comparable to APRI, with AUROCs around 0.8[35], and of 0.73 in a recent study of 388 patients[23].

Fibrotest: "Fibrosure in the US" patented by Biopredictive, Paris, France is probably the most validated of the established panels. It is a proprietary combination of five serum biochemical markers (alpha-2-macroglobulin, apolipoprotein A1, haptoglobin, γ-glut amyltranspeptidase, and bilirubin) that are altered with liver fibrosis[36]. Its score is correlated with the degree of liver damage. A meta-analysis of eight studies, including 1842 patients, showed a median AUROC of 0.84 for the diagnosis of advanced fibrosis[37], confirming previous studies that indicated the validity of the test for the diagnosis of advanced fibrosis and cirrhosis but not of mild or intermediate fibrosis[38]. Scores may be influenced by acute inflammation, which leads to increases in serum α 2-macroglobulin and haptoglobin levels[39]. Reduction in the Fibrotest score was also observed after treatment of patients[25,40], although in a recent study the change was not as significant with Fibrotest than with other tests[24].

The Forns index combines four simple variables: platelet count, cholesterol levels, age, and gamma glutamyltransferase (GGT)[41]. In a recent review of 22 studies, the median AUROC obtained for significant fibrosis for the Forns index was 0.76 for significant fibrosis and 0.87 for cirrhosis, similar to that obtained with APRI[42]. The score was also reduced significantly during antiviral treatment[24,25].

Hyaluronan is a high molecular weight glycosaminoglycan that is found in the ECM. It enters the circulation during ECM turnover and is rapidly taken up and degraded in the liver through hepatic endothelial cells. Elevated HA levels may reflect increased production of HA, or reduced clearance of circulating HA and, therefore, may correlate with inflammatory activity and fibrosis. In chronic HCV patients, the AUROC of HA was 0.79 for cirrhosis[43] but was less satisfactory for less severe fibrosis. The AUROC was 0.72 in a recent study of 89 patients[34].

Hepascore combines HA with several other parameters: bilirubin, GGT, alpha-2 macroglobulin, age, and gender[44]. The AUROC for diagnosis of cirrhosis was

Table 2 Diagnostic accuracy of selected experimental serum markers

Marker	Prognosis	Sensitivity	Specificity	AUROC
PIIINP	Significant fibrosis	74	75	0.72
	Cirrhosis	64	66	0.76
PINP	Significant fibrosis	70	73	-
	Cirrhosis	63	73	-
YKL-40	Significant fibrosis	78	81	0.81
	Cirrhosis	80	71	0.80
TIMP	Significant fibrosis	66	72	0.71
	Cirrhosis	91	65	0.90
sH2a + ALT[1]	Significant fibrosis	65	85	0.79
	Advanced fibrosis and cirrhosis	-	-	0.86

[1]Except for these values, which were taken from Ref. [87], all other values are medians from Ref. [11]. PⅢNP: N-terminal pro-peptide of collagen type Ⅲ; PINP: N-terminal propeptide of collagen type Ⅰ; TIMP: Tissue inhibitor of metalloproteinase; sH2a: Soluble H2a; ALT: Alanine aminotransferase.

high, 0.89, but it was not better than other tests for significant fibrosis[42].

Fibrometer, patented by Echosens (Paris, France) combines glucose, AST, ferritin, platelet, ALT, body weight, and age by a proprietary formula[45]. In a recent review, the median AUROC for Fibrometer was 0.82 for significant fibrosis and 0.91 for cirrhosis[42], better when compared directly with APRI and FibroTest. It also showed improvement during antiviral treatment[25].

Cirrhometer, patented by Echosens combines the same parameters as Fibrometer but with specific coefficients targeted for the diagnosis of cirrhosis and was developed by the same group of investigators from Angers, France. Boursier *et al*[46] from that group published a long term (mean of 9.5 years) follow up of 373 patients, amounting to 3508 person years. FIB-4, APRI, and Fibrometer at baseline were actually better than a Metavir fibrosis score at baseline at predicting serious liver related events. Cirrhometer was the only predictor of liver related death. Combining Fibrometer and Cirrhometer yielded a better index than Metavir fibrosis score, FIB4, APRI, Fibrotest, and Hepascore. This is an important paper because of two reasons: first, it shows that serum markers can be better than biopsy, and second, it does not compare the different parameters at one point in time but follows a group of patients longitudinally and then, at the end of follow up, determines which parameters were better prognosticators. These kinds of long term follow up longitudinal studies will most probably yield better prognosis, because until now most studies compared a non-invasive marker against an imperfect standard. Still, as the authors themselves acknowledge, the Fibrometer and Cirrhometer need to be further evaluated.

It has been proposed that combination of several of the tests mentioned above could reduce the need for biopsy[47]. Non-invasive markers for the staging of liver fibrosis are at the edge of replacing liver histology as the gold standard, at least for hepatitis C[48].

Experimental serum markers

Direct experimental markers: Most of the experimental serum markers proposed for the diagnosis of fibrosis and cirrhosis are direct markers related to ECM metabolism. They can be classified as experimental as they are still not widely accepted clinically. The large increase in collagen synthesis by activated HSCs can be an indicator of the fibrogenic process. Collagen is synthesized as a precursor with propeptide extensions at both the N- and C-terminal ends[49]. Before collagen deposition in the ECM, the propeptides are cleaved by N- and C-terminal proteases. The N-terminal pro-peptide of collagen type Ⅲ (PⅢNP) has been the subject of many studies as a marker of liver fibrosis[50,51]. It was reported to detect cirrhosis with a sensitivity of about 94% and specificity of about 81%[52], although other studies showed lower values (Table 2). In a recent study comparing pediatric and adult HCV patients, a significant correlation with advanced fibrosis was obtained in adults (AUROC 0.894) but not in children[53]. Procollagen type Ⅲ amino terminal peptide (PⅢNP) levels are elevated in hepatitis and correlate with aminotransferase levels, and it is more likely a marker of inflammation than of fibrosis[54,55]. The main problem with PⅢNP, as well as with all other ECM-related markers, is that they are not specific for the liver, and their increase can reflect fibrosis or inflammation in other organs. The N-terminal propeptide of collagen type Ⅰ (PINP) has also been studied for the diagnosis of liver fibrosis, but similar to PⅢNP, it may also relate more to inflammation[56]. Type Ⅳ collagen levels have also been correlated to liver fibrosis[57]. The glycoprotein YKL-40, involved in remodeling of the ECM[58], is expressed in liver tissue, particularly in HSCs. Serum concentrations of YKL-40 correlated with other ECM-related markers, such as PⅢNP and HA. Several studies have shown elevated YKL-40 concentrations in the sera of patients with liver diseases. An AUROC of 0.81 was reported for advanced fibrosis in HCV patients[59]. As with other ECM components, YKL-40 can also originate in tissues other than the liver[60]. Laminin levels have also been evaluated for diagnosis of fibrosis, and in a recent study of 87 patients with chronic HBV, it gave 71.9% sensitivity and 80.0% specificity for significant fibrosis[61].

As mentioned above, some cytokines mediate hepatic fibrogenesis and have been investigated as potential markers of fibrosis. TGF-β stimulates ECM synthesis in HSCs. TGF-β levels correlate with the presence of liver fibrosis in patients with alcoholic liver disease (ALD) and HCV[62], and in a recent study, the AUROC obtained for advanced fibrosis was 0.835[53]. TNF-α was associated with fibrosis in ALD[63] and in chronic HBV patients[64]. Platelet-derived growth factor

(PDGF) has also been proposed as a potential marker for fibrosis progression[65]. Connective tissue growth factor (CTGF) is synthesized by HSCs and hepatocytes and is strongly dependent on TGF-β[66,67] and is also related to the fibrogenic process[66]. Its levels also correlate with fibrosis and are decreased in cirrhosis, when fibrogenesis is finally reduced. In studies of CTGF, it gave an AUROC for cirrhosis and fibrosis of 0.955 and 0.887, respectively[68].

The fibrolytic process in the liver is reflected by the serum levels of MMPs and TIMPs. MMP-1 concentrations decrease, while TIMP-1 levels increase during fibrosis in HCV patients[69]. TIMP-1 and MMP-2 (secreted by activated HSCs) correlate well with cirrhosis but the correlation with fibrosis is less clear[69-71].

Indirect experimental markers: Recently, indirect experimental markers have been described and evaluated. Markers of cell damage and death include CK18, evaluated in a group of 143 alcoholics, which could predict severe fibrosis with an AUROC of 0.84[72]. Release of Golgi protein-73 (GP73) was measured in two studies involving 229 and 296 patients with different types of liver disease, showing an AUROC of 0.9 for cirrhosis but much less significant results for fibrosis[73,74]. In a study including 111 individuals with NAFLD, ferritin levels were measured, giving an AUROC of 0.87 for advanced fibrosis and cirrhosis in combination with the BMI. Indicators of oxidative stress, such as malondialdhyde (MDA) and superoxide dismutase (SOD), were found to correlate with fibrosis in a study involving 150 HCV patients, giving AUROCs of 0.9 and 0.8, respectively, for advanced fibrosis and cirrhosis. Again, as mentioned above, the main drawback of all these markers is the lack of liver specificity, as they can be released from other damaged tissues. Interferon (IFN)-L3 expression was reported to be somewhat more restricted to the liver upon viral infection[75]. Changes in IFN-L3 levels were reported to correlate with the response to HCV. In a recent study of 119 chronic HCV patients, serum IFN-L3 increased with advanced fibrosis[76].

An empiric approach has been used in several studies to find differences in the proteome with the development of fibrosis and cirrhosis. In this way, a series of potential markers was identified, *e.g.,* microfibril-associated protein 4 (MFAP-4), which gave an AUROC of 0.97 for cirrhosis and 0.76 for advanced fibrosis[77]. Other identified possible markers in a study of chronic hepatitis C patients were alpha 2 macroglobulin (A2M)/hemopexin with AUROC 0.80 for the detection of significant fibrosis and 0.92 for advanced fibrosis[78]. Also identified in another study as a potential marker was vitamin D binding protein (VDBP) in addition to the established A2M and apolipoprotein (AI)[79]. Similarly, differences in the glycome of patients were investigated. Analyzing binding of serum glycoproteins to a panel of multiple lectins, 183 chronic HCV patients were tested, giving an AUROC of 0.80 for significant fibrosis; 0.88 for severe fibrosis; and 0.93 for cirrhosis, higher than those obtained in direct comparison with several established markers[80]. In a different glycomic approach, the serum N-glycome of 128 chronic HBV patients was analyzed using DNA sequencer-assisted fluorophore-assisted carbohydrate electrophoresis (DSA-FACE). Selected peak ratios gave correlation with fibrosis, obtaining AUROC of 0.675, 0.736, and 0.754 in the diagnosis of significant fibrosis, advanced fibrosis, and early cirrhosis, respectively[81]. These empiric -omic approaches have the drawback of the complexity of the analysis.

Finally, a series of experimental markers have been identified that are liver specific, an attribute that holds promise for a more specific diagnosis. In a recent study of 293 HBV patients, serum transferrin levels were lower in advanced fibrosis and cirrhosis (F3, F4) than in mild fibrosis (F1, F2). There was, however, an increase in F1, F2, so the difference between no fibrosis (F0) and F3, F4 was very small[82]. The serum levels of complement C3 and C4 beta chains (synthesized in the liver), analyzed by two dimensional gel electrophoresis were found to decrease in HCV patients with cirrhosis[83]. The hepatocyte levels of the asialoglycoprotein receptor are significantly reduced with fibrosis and cirrhosis[84,85]. A soluble form of this receptor (sH2a) is secreted to the plasma and showed very constant levels in healthy individuals and a significant, 3 fold decrease in cirrhosis[86]. A study in HCV patients yielded an AUROC of 0.72 for advanced fibrosis. In a combination with ALT, the AUROCs were 0.86 for advanced fibrosis and cirrhosis and 0.79 for significant fibrosis[87].

EVALUATION OF LIVER FIBROSIS BY IMAGING METHODS

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is used routinely to assess cirrhosis and its complications. However, detection of less advanced stages of fibrosis is more challenging, and several novel MR imaging techniques were used for this purpose[88].

Established modalities

Conventional MRI: Morphologic changes related to cirrhosis can be evaluated with conventional MRI. Macro-structural changes include surface nodularity, widening of fissures, expansion of the gallbladder fossa, notching of the right lobe, and enlargement of the lateral segments of the left lobe and caudate lobe. Parenchymal changes include fibrotic septa and bridges, regenerative nodules, and siderotic nodules or steatotic nodules. Other notable changes in some of the cases are related to portal hypertension, including splenomegaly, porto-systemic varices, ascites, and bowel wall thickening. Administration of intravenous (iv) contrast material improves the visibility of fibrosis

and cirrhosis-related changes and complications of cirrhosis. Fibrosis has a specific enhancement pattern with peak enhancement at the late phases (venous/equilibrium phases). This distinctive enhancement pattern and the reticular appearance enable the differentiation of it from other vascular lesions related to cirrhosis (*e.g.,* arterio-portal shunts, HCC)[89].

Innovative techniques

MR elastography: Similar to sonographic transient elastography (TE), MR elastography is based on the fact that the velocity and wavelength of the wave propagating in the tissue increases as the stiffness of the medium increases, *e.g.*, the fibrotic liver. Specific software and hardware are required to perform MR elastography. A driver device is placed over the patient's right upper abdomen and generates acoustic pressure waves at 40-120 Hz. These waves create shear waves in the liver. The images depict the propagating mechanical wave and a specific algorithm generates a quantitative stiffness map.

In several studies, MR elastography detected advanced fibrosis and cirrhosis in NAFLD patients and chronic hepatitis B patients. The quantitative assessment correlated significantly with the stage of fibrosis. It also proved to be an efficient tool for differentiating lower and higher grades of cirrhosis.

Compared with other MRI techniques, MR elastography is more sensitive for the assessment of liver fibrosis and cirrhosis compared with morphological features detected with conventional MRI. It has also much higher inter-observer agreement compared with MRI and diffusion weighted images (DWI). As opposed to ultrasonography, MR elastography is not affected by lack of acoustic window, obesity or presence of ascites and it is not operator dependent. Huwart *et al*[90] showed that MR elastography is more accurate than US elastography, APRI, or a combination of both, and its coefficient repeatability was better than US elastography.

In a meta-analysis of 12 studies done by Singh *et al*[91] using LB as a standard, MR elastography was found to be highly accurate for the diagnosis of advanced fibrosis independent age, sex, BMI, inflammation, and etiology of the liver disease.

Limitations of MR elastography are its cost and the fact that it is time consuming. Liver stiffness may be affected also from hepatic iron overload, steatosis, vascular congestion, cholestasis, and portal hypertension. In these cases, the accuracy of MR elastography may be altered[90-100].

T1 mapping of the liver: In this method, T1 relaxation time images are acquired and T1 maps are created using the scanner's software. Haimerl *et al*[101] showed that T1 maps after the administration of liver specific contrast medium (Gd-EOB-DTPA) correlated with the stage of cirrhosis, but no correlation was found between fibrosis and the non-contrast enhanced images. Other studies by Allkemper *et al*[102] and Rauscher *et al*[103] found a correlation between cirrhosis and T1 relaxation times in non-contrast enhanced MR.

A study by Banerjee *et al*[104] used T1 mapping for assessment of fibrosis, 1H MR spectroscopy for quantifying lipid content, and T2* sequence for assessing iron overload. An algorithm created an iron corrected T1 value, removing the effect of elevated iron on the T1 value. MR values were compared to the histology data. The corrected T1 value identified fibrosis with sensitivity of 86% and specificity of 93% and correlated strongly with different stages of fibrosis, except for an overlap between mild and moderate fibrosis. Additionally, 1H MR spectroscopy correlated strongly with hepatic steatosis. Hepatic iron content had a strong negative correlation with T2*. In this study, the data for all three parameters- fibrosis, steatosis and iron content- was acquired in a 23 min scan[101-104].

Experimental techniques

Reticuloendothelial specific contrast agents: Few studies have been performed with reticuloendothelial system-specific contrast agents. Superparamagnetic iron oxide (SPIO) causes signal drop in the hepatocyte containing liver parenchyma, and as a consequence, it increases the conspicuity of the detection of fibrotic tissue that is less affected by this contrast agent.

Other studies investigated the double contrast enhanced MRI technique. This technique combines a gadolinium based contrast agent and SPIO in the same study. The synergistic effect of both contrast agents increases the visibility of the fibrotic tissue and helps in differentiating advanced hepatic fibrosis from mild fibrosis. This technique also enabled the quantification of liver texture and its correlation with the stage of fibrosis. However, these contrast agents are not clinically available anymore[105-109].

Susceptibility-weighted MRI: Susceptibility-weighted imaging is a gradient echo sequence with increased sensitivity to the presence of iron, hemoglobin, and calcifications. Measurement of liver to muscle signal intensity ratio was shown to correlate with liver fibrosis with high inter-observer agreement[110].

Diffusion weighted MRI: DWI sequences assess the ability of protons to diffuse within a tissue. This sequence is being used routinely for oncology purposes. The apparent diffusion coefficient (ADC) map is a calculated map derived from the DWI images and correlates with the proton's diffusion ability. Preliminary studies using various hardware and different sequences have attempted to correlate between the reduced ADC value that appears in fibrosis and the degree of fibrosis, but the results were not consistent. In studies by Razek *et al*[111] and Lewin

et al[112], DWI correlated with fibrosis in children and adults. In another study, DWI correlated with stages of fibrosis with sensitivity of 75%-85% (depends on the stage of the fibrosis) and specificity of 68%-94%. In this study, the ability to identify fibrosis was significantly higher for MR elastography than DWI.

The limitations of DWI in the assessment of fibrosis are due to the fact that diffusion is affected by perfusion changes, hepatic steatosis, presence of iron in the tissue, and inflammatory changes. Moreover, the sequence is sensitive to susceptibility and motion related artifacts; and since the quantitative analysis is based on the images, it is also very limited[89,93,111,112].

Perfusion MRI: Parenchymal changes in fibrosis cause gradual obliteration of intrahepatic vessels and sinusoids and slow the passage of blood within the parenchyma. In addition, in portal hypertension, portal flow to the liver decreases and arterial flow takes place. These kinetic flow changes related to fibrosis and cirrhosis can be assessed with dynamic contrast enhanced MRI. This technique was shown to be reliable in the staging of liver fibrosis in patients with chronic hepatitis.

Limitations of the study are related to the fact that perfusion is affected also by the cardiac status, fasting state, hepatic congestion, inflammation, liver masses, and hepatic portal venous flow, and, therefore, the kinetic changes are not reflecting the fibrosis exclusively. Image analysis is a time consuming process; and image quality is not sufficient for assessment of nodules, resulting in two injections of contrast material during the scan[89,113,114].

MR spectroscopy: Assessments of liver fibrosis using MR spectroscopy achieved non-uniform results in different studies. PDE (phophodiester) can be measured by MR spectroscopy with sensitivity and specificity of 81% and 69%, respectively, for differentiating advanced from mild fibrosis. A study by Godfrey *et al*[96] showed poor correlation between the phosphomonoester:phophodiester (PME:PDE) ratio and the stage of cirrhosis. The limitations of this technique are that it is time consuming and requires special hardware and software[115,116].

Computed tomography

Morphological liver changes, signs of cirrhosis and signs of portal hypertension can be detected by computed tomography (CT, splenomegaly, collateral venous circulation, and enlarged portal vein), but CT is less sensitive for less advanced cirrhosis.

Perfusion CT: Perfusion CT may help differentiate minimal fibrosis from intermediate fibrosis in patients with chronic liver disease. Mean transient time is the most sensitive parameter, but there is still large overlap between the different parameters.

Fibro CT: An experimental processing method of conventional CT scan images, which are analyzed by additional software. Optical analysis of CT images of the liver utilizing this technique detected the stage and distribution of liver fibrosis in patients with chronic hepatitis C[117,118].

OTHER PHYSICAL METHODS

Ultrasonography

Conventional US: US is a widely available and low cost modality that has no ionizing radiation, allowing for repeated examinations. For these reasons, it is often performed as the initial modality for evaluation of patients with suspected diffuse liver disease and for non-invasive diagnosis of liver fibrosis.

US findings that suggest progression of fibrosis in patients with chronic liver disease include altered parenchymal echogenicity with coarsened echotexture and surface nodularity that reflects the presence of regenerative nodules and fibrous septa. As cirrhosis progresses, characteristic hypertrophy of the caudate and lateral segment with volume loss of the right lobe of the liver is observed, while in the advanced phase liver atrophy is complete[119]. These findings may lack high sensitivity and specificity, and liver morphology may be normal in the early stage of cirrhosis.

In a prospective comparative study of 85 patients with histologically assessed liver conditions, fibrosis was reliably detected on US examination with a sensitivity of 57% and a specificity of 88%[120].

Several studies have evaluated the performance of US features. Early studies of US criteria accuracy found that by using a ratio of transverse caudate lobe width to transverse right lobe width, cirrhotic livers could be separated from non-cirrhotic liver with a sensitivity of 84%, a specificity of 100%, and an accuracy of 94%[121]. A later study examined the performance of a 2 (nodularity and portal velocity) or 7 (nodularity, portal velocity, liver size, caudate hypertrophy, echogenicity, portal vein diameter, and spleen size) component score for the diagnosis of cirrhosis. The sensitivity was 82.2% and 78.7%, while specificity was 79.9% and 80.1%, respectively. Liver surface nodularity is considered one of the most sensitive and more reproducible US signs when associated with reduction in portal velocity[122,123].

In a more recent study, three US parameters were investigated, liver surface nodularity, caudate lobe hypertrophy, and pattern of hepatic venous blood flow, and compared to histological findings on LB. Hepatic surface nodularity was shown to be the most direct sign of advanced fibrosis, with reported sensitivity and specificity of 54% and 95%, respectively. The addition of other signs, such as caudate lobe hypertrophy, increased the sensitivity but diminished the specificity of US[124].

Doppler and US can also detect the development

of portal hypertension by measuring portal vein diameter, which should not exceed 13 mm in quiet respiration, velocity of flow, hepatofugal flow, ascites, and splenomegaly.

Contrast enhanced US: Contrast enhanced US (CEUS) is used in the characterization of liver tumors. However, in recent years, it has also been used to evaluate liver fibrosis, because of changes in intra hepatic microcirculation (intrahepatic shunts) that can occur in chronic liver diseases with fibrotic evolution. Several measurements have been performed, the arrival time in hepatic veins (HVAT) or more recently the intrahepatic transit time (ITT), which is defined as the time delay between the arrival of contrast in the portal vein and in the hepatic vein, the latter considered as an improved parameter in several studies. In one study, an arrival time of contrast in the hepatic vein below 17 s had 100% sensitivity and 93% specificity for cirrhosis, the HVAT being significantly shorter in cirrhotic patients than in noncirrhotic individuals (chronic liver disease and controls patients)[125,126].

Although HVAT measurement is simple, it has some limitations, *e.g.,* cases with extrahepatic shunts. Staub *et al*[126] used a cut-off of 13 s for the transit time and made the diagnosis of severe fibrosis with a specificity of 78.57%, a sensitivity of 78.95%, a positive predictive value of 78.33%, an NPV of 83.33%, and a performance accuracy of 78.79%[127,128].

CEUS requires additional expertise and adds cost, and this may limit its availability for the routine detection of cirrhosis

Elastography: In the last two decades new US-based methods have been developed. Fibrosis in the liver, as in other tissues, determines a reduction in elasticity or an increase in stiffness. US elastography that can evaluate the tissue stiffness permits a non-invasive estimation of liver fibrosis[129-131]. There are two types of US elastography, strain elastography (SE), also named real time elastography (Hi-RTE), and shear wave elastography (SWE). SE is a qualitative technique and evaluation of the tissue stiffness is obtained after manual compression. SWE is a technique that provides a quantitative measure of stiffness, expressed in meters per second (the shear wave speed) or in kilopascals (Young's Modulus) after an acoustic/mechanical pulse induced by the machine itself.

Among SWE methods, TE (Fibroscan) is the only non-imaging method, while Acoustic Radiation Force Impulse (ARFI) (Siemens, Erlangen, Germany and Philips) and 2D-Real Time Shear Waves Elastography (2D-SWE) (Aixplorer system, Supersonic Imagine, Aix-en-Provence, France) are both imaging methods implanted in US machines.

Real-time elastography - Hi-RTE or SE: Real-time elastography is integrated in a US machine (Hitachi Medical Systems Europe Holding AG, Zug, Switzerland) and is technically different from SWE methods. Hi-RTE relies on tissue deformation induced by operator pressure. Recently, a new linear probe was used to assess the liver parenchyma while the internal compression produced by the heartbeat was considered to stress the tissue.

Hi-RTE is a qualitative method used to assess liver fibrosis, where stiffness is given in the color scale or the semi-quantitative method based on the ratio strain between two regions of interest (ROI). The first data regarding chronic hepatitis evaluated by RTE was published by Friedrich-Rust. RTE was performed in 79 patients with chronic viral hepatitis and compared with histological score after LB. The diagnostic accuracy was 0.75 for the diagnosis of significant fibrosis (fibrosis stage according to METAVIR score > or = F2), 0.73 for severe fibrosis (F > or = F3), and 0.69 for cirrhosis[131]. Tatsumi performed Hi-RTE in 119 patients with chronic liver disease and compared the results with LB, TE, and serum markers. The levels of liver strain measured by real-time TE correlated well with liver stiffness. Hi-RTE showed a negative correlation with fibrotic stages and TE findings, suggesting that RTE is a better test than TE[132]. A very recent study was conducted by Meng in which real-time tissue elastography (TE) and LB were performed in 166 patients with chronic hepatitis B and compared with TE. They found that real-time TE has diagnostic performance similar to that of TE in the assessment of liver fibrosis[133]. Colombo conducted a study that evaluated 45 patients with chronic liver diseases and 27 normal subjects and compared three elastographic methods: TE, ARFI, and Hi-RTE. The AUROCs for predicting significant fibrosis (F $\geqslant$ 2) for TE, RTE, and ARFI were 0.89, 0.75, and 0.81, respectively (TE was significantly better than RTE, and there was no significant difference between TE and ARFI nor between ARFI and RTE). The AUROCs for predicting liver cirrhosis (F = 4) for TE, RTE, and ARFI were 0.92, 0.85, and 0.93 respectively with no significant difference between the three curves[134].

TE: TE is a novel method, and the first clinical data using this technique was published in 2003.

TE (Fibroscan; Echosens, Paris, France) was the first US-based elastographic method to evaluate elasticity by measuring the velocity of elastic shear waves in parenchyma generated by a mechanical push. An Ultrasonic M mode transducer is placed above the right lobe of the liver through an intercostal space and produces a mechanical vibration that generates elastic shear waves that propagate through the tissue. The propagation is followed by pulse-echo US acquisitions, and velocity of the waves is measured and expressed in kilopascals. The velocity of the waves correlates directly with the elasticity of the tissue. The stiffer the tissue, the faster the shear wave propagates. The examination is performed on a non-fasting patient lying on dorsal decubitus with the arm in maximal abduction, and the measurement is taken in the right

intercostal space. TE is rapid, easy to perform, and well-tolerated by patients with results immediately available. The technique is operator-independent. Liver stiffness is computed as the median of 10 validated measurements in accordance with manufacturer instructions. Measurements with an interquartile range of less than 30% of the median value and a success rate of greater than 60% are considered reliable. Several studies have demonstrated the reproducibility of the method[135,136].

TE was first validated for liver fibrosis evaluation in patients with chronic hepatitis C and later evaluated in other etiologies of chronic diffuse liver diseases[137-141]. All these studies have demonstrated that there is no specific cut-off to discriminate liver fibrosis and that it varies according to the etiology of liver disease. Many studies showed that TE is highly sensitive and can differentiate between the absence and mild fibrosis from significant fibrosis and cirrhosis but is not accurate enough to differentiate among stages of mild fibrosis, especially between F0-1 and F2. Using a cut-off value of 6.6 kPa, Sporea *et al*[142] reached the best discrimination between absence of fibrosis/mild fibrosis ($F < 2$) and the presence of moderate to severe fibrosis ($F \geqslant 2$). In the meta-analysis by Friedrich-Rust *et al*[143], the mean AUROC in HCV patients was 0.84 with a suggested optimal cut-off of 7.6 kPa for detecting significant fibrosis ($F \geqslant 2$), and the mean AUROC was 0.94 with an optimal cut-off of 13 kPa for predicting cirrhosis. A more recent meta-analysis published by Tsochatzis *et al*[140] included 40 studies and patients with diverse etiologies of chronic liver disease (chronic hepatitis B, C, alcohol, and other causes of cirrhosis). Data regarding patients with chronic hepatitis C were extracted from 14 studies, and the summary sensitivity and specificity were 0.78 and 0.80, respectively, for predicting significant fibrosis. Data regarding patients with chronic hepatitis B were extracted from four studies, and the summary sensitivity was 0.84 and the summary specificity was 0.78. In this analysis for predicting liver cirrhosis (F4 on biopsy), the summary sensitivity was 0.83 and the summary specificity was 0.89, and the mean optimal cut-off was 15.0 ± 4.1 kPa (median 14.5 kPa). The summary sensitivity and specificity for predicting significant fibrosis were 0.79 and 0.78, respectively. The mean optimal cut-off was 7.3 ± 1.4 kPa (median 7.2 kPa).

Recently the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) issued guidelines in which values above 6.8-7.6 kPa in chronic viral hepatitis may indicate the presence of significant fibrosis ($F \geqslant 2$) with a high probability, while the range 11.0-13.6 kPa may indicate a cirrhotic stage ($F = 4$)[144]. EASL guidelines indicate that TE can be used to assess liver fibrosis (level of recommendation A2) in patients with chronic hepatitis C[145].

TE can also be used to predict complications of cirrhosis, such as portal hypertension, or can have a role in the post-transplant setting[146-148]. The limitations of TE include the requirement for expensive equipment, and lack of standardized cutoffs for diagnosis of fibrosis stages. Moreover, TE cannot be performed in patients with obesity and ascites because of poor penetration.

In the last few years, other US-based SWE methods have been used, integrated into conventional US equipment, enabling visualization of tissue along with assessment of tissue elasticity.

Supersonic shear wave elastography or 2D SWE: Supersonic shear wave elastography (SSWE) use acoustic radiation force to induce microscopic tissue movements, producing shear wave in the tissue. In SWE methods, in contrast with RTE, deformation force and tissue deformation are known and, for that reason, quantitative estimation of tissue stiffness, expressed as Young's modulus (kilopascal) or shear wave velocity (m/s), can be obtained.

Two-dimensional WE is the only method that can provide real-time measurements of liver stiffness[149]. The technique is available on the Aixplorer® system. The patient is placed in the supine position with the right arm in maximum abduction, and a convex probe is placed in the right intercostal space, using the best acoustic window available for liver evaluation. Acquisition is performed on the right liver lobe, and no movement of the probe is recommended in order to avoid motion artifacts and to allow map stabilization. The patient has to hold breath for 3 to 4 s in the expiration phase to acquire a stable image. The SWE box has to be placed in a homogeneous vessel free area away from the Glisson capsule. The elasticity value is displayed on the image, and color mapping in the box is depicted in real time. For quantitative measurements, a round region of interest is placed inside the SWE box, and minimum stiffness and maximum stiffness expressed in kilopascals are recorded. A measurement is considered valid if the region of interest is filled out with color.

Contrary to TE, the method can be used in patients with ascites. The first clinical study was published by Bavu *et al*[150] who evaluated 133 patients with chronic hepatitis C by means of SWE, TE, and, in a subgroup of patients, LB. The AUROCs for elasticity values assessed by SWE were 0.95 for significant fibrosis, 0.96 for severe fibrosis, and 0.97 for liver cirrhosis. In this study, the AUROCs for SWE were better than those from TE performed in the same session for $F \geqslant 2$, $F \geqslant 3$, and F4. Ferraioli *et al*[151] compared SSWE with TE and LB. The cut-off value was 7.4 kPa for $F \geqslant 2$ (AUROC = 0.91), 8.7 kPa for $F \geqslant 3$ (AUROC = 0.99), and 9.2 kPa for $F = 4$ (AUROC = 0.97). The AUROCs were similar to those in the Bavu study. More recently, Leung conducted a study in a cohort of HBV patients, comparing TE and SWE of the liver and of the spleen. SWE of liver had significantly higher accuracy than TE of liver and SWE of spleen in all fibrosis stages. The AUROCs for 2D SWE of liver, TE of liver, and 2D SWE of

spleen were 0.86, 0.80, and 0.81, respectively, for mild fibrosis (F1 stage); 0.88, 0.78, and 0.82, respectively, for moderate fibrosis (F2 stage); 0.93, 0.83, and 0.83, respectively for severe fibrosis (F3 stage); and 0.98, 0.92, and 0.84, respectively, for cirrhosis (F4 stage). Two-dimensional SWE of the liver was the most reliable parameter to assess and evaluate liver fibrosis[152]. A very recent study was conducted by Zheng *et al*[153] that included 198 patients with chronic liver disease from different etiologies (HCV, HBV, autoimmune hepatitis, PBC, drug induced liver disease) using LB as a reference standard for most of them. They evaluated the individual and combined performances of 2D SWE and conventional US in assessing liver fibrosis and cirrhosis to determine when 2D SWE should be added to routine US. Two-dimensional SWE was significantly superior to conventional US in detecting liver fibrosis, but for diagnosis of decompensated cirrhosis, there was no significant difference between 2D SWE and conventional US.

ARFI: ARFI elastography is performed with a Siemens Acuson S2000TM US system. The same principle is used in a Philips system. ARFI imaging is a US-based elastography method integrated in conventional US machines where a region of interest in the liver is mechanically excited with an acoustic pulse inducing localized tissue displacement, which results in shear wave propagation. In this method, a single measurement over a small FOV is obtained (point quantification SWE). As compared with TE, ARFI elastography can be used also in patients with ascites[154]. Usually, 10 valid measurements are performed, and a median value is calculated (expressed in m/s). Compared with TE, ARFI has similar accuracy but lower rates of measurement failures[155].

ARFI was first used and validated in patients with chronic hepatitis C and subsequently in other etiologies of chronic liver diseases[156]. Sporea *et al*[157] found in a large cohort of patients that LS measurement by means of ARFI is a reliable method for predicting fibrosis severity in HCV patients. Similarly to TE, there is a large overlap of ARFI measurements for fibrosis F0-F2, and only severe fibrosis and cirrhosis can be excluded with great certainty. The overall correlation with histological fibrosis was not significantly different for TE in comparison with ARFI elastography. However, TE was better than ARFI for predicting the presence of liver cirrhosis and fibrosis (F $\geqslant$ 1). A meta-analysis that included 36 studies revealed good accuracy of the ARFI imaging for the staging of F $\geqslant$ 2 and F $\geqslant$ 3 with an AUROC of 0.84, and excellent diagnostic accuracy with an AUROC of 0.93 for F = 4[155]. In a retrospective international multicenter study that included 914 patients with chronic hepatitis C (10 centers, five countries from Europe and Asia), all patients were evaluated by means of LB, ARFI and, in a subgroup of patients, also by TE. A highly significant correlation (r = 0.654) was found between ARFI measurements and fibrosis (P < 0.0001), being significantly higher in European as compared with Asian patients (r = 0.756, P < 0.0001 *vs* r = 0.544, P < 0.0001). The predictive values of ARFI for various stages of fibrosis were: F $\geqslant$ 1 - cut-off > 1.19 m/s (AUROC = 0.779); F $\geqslant$ 2 cut-off > 1.33 m/s (AUROC = 0.792); F $\geqslant$ 3 cut-off >1.43 m/s (AUROC = 0.829); and F = 4 cut-off >1.55 m/s (AUROC = 0.842). The cut-offs for predicting significant fibrosis and cirrhosis were different in European *vs* Asian subjects: 1.21 m/s (AUROC = 0.857) and 1.74 m/s (AUROC = 0.892) in European subjects, and 1.32 m/s (AUROC = 0.736) and 1.55 m/s (AUROC = 0.736) in Asian patients[158].

Thirteen studies including 1163 patients with chronic hepatopathies were included in a recent meta-analysis The sensibility and sensitivity were 0.74 and 0.83, respectively, for the detection of significant fibrosis (F $\geqslant$ 2) using ARFI and 0.78 and 0.84, respectively, using TE. For the diagnosis of cirrhosis, the sensitivity and specificity for ARFI were 0.87 and 0.87, respectively, and for TE were 0.89 and 0.87, respectively, for TE. The median optimal cut-off value of liver stiffness assessed by ARFI for the detection of significant fibrosis and cirrhosis were 1.3 m/s and 1.8 m/s, respectively[159]. One study compared the feasibility of three shear waves elastographic methods. In a cohort of 332 patients, with or without hepatopathies, liver stiffness was evaluated by TE, ARFI, and SWE. Reliable measurements were obtained in a significantly higher percentage by means of ARFI as compared with TE and SWE: 92.1% *vs* 72.2% and 92.1% *vs* 71.3%, respectively. In subjects in whom reliable liver stiffness measurements were obtained by all three elastographic methods, the accuracy was similar for ARFI and SWE for diagnosing significant fibrosis and cirrhosis compared with TE[160].

PRACTICAL INTEGRATIVE POINTS AND CONCLUSIONS

We are of the opinion that free, powerful tools like FIB-4, De Ritis Ratio, and APRI, preferably with inexpensive imaging technologies (as discussed above), but possibly without them, should be the first step in the evaluation of liver fibrosis and cirrhosis. A large part, if not an overwhelming majority of liver biopsies could be avoided.

Some of the experimental serum markers, especially those that are liver-specific, combined with novel imaging and physical techniques could create a nearly biopsy-free scenario in the near future.

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