**Name of journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO: 19048**

**Columns:** **TOPIC HIGHLIGHT**

2015 Advances in Cirrhosis

**Non-invasive diagnosis of liver fibrosis and cirrhosis**

Lurie Y *et al*. Non-invasive diagnosis of liver fibrosis

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**Author** **contributions**: All authors contributed to this manuscript.

**Conflict-of-interest statement:** The authors have no conflict of interest to report.

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**Received:** April 28, 2015

**Peer-review started:** May 7, 2015

**First decision:** June 26, 2015

**Revised:** July 23, 2015

**Accepted:** September 14, 2015

**Article in press:**

**Published online:**

**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. In recent years we may perhaps be witnessing the beginning of the end of the first phase for the development of non-invasive markers. There is early evidence 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

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**Core tip:** Liver fibrosis (leading to liver cirrhosis), and not inflammation and cytolysis, is the 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.

Lurie Y, Webb M, Cytter-Kuint R, Shteingert S, Lederkremer GZ. Non-invasive diagnosis of liver fibrosis and cirrhosis. *World J Gastroenterol* 2015; In press

**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]. Then, the first quantifiable noninvasive markers of liver disease – serum enzyme levels - alkaline phosphatase in 1930 and transaminases in 1955-1956 became available. 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 exposing additional hidden parts of the iceberg 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. Neither liver biopsy[3] nor single parameters like ALT or AST or platelet numbers were good enough. Standing on the shoulders of giants - Child Turcotte[4] and of Maddrey[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 to 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 intitiated by a variety of insults leading to the death of hepatocytes, prominently viral infections (HBV, 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 mediator cytokine is transforming growth factor beta (TGF-β)[10]. TGF-β stimulates ECM gene expression and decreases ECM degradation by down-regulation 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, non-alcoholic fatty liver disease, 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) so that it is a good indicator of ill health, but 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 several of them are now established in clinical use, their prognostic value is not clearcut. They are increasingly useful in the exclusion of advanced fibrosis and cirrhosis, but still do not distinguish well early and intermediate stages of fibrosis, a problem shared with the past "gold standard" – liver biopsy[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 clarity's sake 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 two years after these tests were described. Williams and Hoofnagle from the 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 as suffering from chronic hepatitis, and misclassified 7 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 MEGX formation and ICG clearance[16].

It is fascinating to note that sixteen years later, in the EASL 2015 postgraduate course one of the take home messages is 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 liver biopsy 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, FIB-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:



and is one of the simplest marker panels that can diagnose with acceptable accuracy significant fibrosis and cirrhosis[19]. It has been extensively evaluated in hepatitis C (HCV). A meta-analysis including 40 studies and a total of 8739 HCV patients showed that APRI had an 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 WHO guidelines on the assessment of the degree of liver fibrosis and cirrhosis in hepatitis C patients, suggests 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. Being asymptomatic, and lacking serological markers its onset and course are even more insidious than viral and autoimmune liver diseases. Thus, an ultrasound 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 ultrasound detected steatosis had advanced NASH, defined as NAS score > 4. Clearly, ultrasound alone does not suffice, and the authors recommend adding APRI. An APRI value > 1 is the most significant predictor of advanced fibrosis in the study population. The predictors of having advanced NASH are being female, having a BMI of > 30 and an AST > 40. In indeterminate cases, a liver biopsy should be seriously considered[30].

Xiao *et al*[31] from Chengdu compared APRI and FIB-4, the two most validated noninvasive indexes in a meta-analysis of 39 articles published in 2015, including 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 (109/L) × ALT (IU/L)]1/2.

It was initially evaluated in HIV/HCV coinfected patients[32]. FIB-4 performed similarly to FibroTest in the diagnosis of advanced fibrosis and cirrhosis in HCV patients[33], in a more recent study of 89 HBV and HCV patients[34] and also in a comparison 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-glutamyltranspeptidase, and bilirubin) that are altered with liver fibrosis[36]. Its score is correlated with the degree of liver damage. A metaanalysis of 8 studies, including 1842 patients, showed a median AUROC of 0.84 for the diagnosis of advanced fibrosis[37] confirming previous studies, that indicate 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] though 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 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, AUROC 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 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, Paris, France 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 and than 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 the 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 in 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 in the clinic. 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 the children[53]. 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 similarly 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, 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 correlated with the presence of liver fibrosis in patients with alcoholic liver disease (ALD) and HCV[62], 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]. 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 correlated with fibrosis, and 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 body mass index. 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 0.94 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. 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 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 alpha-2-macroglobulin 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). But there was 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, three fold decrease in cirrhosis[86]. A study in HCV patients resulted in 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**

***MRI***

MRI is being used routinely for assessment of 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, siderotic nodules or steatotic nodules. Other changes that can be noted in some of the cases and are related to portal hypertension include splenomegaly, porto-systemic varices, ascites and bowel wall thickening. Administration of IV contrast material improves the visibility of fibrosis and cirrhosis-related changes and complications of cirrosis. 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 to differentiate it from other vascular lesions related to cirrhosis (for example: 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 in stiffer medium, in this case, the fibrotic liver. Specific software and hardware are required. A driver device is placed over the patient's right upper abdomen generating 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 MRS 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 ultrasound elastography, APRI or combination of both and its coefficient repeatability was better than ultrasound elastography.

In a meta-analysis of 12 studies done by Singh *et al*[91] using liver biopsy as a standard, MR elastography was found to be highly accurate for 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 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 an overlap between mild and moderate fibrosis. Additionally, 1H MR spectroscopy correlated strongly with hepatic steatosis. Hepatic iron content had 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 were done with reticuloendothelial system-specific contrast agents. Superparamagnetic iron oxide (SPIO) causes signal drop in the hepatocyte containing liver parenchyma, and as a consequence, 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 ADC (apparent diffusion coefficient) 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 tried to correlate between the reduced ADC value that appears in fibrosis and degree of fibrosis but the results were not consistent. In studies done by Abdel 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 94%-68%. In this study, MR elastography had significantly higher ability in identifying fibrosis.

‏ The limitations of DWI in assessment of fibrosis are related 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 proved to be reliable in 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%, for differentiating advanced from mild fibrosis. A study by Godffrey *et al*. showed correlation between PME:PDE ratio (phosphomoniester:phophodiester) and the stage of cirrhosis.‏ The limitations of this technique are that it is time consuming and requires special hardware and software[95,115,116].

***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 patient with chronic liver disease. Mean transient time is the most sensitive parameter, still with 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 ultrasound:** Ultrasound (US) is a widely available and low cost modality which has no ionizing radiation, allowing repeated examinations. For these reasons, it is most of the time 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 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 diminishes the specificity of US[124].

Doppler and ultrasound 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 in the evaluation of 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 a 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 (chronic liver disease and controls patients)[125,126].

Although HVAT measurement is simple, it has some limitations as for example in 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].

Contrast-enhanced US 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 ultrasound-based methods have been developed. Fibrosis in the liver as in other tissue determines a reduction in elasticity or an increase in stiffness. Ultrasound elastography that can evaluate the tissue stiffness permit a non-invasive estimation of liver fibrosis[129-131]. There are 2 types of ultrasound 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, Philips) and 2D-Real Time Shear Waves Elastography (2D-SWE) (Supersonic Imagine, Aixplorer system) are both imaging methods implanted in ultrasound machines.

**Real-time elastography – Hi-RTE or SE:** Real-time elastography is integrated in an 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 heart beatings was considered to stress the tissue.

Hi-RTE is a qualitative method to assess liver fibrosis where stiffness is given in the color scale or 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 liver biopsy. The diagnostic accuracy were 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 tissue elastography 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 and liver biopsy were performed in 166 patients with chronic hepatitis B and compared with TE. They found that Real-time tissue elastography has diagnostic performance similar to that of TE in the assessment of liver fibrosis[133]. Colombo conducted a study which evaluated 45 patients with chronic liver diseases and 27 normal subjects with comparison of three elastographic methods: TE, ARFI and Hi-RTE. The AUROCs for predicting significant fibrosis (F ≥ 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, RT-E 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 ultrasound-based elastographic method that evaluates 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 (kPa). The velocity of the waves correlates directly with the elasticity of the tissue. The stiffer the tissue is, 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% were considered reliable. Several studies have proved 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 to 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 reached the best discrimination between absence of fibrosis/mild fibrosis (F < 2) and the presence of moderate to severe fibrosis (F ≥ 2)[142]. In the Friedrich-Rust meta-analysis, in HCV patients, the mean AUROC was 0.84 with a suggested optimal cut-off of 7.6 kPa for detecting significant fibrosis (F ≥ 2) and the mean AUROC was 0.94 with an optimal cut-off of 13kPa for predicting cirrhosis[143]. A more recent meta-analysis published by Tsochatzis 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 4 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 ± 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)[140].

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 ≥ 2) with a high probability, while the range 11-13.6 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].

Transient elastography 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 ultrasound-based SWE methods have been used, integrated into conventional ultrasound equipment, enabling visualization of tissue along with assessment of tissue elasticity.

**Supersonic shear wave elastography or 2D SWE:** Supersonic Shear Wave Elastography (SSWE) acoustic radiation force to induce microscopic tissue movements, producing shear wave in the tissue. In SWE methods, in contrast with to RTE, both 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.

2D WE is the only method that can provide real-time measurements of liver stiffness[149]. The technique is available on the Aixplorer® system (SuperSonic Imagine, France). The patient is placed in 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. Elasticity value is displayed on the image. 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 who evaluated 133 patients with chronic hepatitis C by means of SWE, TE and, in a subgroup of patients, also by means of 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 ≥ 2, F ≥ 3 and F4[150]. Ferraioli compared SSWE with TE and LB. The cut-off value found for F ≥ 2 was 7.4 kPa (AUROC = 0.91), for F ≥ 3 it was 8.7 kPa (AUROC = 0.99) and for F = 4 it was 9.2 kPa (AUROC = 0.97). The AUROCs were similar to those in the Bavu study[151]. More recently, Leung conducted a study in a cohort of HBV patients, comparing TE, SWE of the liver and of the spleen. SWE of liver has a 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, respectively, 0.86, 0.80, and 0.81 for mild fibrosis (F1 stage); 0.88, 0.78, and 0.82 for moderate fibrosis (F2 stage); 0.93, 0.83, and 0.83 for severe fibrosis (F3 stage); and 0.98, 0.92, and 0.84 for cirrhosis (F4 stage). 2D SWE of the liver was the most reliable parameter to assess and evaluate liver fibrosis[152]. A very recent study was conducted by Zheng 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 two-dimensional 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 in diagnosis of decompensated cirrhosis there was no significant difference between 2D SWE and conventional US[153].

**Acoustic radiation force impulse elastography:** Acoustic radiation force impulse elastography (ARFI) Elastography is performed with a Siemens Acuson S2000TM ultrasound system (Siemens AG, Erlangen, Germany). The same principle is used in a Philips system. ARFI imaging is an 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 afterwards in other etiologies of chronic liver diseases[156]. Sporea 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 ≥ 1)[157].A meta-analysis that included 36 studies revealed good accuracy of the ARFI imaging for the staging of F ≥ 2 and F ≥ 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, 5 countries from Europe and Asia), all patients were evaluated by means of LB and ARFI, and in a subgroup of patients also by means of 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 ≥ 1 – cut-off >1.19 m/s (AUROC = 0.779); F ≥ 2 cut-off >1.33 m/s (AUROC = 0.792); F ≥ 3 cut-off >1.43 m/s (AUROC = 0.829); 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 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 metaanalysis The sensibility and sensitivity were 0.74 and 0.83 for detection of significant fibrosis (F ≥ 2) using ARFI and respectively 0.78 and 0.84 using TE. For the diagnosis of cirrhosis the sensitivity and specificity were 0.87 and 0.87 for ARFI, and 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 1.31 m/s and 1.8 m/s, respectively[159]. One study tried to compare 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%. 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, considering TE as reference method[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.

**REFERENCES**

1 **Major RH**. Classic Descriptions of Disease. 3rd edition. Charles C Thomas Pub Ltd: 1978, Apr 1

2 **Sherlock S**. Diseases of the liver and biliary system. 3rd edition ed. Oxford: Blackwell Scientific publications, 1962

3 **Bedossa P**, Carrat F. Liver biopsy: the best, not the gold standard. *J Hepatol* 2009; **50**: 1-3 [PMID: 19017551 DOI: 10.1016/j.jhep.2008.10.014]

4 **Child CG**, Turcotte JG. Surgery and portal hypertension. *Major Probl Clin Surg* 1964; **1**: 1-85 [PMID: 4950264]

5 **Maddrey WC**, Boitnott JK, Bedine MS, Weber FL, Mezey E, White RI. Corticosteroid therapy of alcoholic hepatitis. *Gastroenterology* 1978; **75**: 193-199 [PMID: 352788 DOI: S0016508578001584]

6 **Bataller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074 DOI: 10.1172/jci24282]

7 **Tsukada S**, Parsons CJ, Rippe RA. Mechanisms of liver fibrosis. *Clin Chim Acta* 2006; **364**: 33-60 [PMID: 16139830 DOI: 10.1016/j.cca.2005.06.014]

8 **Geerts A**. History, heterogeneity, developmental biology, and functions of quiescent hepatic stellate cells. *Semin Liver Dis* 2001; **21**: 311-335 [PMID: 11586463 DOI: 10.1055/s-2001-17550]

9 **Gressner AM**, Lotfi S, Gressner G, Haltner E, Kropf J. Synergism between hepatocytes and Kupffer cells in the activation of fat storing cells (perisinusoidal lipocytes). *J Hepatol* 1993; **19**: 117-132 [PMID: 8301032]

10 **Inagaki Y**, Okazaki I. Emerging insights into Transforming growth factor beta Smad signal in hepatic fibrogenesis. *Gut* 2007; **56**: 284-292 [PMID: 17303605 DOI: 10.1136/gut.2005.088690]

11 **Ahmad W**, Ijaz B, Gull S, Asad S, Khaliq S, Jahan S, Sarwar MT, Kausar H, Sumrin A, Shahid I, Hassan S. A brief review on molecular, genetic and imaging techniques for HCV fibrosis evaluation. *Virol J* 2011; **8**: 53 [PMID: 21299910 DOI: 1743-422X-8-53]

12 **Liu T**, Wang X, Karsdal MA, Leeming DJ, Genovese F. Molecular serum markers of liver fibrosis. *Biomark Insights* 2012; **7**: 105-117 [PMID: 22872786 DOI: 10.4137/BMI.S10009]

13 **Castera L**, Pinzani M. Biopsy and non-invasive methods for the diagnosis of liver fibrosis: does it take two to tango? *Gut* 2010; **59**: 861-866 [PMID: 20581229 DOI: 10.1136/gut.2010.214650]

14 **Gressner OA**, Gao C. Monitoring fibrogenic progression in the liver. *Clin Chim Acta* 2014; **433**: 111-122 [PMID: 24607331 DOI: 10.1016/j.cca.2014.02.021]

15 **DE RITIS F**, COLTORTI M, GIUSTI G. An enzymic test for the diagnosis of viral hepatitis; the transaminase serum activities. *Clin Chim Acta* 1957; **2**: 70-74 [PMID: 13447217]

16 **Giannini E**, Botta F, Fasoli A, Ceppa P, Risso D, Lantieri PB, Celle G, Testa R. Progressive liver functional impairment is associated with an increase in AST/ALT ratio. *Dig Dis Sci* 1999; **44**: 1249-1253 [PMID: 10389705]

17 **LA A**. Non invasive diagnosis of fibrosis in NAFLD, how reliable is it? EASL postgraduate course metabolic liver disease. The International Liver Congress; 2015: 24-25; Vienna, Austria

18 **McPherson S**, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut* 2010; **59**: 1265-1269 [PMID: 20801772 DOI: 10.1136/gut.2010.216077]

19 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]

20 **Lin ZH**, Xin YN, Dong QJ, Wang Q, Jiang XJ, Zhan SH, Sun Y, Xuan SY. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. *Hepatology* 2011; **53**: 726-736 [PMID: 21319189 DOI: 10.1002/hep.24105]

21 **Zhu X**, Wang LC, Chen EQ, Chen XB, Chen LY, Liu L, Lei XZ, Liu C, Tang H. Prospective evaluation of FibroScan for the diagnosis of hepatic fibrosis compared with liver biopsy/AST platelet ratio index and FIB-4 in patients with chronic HBV infection. *Dig Dis Sci* 2011; **56**: 2742-2749 [PMID: 21399926 DOI: 10.1007/s10620-011-1659-1]

22 **Usluer G**, Erben N, Aykin N, Dagli O, Aydogdu O, Barut S, Cevik F, Ormen B. Comparison of non-invasive fibrosis markers and classical liver biopsy in chronic hepatitis C. *Eur J Clin Microbiol Infect Dis* 2012; **31**: 1873-1878 [PMID: 22231498 DOI: 10.1007/s10096-011-1513-6]

23 **Martin J**, Khatri G, Gopal P, Singal AG. Accuracy of ultrasound and noninvasive markers of fibrosis to identify patients with cirrhosis. *Dig Dis Sci* 2015; **60**: 1841-1847 [PMID: 25586089 DOI: 10.1007/s10620-015-3531-1]

24 **Haseltine EL**, Penney MS, George S, Kieffer TL. Successful treatment with telaprevir-based regimens for chronic hepatitis C results in significant improvements to serum markers of liver fibrosis. *J Viral Hepat* 2015; **22**: 701-707 [PMID: 25582683 DOI: 10.1111/jvh.12382]

25 **Halfon P**, Carrat F, Bédossa P, Lambert J, Pénaranda G, Perronne C, Pol S, Cacoub P. Effect of antiviral treatment on serum markers of liver fibrosis in HIV-hepatitis C virus-coinfected patients: the Fibrovic 2 Study - ANRS HC02. *Antivir Ther* 2009; **14**: 211-219 [PMID: 19430096]

26 **Papastergiou V**, Stampori M, Lisgos P, Pselas C, Prodromidou K, Karatapanis S. Durability of a sustained virological response, late clinical sequelae, and long-term changes in aspartate aminotransferase to the platelet ratio index after successful treatment with peginterferon/ribavirin for chronic hepatitis C: a prospective study. *Eur J Gastroenterol Hepatol* 2013; **25**: 798-805 [PMID: 23395996 DOI: 10.1097/MEG.0b013e32835eb8bf]

27 **Shaheen AA**, Myers RP. Diagnostic accuracy of the aspartate aminotransferase-to-platelet ratio index for the prediction of hepatitis C-related fibrosis: a systematic review. *Hepatology* 2007; **46**: 912-921 [PMID: 17705266 DOI: 10.1002/hep.21835]

28 . 2014; : [PMID: 25535634 DOI: NBK263483]

29 **Reuben A**. Leave gourmandising. *Hepatology* 2002; **36**: 1303-1306 [PMID: 12395350 DOI: S0270913902001520]

30 **Tapper EB**, Krajewski K, Lai M, Challies T, Kane R, Afdhal N, Lau D. Simple non-invasive biomarkers of advanced fibrosis in the evaluation of non-alcoholic fatty liver disease. *Gastroenterol Rep (Oxf)* 2014; **2**: 276-280 [PMID: 25002154 DOI: 10.1093/gastro/gou034]

31 **Xiao G**, Yang J, Yan L. Comparison of diagnostic accuracy of aspartate aminotransferase to platelet ratio index and fibrosis-4 index for detecting liver fibrosis in adult patients with chronic hepatitis B virus infection: a systemic review and meta-analysis. *Hepatology* 2015; **61**: 292-302 [PMID: 25132233 DOI: 10.1002/hep.27382]

32 **Sterling RK**, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317-1325 [PMID: 16729309 DOI: 10.1002/hep.21178]

33 **Vallet-Pichard A**, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology* 2007; **46**: 32-36 [PMID: 17567829 DOI: 10.1002/hep.21669]

34 **Stibbe KJ**, Verveer C, Francke J, Hansen BE, Zondervan PE, Kuipers EJ, de Knegt RJ, van Vuuren AJ. Comparison of non-invasive assessment to diagnose liver fibrosis in chronic hepatitis B and C patients. *Scand J Gastroenterol* 2011; **46**: 962-972 [PMID: 21623677 DOI: 10.3109/00365521.2011.574725]

35 **Amorim TG**, Staub GJ, Lazzarotto C, Silva AP, Manes J, Ferronato Mda G, Shiozawa MB, Narciso-Schiavon JL, Dantas-Correa EB, Schiavon Lde L. Validation and comparison of simple noninvasive models for the prediction of liver fibrosis in chronic hepatitis C. *Ann Hepatol* 2012; **11**: 855-861 [PMID: 23109448]

36 **Poynard T**, Imbert-Bismut F, Munteanu M, Messous D, Myers RP, Thabut D, Ratziu V, Mercadier A, Benhamou Y, Hainque B. Overview of the diagnostic value of biochemical markers of liver fibrosis (FibroTest, HCV FibroSure) and necrosis (ActiTest) in patients with chronic hepatitis C. *Comp Hepatol* 2004; **3**: 8 [PMID: 15387887 DOI: 10.1186/1476-5926-3-8]

37 **Poynard T**, Ngo Y, Munteanu M, Thabut D, Ratziu V. Noninvasive Markers of Hepatic Fibrosis in Chronic Hepatitis B. *Curr Hepat Rep* 2011; **10**: 87-97 [PMID: 21654911 DOI: 10.1007/s11901-011-0096-0]

38 **Poynard T**, Morra R, Halfon P, Castera L, Ratziu V, Imbert-Bismut F, Naveau S, Thabut D, Lebrec D, Zoulim F, Bourliere M, Cacoub P, Messous D, Munteanu M, de Ledinghen V. Meta-analyses of FibroTest diagnostic value in chronic liver disease. *BMC Gastroenterol* 2007; **7**: 40 [PMID: 17937811 DOI: 10.1186/1471-230x-7-40]

39 **Ratziu V**, Massard J, Charlotte F, Messous D, Imbert-Bismut F, Bonyhay L, Tahiri M, Munteanu M, Thabut D, Cadranel JF, Le Bail B, de Ledinghen V, Poynard T. Diagnostic value of biochemical markers (FibroTest-FibroSURE) for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease. *BMC Gastroenterol* 2006; **6**: 6 [PMID: 16503961 DOI: 10.1186/1471-230x-6-6]

40 **Poynard T**, Moussalli J, Munteanu M, Thabut D, Lebray P, Rudler M, Ngo Y, Thibault V, Mkada H, Charlotte F, Bismut FI, Deckmyn O, Benhamou Y, Valantin MA, Ratziu V, Katlama C. Slow regression of liver fibrosis presumed by repeated biomarkers after virological cure in patients with chronic hepatitis C. *J Hepatol* 2013; **59**: 675-683 [PMID: 23712051 DOI: 10.1016/j.jhep.2013.05.015]

41 **Forns X**, Ampurdanès S, Llovet JM, Aponte J, Quintó L, Martínez-Bauer E, Bruguera M, Sánchez-Tapias JM, Rodés J. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**: 986-992 [PMID: 12297848 DOI: 10.1053/jhep.2002.36128]

42 **Chou R**, Wasson N. Blood tests to diagnose fibrosis or cirrhosis in patients with chronic hepatitis C virus infection: a systematic review. *Ann Intern Med* 2013; **158**: 807-820 [PMID: 23732714 DOI: 10.7326/0003-4819-158-11-201306040-00005]

43 **McHutchison JG**, Blatt LM, de Medina M, Craig JR, Conrad A, Schiff ER, Tong MJ. Measurement of serum hyaluronic acid in patients with chronic hepatitis C and its relationship to liver histology. Consensus Interferon Study Group. *J Gastroenterol Hepatol* 2000; **15**: 945-951 [PMID: 11022838]

44 **Adams LA**, Bulsara M, Rossi E, DeBoer B, Speers D, George J, Kench J, Farrell G, McCaughan GW, Jeffrey GP. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. *Clin Chem* 2005; **51**: 1867-1873 [PMID: 16055434 DOI: 10.1373/clinchem.2005.048389]

45 **Calès P**, Oberti F, Michalak S, Hubert-Fouchard I, Rousselet MC, Konaté A, Gallois Y, Ternisien C, Chevailler A, Lunel F. A novel panel of blood markers to assess the degree of liver fibrosis. *Hepatology* 2005; **42**: 1373-1381 [PMID: 16317693 DOI: 10.1002/hep.20935]

46 **Boursier J**, Brochard C, Bertrais S, Michalak S, Gallois Y, Fouchard-Hubert I, Oberti F, Rousselet MC, Calès P. Combination of blood tests for significant fibrosis and cirrhosis improves the assessment of liver-prognosis in chronic hepatitis C. *Aliment Pharmacol Ther* 2014; **40**: 178-188 [PMID: 24889599 DOI: 10.1111/apt.12813]

47 **Sebastiani G**, Vario A, Guido M, Noventa F, Plebani M, Pistis R, Ferrari A, Alberti A. Stepwise combination algorithms of non-invasive markers to diagnose significant fibrosis in chronic hepatitis C. *J Hepatol* 2006; **44**: 686-693 [PMID: 16490278 DOI: http: //dx.doi.org/10.1016/j.jhep.2006.01.007]

48 **Mauss B**, Rockstroh, Sarrazin, Wedemeyer. Hepatology 2015: A clinical textbook. 6th ed. Flying publisher, 2015: 655

49 **Gelse K**, Pöschl E, Aigner T. Collagens--structure, function, and biosynthesis. *Adv Drug Deliv Rev* 2003; **55**: 1531-1546 [PMID: 14623400]

50 **Nøjgaard C**, Johansen JS, Christensen E, Skovgaard LT, Price PA, Becker U. Serum levels of YKL-40 and PIIINP as prognostic markers in patients with alcoholic liver disease. *J Hepatol* 2003; **39**: 179-186 [PMID: 12873813]

51 **Rosenberg WM**, Voelker M, Thiel R, Becka M, Burt A, Schuppan D, Hubscher S, Roskams T, Pinzani M, Arthur MJ. Serum markers detect the presence of liver fibrosis: a cohort study. *Gastroenterology* 2004; **127**: 1704-1713 [PMID: 15578508]

52 **Teare JP**, Sherman D, Greenfield SM, Simpson J, Bray G, Catterall AP, Murray-Lyon IM, Peters TJ, Williams R, Thompson RP. Comparison of serum procollagen III peptide concentrations and PGA index for assessment of hepatic fibrosis. *Lancet* 1993; **342**: 895-898 [PMID: 8105167]

53 **Valva P**, Casciato P, Diaz Carrasco JM, Gadano A, Galdame O, Galoppo MC, Mullen E, De Matteo E, Preciado MV. The role of serum biomarkers in predicting fibrosis progression in pediatric and adult hepatitis C virus chronic infection. *PLoS One* 2011; **6**: e23218 [PMID: 21858035 DOI: 10.1371/journal.pone.0023218]

54 **Guéchot J**, Laudat A, Loria A, Serfaty L, Poupon R, Giboudeau J. Diagnostic accuracy of hyaluronan and type III procollagen amino-terminal peptide serum assays as markers of liver fibrosis in chronic viral hepatitis C evaluated by ROC curve analysis. *Clin Chem* 1996; **42**: 558-563 [PMID: 8605673]

55 **Montalto G**, Soresi M, Aragona F, Tripi S, Carroccio A, Anastasi G, Magliarisi C, Barresi E, Notarbartolo A. [Procollagen III and laminin in chronic viral hepatopathies]. *Presse Med* 1996; **25**: 59-62 [PMID: 8745719]

56 **Schytte S**, Hansen M, Møller S, Junker P, Henriksen JH, Hillingsø J, Teisner B. Hepatic and renal extraction of circulating type I procollagen aminopropeptide in patients with normal liver function and in patients with alcoholic cirrhosis. *Scand J Clin Lab Invest* 1999; **59**: 627-633 [PMID: 10691054]

57 **Maruyama K**, Okazaki I, Takagi T, Ishii H. Formation and degradation of basement membrane collagen. *Alcohol Alcohol Suppl* 1991; **1**: 369-374 [PMID: 1845565]

58 **Tran A**, Benzaken S, Saint-Paul MC, Guzman-Granier E, Hastier P, Pradier C, Barjoan EM, Demuth N, Longo F, Rampal P. Chondrex (YKL-40), a potential new serum fibrosis marker in patients with alcoholic liver disease. *Eur J Gastroenterol Hepatol* 2000; **12**: 989-993 [PMID: 11007134]

59 **Saitou Y**, Shiraki K, Yamanaka Y, Yamaguchi Y, Kawakita T, Yamamoto N, Sugimoto K, Murata K, Nakano T. Noninvasive estimation of liver fibrosis and response to interferon therapy by a serum fibrogenesis marker, YKL-40, in patients with HCV-associated liver disease. *World J Gastroenterol* 2005; **11**: 476-481 [PMID: 15641129]

60 **Mylin AK**, Rasmussen T, Johansen JS, Knudsen LM, Nørgaard PH, Lenhoff S, Dahl IM, Johnsen HE. Serum YKL-40 concentrations in newly diagnosed multiple myeloma patients and YKL-40 expression in malignant plasma cells. *Eur J Haematol* 2006; **77**: 416-424 [PMID: 16930142 DOI: 10.1111/j.0902-4441.2006.t01-1-EJH2879.x]

61 **Li F**, Zhu CL, Zhang H, Huang H, Wei Q, Zhu X, Cheng XY. Role of hyaluronic acid and laminin as serum markers for predicting significant fibrosis in patients with chronic hepatitis B. *Braz J Infect Dis* 2012; **16**: 9-14 [PMID: 22358349 DOI: S1413-86702012000100002]

62 **Kanzler S**, Baumann M, Schirmacher P, Dries V, Bayer E, Gerken G, Dienes HP, Lohse AW. Prediction of progressive liver fibrosis in hepatitis C infection by serum and tissue levels of transforming growth factor-beta. *J Viral Hepat* 2001; **8**: 430-437 [PMID: 11703574]

63 **McClain C**, Barve S, Joshi-Barve S, Song Z, Deaciuc I, Chen T, Hill D. Dysregulated cytokine metabolism, altered hepatic methionine metabolism and proteasome dysfunction in alcoholic liver disease. *Alcohol Clin Exp Res* 2005; **29**: 180S-188S [PMID: 16344606]

64 **Akpolat N**, Yahsi S, Godekmerdan A, Demirbag K, Yalniz M. Relationship between serum cytokine levels and histopathological changes of liver in patients with hepatitis B. *World J Gastroenterol* 2005; **11**: 3260-3263 [PMID: 15929178]

65 **Zhang BB**, Cai WM, Weng HL, Hu ZR, Lu J, Zheng M, Liu RH. Diagnostic value of platelet derived growth factor-BB, transforming growth factor-beta1, matrix metalloproteinase-1, and tissue inhibitor of matrix metalloproteinase-1 in serum and peripheral blood mononuclear cells for hepatic fibrosis. *World J Gastroenterol* 2003; **9**: 2490-2496 [PMID: 14606082]

66 **Gressner OA**, Gressner AM. Connective tissue growth factor: a fibrogenic master switch in fibrotic liver diseases. *Liver Int* 2008; **28**: 1065-1079 [PMID: 18783549 DOI: 10.1111/j.1478-3231.2008.01826.x]

67 **Gressner OA**, Lahme B, Demirci I, Gressner AM, Weiskirchen R. Differential effects of TGF-beta on connective tissue growth factor (CTGF/CCN2) expression in hepatic stellate cells and hepatocytes. *J Hepatol* 2007; **47**: 699-710 [PMID: 17629588 DOI: 10.1016/j.jhep.2007.05.015]

68 **Gressner AM**, Yagmur E, Lahme B, Gressner O, Stanzel S. Connective tissue growth factor in serum as a new candidate test for assessment of hepatic fibrosis. *Clin Chem* 2006; **52**: 1815-1817 [PMID: 16858074 DOI: 10.1373/clinchem.2006.070466]

69 **Leroy V**, Monier F, Bottari S, Trocme C, Sturm N, Hilleret MN, Morel F, Zarski JP. Circulating matrix metalloproteinases 1, 2, 9 and their inhibitors TIMP-1 and TIMP-2 as serum markers of liver fibrosis in patients with chronic hepatitis C: comparison with PIIINP and hyaluronic acid. *Am J Gastroenterol* 2004; **99**: 271-279 [PMID: 15046217]

70 **Boeker KH**, Haberkorn CI, Michels D, Flemming P, Manns MP, Lichtinghagen R. Diagnostic potential of circulating TIMP-1 and MMP-2 as markers of liver fibrosis in patients with chronic hepatitis C. *Clin Chim Acta* 2002; **316**: 71-81 [PMID: 11750276]

71 **Walsh KM**, Timms P, Campbell S, MacSween RN, Morris AJ. Plasma levels of matrix metalloproteinase-2 (MMP-2) and tissue inhibitors of metalloproteinases -1 and -2 (TIMP-1 and TIMP-2) as noninvasive markers of liver disease in chronic hepatitis C: comparison using ROC analysis. *Dig Dis Sci* 1999; **44**: 624-630 [PMID: 10080160]

72 **Lavallard VJ**, Bonnafous S, Patouraux S, Saint-Paul MC, Rousseau D, Anty R, Le Marchand-Brustel Y, Tran A, Gual P. Serum markers of hepatocyte death and apoptosis are non invasive biomarkers of severe fibrosis in patients with alcoholic liver disease. *PLoS One* 2011; **6**: e17599 [PMID: 21445263 DOI: 10.1371/journal.pone.0017599]

73 **Liu X**, Wan X, Li Z, Lin C, Zhan Y, Lu X. Golgi protein 73(GP73), a useful serum marker in liver diseases. *Clin Chem Lab Med* 2011; **49**: 1311-1316 [PMID: 21663469 DOI: 10.1515/CCLM.2011.640]

74 **Morota K**, Nakagawa M, Sekiya R, Hemken PM, Sokoll LJ, Elliott D, Chan DW, Dowell BL. A comparative evaluation of Golgi protein-73, fucosylated hemopexin, α-fetoprotein, and PIVKA-II in the serum of patients with chronic hepatitis, cirrhosis, and hepatocellular carcinoma. *Clin Chem Lab Med* 2011; **49**: 711-718 [PMID: 21231906 DOI: 10.1515/CCLM.2011.097]

75 **Sommereyns C**, Paul S, Staeheli P, Michiels T. IFN-lambda (IFN-lambda) is expressed in a tissue-dependent fashion and primarily acts on epithelial cells in vivo. *PLoS Pathog* 2008; **4**: e1000017 [PMID: 18369468 DOI: 10.1371/journal.ppat.1000017]

76 **Aoki Y**, Sugiyama M, Murata K, Yoshio S, Kurosaki M, Hashimoto S, Yatsuhashi H, Nomura H, Kang JH, Takeda T, Naito S, Kimura T, Yamagiwa Y, Korenaga M, Imamura M, Masaki N, Izumi N, Kage M, Mizokami M, Kanto T. Association of serum IFN-λ3 with inflammatory and fibrosis markers in patients with chronic hepatitis C virus infection. *J Gastroenterol* 2015; **50**: 894-902 [PMID: 25501286 DOI: 10.1007/s00535-014-1023-2]

77 **Mölleken C**, Sitek B, Henkel C, Poschmann G, Sipos B, Wiese S, Warscheid B, Broelsch C, Reiser M, Friedman SL, Tornøe I, Schlosser A, Klöppel G, Schmiegel W, Meyer HE, Holmskov U, Stühler K. Detection of novel biomarkers of liver cirrhosis by proteomic analysis. *Hepatology* 2009; **49**: 1257-1266 [PMID: 19177598 DOI: 10.1002/hep.22764]

78 **Cheung KJ**, Tilleman K, Deforce D, Colle I, Moreno C, Gustot T, Van Vlierberghe H. Usefulness of a novel serum proteome-derived index FI-PRO (fibrosis-protein) in the prediction of fibrosis in chronic hepatitis C. *Eur J Gastroenterol Hepatol* 2011; **23**: 701-710 [PMID: 21623191 DOI: 10.1097/MEG.0b013e3283471b74]

79 **Ho AS**, Cheng CC, Lee SC, Liu ML, Lee JY, Wang WM, Wang CC. Novel biomarkers predict liver fibrosis in hepatitis C patients: alpha 2 macroglobulin, vitamin D binding protein and apolipoprotein AI. *J Biomed Sci* 2010; **17**: 58 [PMID: 20630109 DOI: 10.1186/1423-0127-17-58]

80 **Ito K**, Kuno A, Ikehara Y, Sugiyama M, Saito H, Aoki Y, Matsui T, Imamura M, Korenaga M, Murata K, Masaki N, Tanaka Y, Hige S, Izumi N, Kurosaki M, Nishiguchi S, Sakamoto M, Kage M, Narimatsu H, Mizokami M. LecT-Hepa, a glyco-marker derived from multiple lectins, as a predictor of liver fibrosis in chronic hepatitis C patients. *Hepatology* 2012; **56**: 1448-1456 [PMID: 22535703 DOI: 10.1002/hep.25815]

81 **Qu Y**, Gao CF, Zhou K, Zhao YP, Xu MY, Lu LG. Serum N-glycomic markers in combination with panels improves the diagnosis of chronic hepatitis B. *Ann Hepatol* 2012; **11**: 202-212 [PMID: 22345337 DOI: 982811]

82 **Cho HJ**, Kim SS, Ahn SJ, Park JH, Kim DJ, Kim YB, Cho SW, Cheong JY. Serum transferrin as a liver fibrosis biomarker in patients with chronic hepatitis B. *Clin Mol Hepatol* 2014; **20**: 347-354 [PMID: 25548740 DOI: 10.3350/cmh.2014.20.4.347]

83 **Gangadharan B**, Antrobus R, Chittenden D, Rossa J, Bapat M, Klenerman P, Barnes E, Dwek RA, Zitzmann N. New approaches for biomarker discovery: the search for liver fibrosis markers in hepatitis C patients. *J Proteome Res* 2011; **10**: 2643-2650 [PMID: 21410221 DOI: 10.1021/pr101077c]

84 **Du S**, Mao Y, Tong J, Li F, Che L, Li S, Yang H, Ba J, Hu N, Xing H, Lu X, Sang X, Zhang X, Wang X, Zhong S. A novel liver function evaluation system using radiopharmacokinetic modeling of technetium-99m-DTPA-galactosyl human serum albumin. *Nucl Med Commun* 2013; **34**: 893-899 [PMID: 23744410 DOI: 10.1097/MNM.0b013e328362e7c7]

85 **Pimstone NR**, Stadalnik RC, Vera DR, Hutak DP, Trudeau WL. Evaluation of hepatocellular function by way of receptor-mediated uptake of a technetium-99m-labeled asialoglycoprotein analog. *Hepatology* 1994; **20**: 917-923 [PMID: 7927233]

86 **Benyair R**, Kondratyev M, Veselkin E, Tolchinsky S, Shenkman M, Lurie Y, Lederkremer GZ. Constant serum levels of secreted asialoglycoprotein receptor sH2a and decrease with cirrhosis. *World J Gastroenterol* 2011; **17**: 5305-5309 [PMID: 22219600 DOI: 10.3748/wjg.v17.i48.5305]

87 **Veselkin E**, Kondratyev M, Lurie Y, Ron E, Santo M, Reif S, Elashvili I, Bar L, Lederkremer GZ. A secreted form of the asialoglycoprotein receptor, sH2a, as a novel potential noninvasive marker for liver fibrosis. *PLoS One* 2011; **6**: e27210 [PMID: 22096539 DOI: 10.1371/journal.pone.0027210]

88 **Hussain SM**, Reinhold C, Mitchell DG. Cirrhosis and lesion characterization at MR imaging. *Radiographics* 2009; **29**: 1637-1652 [PMID: 19959512 DOI: 10.1148/rg.296095508]

89 **Faria SC**, Ganesan K, Mwangi I, Shiehmorteza M, Viamonte B, Mazhar S, Peterson M, Kono Y, Santillan C, Casola G, Sirlin CB. MR imaging of liver fibrosis: current state of the art. *Radiographics* 2009; **29**: 1615-1635 [PMID: 19959511 DOI: 10.1148/rg.296095512]

90 **Huwart L**, Sempoux C, Vicaut E, Salameh N, Annet L, Danse E, Peeters F, ter Beek LC, Rahier J, Sinkus R, Horsmans Y, Van Beers BE. Magnetic resonance elastography for the noninvasive staging of liver fibrosis. *Gastroenterology* 2008; **135**: 32-40 [PMID: 18471441 DOI: 10.1053/j.gastro.2008.03.076]

91 **Singh S**, Venkatesh SK, Wang Z, Miller FH, Motosugi U, Low RN, Hassanein T, Asbach P, Godfrey EM, Yin M, Chen J, Keaveny AP, Bridges M, Bohte A, Murad MH, Lomas DJ, Talwalkar JA, Ehman RL. Diagnostic performance of magnetic resonance elastography in staging liver fibrosis: a systematic review and meta-analysis of individual participant data. *Clin Gastroenterol Hepatol* 2015; **13**: 440-451.e6 [PMID: 25305349 DOI: 10.1016/j.cgh.2014.09.046]

92 **Loomba R**, Wolfson T, Ang B, Hooker J, Behling C, Peterson M, Valasek M, Lin G, Brenner D, Gamst A, Ehman R, Sirlin C. Magnetic resonance elastography predicts advanced fibrosis in patients with nonalcoholic fatty liver disease: a prospective study. *Hepatology* 2014; **60**: 1920-1928 [PMID: 25103310 DOI: 10.1002/hep.27362]

93 **Venkatesh SK**, Wang G, Lim SG, Wee A. Magnetic resonance elastography for the detection and staging of liver fibrosis in chronic hepatitis B. *Eur Radiol* 2014; **24**: 70-78 [PMID: 23928932 DOI: 10.1007/s00330-013-2978-8]

94 **Venkatesh SK**, Yin M, Takahashi N, Glockner JF, Talwalkar JA, Ehman RL. Non-invasive detection of liver fibrosis: MR imaging features vs. MR elastography. *Abdom Imaging* 2015; **40**: 766-775 [PMID: 25805619 DOI: 10.1007/s00261-015-0347-6]

95 **Wang Y**, Ganger DR, Levitsky J, Sternick LA, McCarthy RJ, Chen ZE, Fasanati CW, Bolster B, Shah S, Zuehlsdorff S, Omary RA, Ehman RL, Miller FH. Assessment of chronic hepatitis and fibrosis: comparison of MR elastography and diffusion-weighted imaging. *AJR Am J Roentgenol* 2011; **196**: 553-561 [PMID: 21343496 DOI: 10.2214/AJR.10.4580]

96 **Godfrey EM**, Patterson AJ, Priest AN, Davies SE, Joubert I, Krishnan AS, Griffin N, Shaw AS, Alexander GJ, Allison ME, Griffiths WJ, Gimson AE, Lomas DJ. A comparison of MR elastography and 31P MR spectroscopy with histological staging of liver fibrosis. *Eur Radiol* 2012; **22**: 2790-2797 [PMID: 22752441 DOI: 10.1007/s00330-012-2527-x]

97 **Yin M**, Talwalkar JA, Glaser KJ, Manduca A, Grimm RC, Rossman PJ, Fidler JL, Ehman RL. Assessment of hepatic fibrosis with magnetic resonance elastography. *Clin Gastroenterol Hepatol* 2007; **5**: 1207-1213.e2 [PMID: 17916548 DOI: S1542-3565(07)00628-3]

98 **Batheja M**, Vargas H, Silva AM, Walker F, Chang YH, De Petris G, Silva AC. Magnetic resonance elastography (MRE) in assessing hepatic fibrosis: performance in a cohort of patients with histological data. *Abdom Imaging* 2015; **40**: 760-765 [PMID: 25542217 DOI: 10.1007/s00261-014-0321-8]

99 **Martínez SM**, Crespo G, Navasa M, Forns X. Noninvasive assessment of liver fibrosis. *Hepatology* 2011; **53**: 325-335 [PMID: 21254180 DOI: 10.1002/hep.24013]

100 **Papastergiou V**, Tsochatzis E, Burroughs AK. Non-invasive assessment of liver fibrosis. *Ann Gastroenterol* 2012; **25**: 218-231 [PMID: 24714123]

101 **Haimerl M**, Verloh N, Zeman F, Fellner C, Müller-Wille R, Schreyer AG, Stroszczynski C, Wiggermann P. Assessment of clinical signs of liver cirrhosis using T1 mapping on Gd-EOB-DTPA-enhanced 3T MRI. *PLoS One* 2013; **8**: e85658 [PMID: 24392025 DOI: 10.1371/journal.pone.0085658]

102 **Allkemper T**, Sagmeister F, Cicinnati V, Beckebaum S, Kooijman H, Kanthak C, Stehling C, Heindel W. Evaluation of fibrotic liver disease with whole-liver T1ρ MR imaging: a feasibility study at 1.5 T. *Radiology* 2014; **271**: 408-415 [PMID: 24475807 DOI: 10.1148/radiol.13130342]

103 **Rauscher I**, Eiber M, Ganter C, Martirosian P, Safi W, Umgelter A, Rummeny EJ, Holzapfel K. Evaluation of T1ρ as a potential MR biomarker for liver cirrhosis: comparison of healthy control subjects and patients with liver cirrhosis. *Eur J Radiol* 2014; **83**: 900-904 [PMID: 24661616 DOI: 10.1016/j.ejrad.2014.02.017]

104 **Banerjee R**, Pavlides M, Tunnicliffe EM, Piechnik SK, Sarania N, Philips R, Collier JD, Booth JC, Schneider JE, Wang LM, Delaney DW, Fleming KA, Robson MD, Barnes E, Neubauer S. Multiparametric magnetic resonance for the non-invasive diagnosis of liver disease. *J Hepatol* 2014; **60**: 69-77 [PMID: 24036007 DOI: 10.1016/j.jhep.2013.09.002]

105 **Gandhi SN**, Brown MA, Wong JG, Aguirre DA, Sirlin CB. MR contrast agents for liver imaging: what, when, how. *Radiographics* 2006; **26**: 1621-1636 [PMID: 17102040 DOI: 26/6/1621]

106 **Lucidarme O**, Baleston F, Cadi M, Bellin MF, Charlotte F, Ratziu V, Grenier PA. Non-invasive detection of liver fibrosis: Is superparamagnetic iron oxide particle-enhanced MR imaging a contributive technique? *Eur Radiol* 2003; **13**: 467-474 [PMID: 12594548 DOI: 10.1007/s00330-002-1667-9]

107 **Aguirre DA**, Behling CA, Alpert E, Hassanein TI, Sirlin CB. Liver fibrosis: noninvasive diagnosis with double contrast material-enhanced MR imaging. *Radiology* 2006; **239**: 425-437 [PMID: 16641352 DOI: 239/2/425]

108 **Hughes-Cassidy F**, Chavez AD, Schlang A, Hassanein T, Gamst A, Wolfson T, Sirlin C. Superparamagnetic iron oxides and low molecular weight gadolinium chelates are synergistic for direct visualization of advanced liver fibrosis. *J Magn Reson Imaging* 2007; **26**: 728-737 [PMID: 17685418 DOI: 10.1002/jmri.21066]

109 **Bahl G**, Cruite I, Wolfson T, Gamst AC, Collins JM, Chavez AD, Barakat F, Hassanein T, Sirlin CB. Noninvasive classification of hepatic fibrosis based on texture parameters from double contrast-enhanced magnetic resonance images. *J Magn Reson Imaging* 2012; **36**: 1154-1161 [PMID: 22851409 DOI: 10.1002/jmri.23759]

110 **Balassy C**, Feier D, Peck-Radosavljevic M, Wrba F, Witoszynskyj S, Kiefer B, Reiter G, Dai Y, Ba-Ssalamah A. Susceptibility-weighted MR imaging in the grading of liver fibrosis: a feasibility study. *Radiology* 2014; **270**: 149-158 [PMID: 23925270 DOI: 10.1148/radiol.13122440]

111 **Razek AA**, Abdalla A, Omran E, Fathy A, Zalata K. Diagnosis and quantification of hepatic fibrosis in children with diffusion weighted MR imaging. *Eur J Radiol* 2011; **78**: 129-134 [PMID: 19926420 DOI: 10.1016/j.ejrad.2009.10.012]

112 **Lewin M**, Poujol-Robert A, Boëlle PY, Wendum D, Lasnier E, Viallon M, Guéchot J, Hoeffel C, Arrivé L, Tubiana JM, Poupon R. Diffusion-weighted magnetic resonance imaging for the assessment of fibrosis in chronic hepatitis C. *Hepatology* 2007; **46**: 658-665 [PMID: 17663420 DOI: 10.1002/hep.21747]

113 **Hagiwara M**, Rusinek H, Lee VS, Losada M, Bannan MA, Krinsky GA, Taouli B. Advanced liver fibrosis: diagnosis with 3D whole-liver perfusion MR imaging--initial experience. *Radiology* 2008; **246**: 926-934 [PMID: 18195377 DOI: 10.1148/radiol.2463070077]

114 **Chen BB**, Hsu CY, Yu CW, Wei SY, Kao JH, Lee HS, Shih TT. Dynamic contrast-enhanced magnetic resonance imaging with Gd-EOB-DTPA for the evaluation of liver fibrosis in chronic hepatitis patients. *Eur Radiol* 2012; **22**: 171-180 [PMID: 21879400 DOI: 10.1007/s00330-011-2249-5]

115 **Noren B**, Dahlqvist O, Lundberg P, Almer S, Kechagias S, Ekstedt M, Franzén L, Wirell S, Smedby O. Separation of advanced from mild fibrosis in diffuse liver disease using 31P magnetic resonance spectroscopy. *Eur J Radiol* 2008; **66**: 313-320 [PMID: 17646074 DOI: S0720-048X(07)00302-6]

116 **Sangwaiya MJ**, Sherman DI, Lomas DJ, Shorvon PJ. Latest developments in the imaging of fibrotic liver disease. *Acta Radiol* 2014; **55**: 802-813 [PMID: 24226293 DOI: 10.1177/0284185113510159]

117 **Ronot M**, Asselah T, Paradis V, Michoux N, Dorvillius M, Baron G, Marcellin P, Van Beers BE, Vilgrain V. Liver fibrosis in chronic hepatitis C virus infection: differentiating minimal from intermediate fibrosis with perfusion CT. *Radiology* 2010; **256**: 135-142 [PMID: 20574090 DOI: 10.1148/radiol.10091295]

118 **Romero-Gómez M**, Gómez-González E, Madrazo A, Vera-Valencia M, Rodrigo L, Pérez-Alvarez R, Pérez-López R, Castellano-Megias VM, Nevado-Santos M, Alcón JC, Solá R, Pérez-Moreno JM, Navarro JM, Andrade RJ, Salmerón J, Fernández-López M, Aznar R, Diago M. Optical analysis of computed tomography images of the liver predicts fibrosis stage and distribution in chronic hepatitis C. *Hepatology* 2008; **47**: 810-816 [PMID: 18098299 DOI: 10.1002/hep.22112]

119 **Heller MT**, Tublin ME. The role of ultrasonography in the evaluation of diffuse liver disease. *Radiol Clin North Am* 2014; **52**: 1163-1175 [PMID: 25444098 DOI: 10.1016/j.rcl.2014.07.013]

120 **Saverymuttu SH**, Joseph AE, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. *Br Med J (Clin Res Ed)* 1986; **292**: 13-15 [PMID: 3080046]

121 **Harbin WP**, Robert NJ, Ferrucci JT. Diagnosis of cirrhosis based on regional changes in hepatic morphology: a radiological and pathological analysis. *Radiology* 1980; **135**: 273-283 [PMID: 7367613 DOI: 10.1148/radiology.135.2.7367613]

122 **Gaiani S**, Gramantieri L, Venturoli N, Piscaglia F, Siringo S, D'Errico A, Zironi G, Grigioni W, Bolondi L. What is the criterion for differentiating chronic hepatitis from compensated cirrhosis? A prospective study comparing ultrasonography and percutaneous liver biopsy. *J Hepatol* 1997; **27**: 979-985 [PMID: 9453422 DOI: S0168-8278(97)80140-7]

123 **Di Lelio A**, Cestari C, Lomazzi A, Beretta L. Cirrhosis: diagnosis with sonographic study of the liver surface. *Radiology* 1989; **172**: 389-392 [PMID: 2526349 DOI: 10.1148/radiology.172.2.2526349]

124 **Colli A**, Fraquelli M, Andreoletti M, Marino B, Zuccoli E, Conte D. Severe liver fibrosis or cirrhosis: accuracy of US for detection--analysis of 300 cases. *Radiology* 2003; **227**: 89-94 [PMID: 12601199 DOI: 10.1148/radiol.2272020193]

125 **Abbattista T**, Ridolfi F, Ciabattoni E, Marini F, Bendia E, Brunelli E, Busilacchi P. Diagnosis of liver cirrhosis by transit-time analysis at contrast-enhanced ultrasonography. *Radiol Med* 2008; **113**: 860-874 [PMID: 18587531 DOI: 10.1007/s11547-008-0292-3]

126 **Staub F**, Tournoux-Facon C, Roumy J, Chaigneau C, Morichaut-Beauchant M, Levillain P, Prevost C, Aubé C, Lebigot J, Oberti F, Galtier JB, Laumonier H, Trillaud H, Bernard PH, Blanc JF, Sironneau S, Machet F, Drouillard J, de Ledinghen V, Couzigou P, Foucher P, Castéra L, Tranquard F, Bacq Y, d'Altéroche L, Ingrand P, Tasu JP. Liver fibrosis staging with contrast-enhanced ultrasonography: prospective multicenter study compared with METAVIR scoring. *Eur Radiol* 2009; **19**: 1991-1997 [PMID: 19259683 DOI: 10.1007/s00330-009-1313-x]

127 **Goyal N**, Jain N, Rachapalli V, Cochlin DL, Robinson M. Non-invasive evaluation of liver cirrhosis using ultrasound. *Clin Radiol* 2009; **64**: 1056-1066 [PMID: 19822238 DOI: 10.1016/j.crad.2009.05.010]

128 **Li N**, Ding H, Fan P, Lin X, Xu C, Wang W, Xu Z, Wang J. Intrahepatic transit time predicts liver fibrosis in patients with chronic hepatitis B: quantitative assessment with contrast-enhanced ultrasonography. *Ultrasound Med Biol* 2010; **36**: 1066-1075 [PMID: 20620694 DOI: 10.1016/j.ultrasmedbio.2010.04.012]

129 **Gennisson JL**, Deffieux T, Fink M, Tanter M. Ultrasound elastography: principles and techniques. *Diagn Interv Imaging* 2013; **94**: 487-495 [PMID: 23619292 DOI: 10.1016/j.diii.2013.01.022]

130 **Bamber J**, Cosgrove D, Dietrich CF, Fromageau J, Bojunga J, Calliada F, Cantisani V, Correas JM, D'Onofrio M, Drakonaki EE, Fink M, Friedrich-Rust M, Gilja OH, Havre RF, Jenssen C, Klauser AS, Ohlinger R, Saftoiu A, Schaefer F, Sporea I, Piscaglia F. EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 1: Basic principles and technology. *Ultraschall Med* 2013; **34**: 169-184 [PMID: 23558397 DOI: 10.1055/s-0033-1335205]

131 **Dietrich CF**, Cantisani V. Current status and perspectives of elastography. *Eur J Radiol* 2014; **83**: 403-404 [PMID: 23540945 DOI: 10.1016/j.ejrad.2013.02.028]

132 **Tatsumi C**, Kudo M, Ueshima K, Kitai S, Takahashi S, Inoue T, Minami Y, Chung H, Maekawa K, Fujimoto K, Akiko T, Takeshi M. Noninvasive evaluation of hepatic fibrosis using serum fibrotic markers, transient elastography (FibroScan) and real-time tissue elastography. *Intervirology* 2008; **51 Suppl 1**: 27-33 [PMID: 18544945 DOI: 10.1159/000122602]

133 **Meng F**, Zheng Y, Zhang Q, Mu X, Xu X, Zhang H, Ding L. Noninvasive evaluation of liver fibrosis using real-time tissue elastography and transient elastography (FibroScan). *J Ultrasound Med* 2015; **34**: 403-410 [PMID: 25715361 DOI: 10.7863/ultra.34.3.403]

134 **Colombo S**, Buonocore M, Del Poggio A, Jamoletti C, Elia S, Mattiello M, Zabbialini D, Del Poggio P. Head-to-head comparison of transient elastography (TE), real-time tissue elastography (RTE), and acoustic radiation force impulse (ARFI) imaging in the diagnosis of liver fibrosis. *J Gastroenterol* 2012; **47**: 461-469 [PMID: 22223175 DOI: 10.1007/s00535-011-0509-4]

135 **Sandrin L**, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, Christidis C, Ziol M, Poulet B, Kazemi F, Beaugrand M, Palau R. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; **29**: 1705-1713 [PMID: 14698338 DOI: S0301562903010718]

136 **Fraquelli M**, Rigamonti C, Casazza G, Conte D, Donato MF, Ronchi G, Colombo M. Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. *Gut* 2007; **56**: 968-973 [PMID: 17255218 DOI: gut.2006.111302]

137 **Castéra L**, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Lédinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343-350 [PMID: 15685546 DOI: S0016508504020293]

138 **Ziol M**, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, de Lédinghen V, Marcellin P, Dhumeaux D, Trinchet JC, Beaugrand M. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; **41**: 48-54 [PMID: 15690481 DOI: 10.1002/hep.20506]

139 **Castera L**, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. *J Hepatol* 2008; **48**: 835-847 [PMID: 18334275 DOI: 10.1016/j.jhep.2008.02.008]

140 **Tsochatzis EA**, Gurusamy KS, Ntaoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. *J Hepatol* 2011; **54**: 650-659 [PMID: 21146892 DOI: 10.1016/j.jhep.2010.07.033]

141 **Chon YE**, Choi EH, Song KJ, Park JY, Kim do Y, Han KH, Chon CY, Ahn SH, Kim SU. Performance of transient elastography for the staging of liver fibrosis in patients with chronic hepatitis B: a meta-analysis. *PLoS One* 2012; **7**: e44930 [PMID: 23049764 DOI: 10.1371/journal.pone.0044930]

142 **Sporea I**, Sirli R, Deleanu A, Tudora A, Curescu M, Cornianu M, Lazar D. Comparison of the liver stiffness measurement by transient elastography with the liver biopsy. *World J Gastroenterol* 2008; **14**: 6513-6517 [PMID: 19030204]

143 **Friedrich-Rust M**, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960-974 [PMID: 18395077 DOI: 10.1053/j.gastro.2008.01.034]

144 **Cosgrove D**, Piscaglia F, Bamber J, Bojunga J, Correas JM, Gilja OH, Klauser AS, Sporea I, Calliada F, Cantisani V, D'Onofrio M, Drakonaki EE, Fink M, Friedrich-Rust M, Fromageau J, Havre RF, Jenssen C, Ohlinger R, Săftoiu A, Schaefer F, Dietrich CF. EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 2: Clinical applications. *Ultraschall Med* 2013; **34**: 238-253 [PMID: 23605169 DOI: 10.1055/s-0033-1335375]

145 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245-264 [PMID: 21371579 DOI: 10.1016/j.jhep.2011.02.023]

146 **Shi KQ**, Fan YC, Pan ZZ, Lin XF, Liu WY, Chen YP, Zheng MH. Transient elastography: a meta-analysis of diagnostic accuracy in evaluation of portal hypertension in chronic liver disease. *Liver Int* 2013; **33**: 62-71 [PMID: 22973991 DOI: 10.1111/liv.12003]

147 **Calvaruso V**, Bronte F, Conte E, Simone F, Craxì A, Di Marco V. Modified spleen stiffness measurement by transient elastography is associated with presence of large oesophageal varices in patients with compensated hepatitis C virus cirrhosis. *J Viral Hepat* 2013; **20**: 867-874 [PMID: 24304456 DOI: 10.1111/jvh.12114]

148 **Adebajo CO**, Talwalkar JA, Poterucha JJ, Kim WR, Charlton MR. Ultrasound-based transient elastography for the detection of hepatic fibrosis in patients with recurrent hepatitis C virus after liver transplantation: a systematic review and meta-analysis. *Liver Transpl* 2012; **18**: 323-331 [PMID: 22140010 DOI: 10.1002/lt.22460]

149 **Poynard T**, Munteanu M, Luckina E, Perazzo H, Ngo Y, Royer L, Fedchuk L, Sattonnet F, Pais R, Lebray P, Rudler M, Thabut D, Ratziu V. Liver fibrosis evaluation using real-time shear wave elastography: applicability and diagnostic performance using methods without a gold standard. *J Hepatol* 2013; **58**: 928-935 [PMID: 23321316 DOI: 10.1016/j.jhep.2012.12.021]

150 **Bavu E**, Gennisson JL, Couade M, Bercoff J, Mallet V, Fink M, Badel A, Vallet-Pichard A, Nalpas B, Tanter M, Pol S. Noninvasive in vivo liver fibrosis evaluation using supersonic shear imaging: a clinical study on 113 hepatitis C virus patients. *Ultrasound Med Biol* 2011; **37**: 1361-1373 [PMID: 21775051 DOI: 10.1016/j.ultrasmedbio.2011.05.016]

151 **Ferraioli G**, Tinelli C, Dal Bello B, Zicchetti M, Filice G, Filice C. Accuracy of real-time shear wave elastography for assessing liver fibrosis in chronic hepatitis C: a pilot study. *Hepatology* 2012; **56**: 2125-2133 [PMID: 22767302 DOI: 10.1002/hep.25936]

152 **Leung VY**, Shen J, Wong VW, Abrigo J, Wong GL, Chim AM, Chu SH, Chan AW, Choi PC, Ahuja AT, Chan HL, Chu WC. Quantitative elastography of liver fibrosis and spleen stiffness in chronic hepatitis B carriers: comparison of shear-wave elastography and transient elastography with liver biopsy correlation. *Radiology* 2013; **269**: 910-918 [PMID: 23912619 DOI: 10.1148/radiol.13130128]

153 **Zheng J**, Guo H, Zeng J, Huang Z, Zheng B, Ren J, Xu E, Li K, Zheng R. Two-dimensional shear-wave elastography and conventional US: the optimal evaluation of liver fibrosis and cirrhosis. *Radiology* 2015; **275**: 290-300 [PMID: 25575116 DOI: 10.1148/radiol.14140828]

154 **Palmeri ML**, Wang MH, Dahl JJ, Frinkley KD, Nightingale KR. Quantifying hepatic shear modulus in vivo using acoustic radiation force. *Ultrasound Med Biol* 2008; **34**: 546-558 [PMID: 18222031 DOI: 10.1016/j.ultrasmedbio.2007.10.009]

155 **Friedrich-Rust M**, Nierhoff J, Lupsor M, Sporea I, Fierbinteanu-Braticevici C, Strobel D, Takahashi H, Yoneda M, Suda T, Zeuzem S, Herrmann E. Performance of Acoustic Radiation Force Impulse imaging for the staging of liver fibrosis: a pooled meta-analysis. *J Viral Hepat* 2012; **19**: e212-e219 [PMID: 22239521 DOI: 10.1111/j.1365-2893.2011.01537.x]

156 **Friedrich-Rust M**, Wunder K, Kriener S, Sotoudeh F, Richter S, Bojunga J, Herrmann E, Poynard T, Dietrich CF, Vermehren J, Zeuzem S, Sarrazin C. Liver fibrosis in viral hepatitis: noninvasive assessment with acoustic radiation force impulse imaging versus transient elastography. *Radiology* 2009; **252**: 595-604 [PMID: 19703889 DOI: 10.1148/radiol.2523081928]

157 **Sporea I**, Sirli RL, Deleanu A, Popescu A, Focsa M, Danila M, Tudora A. Acoustic radiation force impulse elastography as compared to transient elastography and liver biopsy in patients with chronic hepatopathies. *Ultraschall Med* 2011; **32 Suppl 1**: S46-S52 [PMID: 20603783 DOI: 10.1055/s-0029-1245360]

158 **Sporea I**, Bota S, Peck-Radosavljevic M, Sirli R, Tanaka H, Iijima H, Badea R, Lupsor M, Fierbinteanu-Braticevici C, Petrisor A, Saito H, Ebinuma H, Friedrich-Rust M, Sarrazin C, Takahashi H, Ono N, Piscaglia F, Borghi A, D'Onofrio M, Gallotti A, Ferlitsch A, Popescu A, Danila M. Acoustic Radiation Force Impulse elastography for fibrosis evaluation in patients with chronic hepatitis C: an international multicenter study. *Eur J Radiol* 2012; **81**: 4112-4118 [PMID: 23000186 DOI: 10.1016/j.ejrad.2012.08.018]

159 **Bota S**, Herkner H, Sporea I, Salzl P, Sirli R, Neghina AM, Peck-Radosavljevic M. Meta-analysis: ARFI elastography versus transient elastography for the evaluation of liver fibrosis. *Liver Int* 2013; **33**: 1138-1147 [PMID: 23859217 DOI: 10.1111/liv.12240]

160 **Sporea I**, Bota S, Jurchis A, Sirli R, Grădinaru-Tascău O, Popescu A, Ratiu I, Szilaski M. Acoustic radiation force impulse and supersonic shear imaging versus transient elastography for liver fibrosis assessment. *Ultrasound Med Biol* 2013; **39**: 1933-1941 [PMID: 23932281 DOI: 10.1016/j.ultrasmedbio.2013.05.003]

**P-Reviewer:** Marginean CO, Petersen JR **S-Editor:** Yu J **L-Editor:** **E-Editor:**

**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 |
|  |  | cirrhosis1 | 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 |
|  |  | Cirrhosis1 | 62 | 87 | 0.90 |

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

**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 + ALT1 | significant fibrosis | 65 | 85 | 0.79 |
|  | advanced fibrosis and cirrhosis | - | - | 0.86 |

1Except for these values, which were taken from[87], all other values are medians from[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.