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TOPIC HIGHLIGHT

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Non-invasive assessment of liver fibrosis in chronic liver diseases: Implementation in clinical practice and decisional algorithms

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Abstract

Chronic hepatitis B and C together with alcoholic and non-alcoholic fatty liver diseases represent the major causes of progressive liver disease that can eventually evolve into cirrhosis and its end-stage complications, including decompensation, bleeding and liver cancer. Formation and accumulation of fibrosis in the liver is the common pathway that leads to an evolutive liver disease. Precise definition of liver fibrosis stage is essential for management of the patient in clinical practice since the presence of bridging fibrosis represents a strong indication for antiviral therapy for chronic viral hepatitis, while cirrhosis requires a specific follow-up including screening for esophageal varices and hepatocellular carcinoma. Liver biopsy has always represented the standard of reference for assessment of hepatic fibrosis but it has some limitations being invasive, costly and prone to sampling errors. Recently, blood markers and instrumental methods have been proposed for the non-invasive assessment of liver fibrosis. However, there are still some doubts as to their implementation in clinical practice and a real consensus on how and when to use them is not still available. This is due to an unsatisfactory accuracy for some of them, and to an incomplete validation for others. Some studies suggest that performance of non-invasive methods for liver fibrosis assessment may increase when they are combined. Combination algorithms of non-invasive methods for assessing liver fibrosis may represent a rational and reliable approach to implement non-invasive assessment of liver fibrosis in clinical practice and to reduce rather than abolish liver biopsies.

Key words: Chronic liver diseases; Hepatic fibrosis; Liver biopsy; Non-invasive methods for liver fibrosis assessment; Combination algorithms; Decisional tree

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INTRODUCTION

Chronic liver diseases (CLDs) represent a major cause of morbidity and mortality worldwide. The major etiologies are chronic infection with hepatitis B (HBV) and C (HCV) viruses, and alcoholic and non-alcoholic fatty liver disease. Chronic hepatitis B and C are the leading causes of cirrhosis and of hepatocellular carcinoma (HCC) worldwide. Approximately 400 million people are chronically infected with HBV and 25%-40% of them die of cirrhosis and of its end-stage complications[1]. HBV is the most important carcinogen after tobacco and the incidence of HCC is 300 000 cases per year[2]. Chronic hepatitis C is a major health concern with around 200 million individuals affected worldwide, with a greater prevalence in Western countries[3]. Natural history studies indicate that advanced fibrosis and cirrhosis develop in about 20%-40% of patients with chronic viral hepatitis[4,5]. Alcoholic liver disease (ALD) is one of the leading causes of end-stage CLD. It is well established that only a minority of heavy drinkers, estimated at between 10% and 30%, will ever develop advanced ALD and that the risk increases with cumulative alcohol intake[6,7]. Non-

alcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease and impaired liver function in industrialized countries, where 10%-23% of the adult population is estimated to be affected[8,9]. The disease has a spectrum ranging from fatty liver alone to non-alcoholic steatohepatitis (NASH), and progressive steatofibrosis. Many cases of cryptogenic cirrhosis may be end-stage forms of NASH[10]. Hepatic steatosis is currently considered a manifestation of metabolic syndrome[11,12], which is defined as an association of at least 3 of the following disturbances: insulin resistance, central obesity, arterial hypertension, and dyslipidemia, whether hypertriglyceridemia or low HDL-cholesterol levels. Only a percentage of individuals with liver steatosis progress to more advanced stages of the disease[8-10]. The pathogenesis of NAFLD and the reasons why some patients with fatty liver develop NASH and have progressive liver disease are not entirely understood. The most widely supported theory implicates insulin resistance as the key mechanism in NAFLD, leading to hepatic steatosis, and perhaps also to NASH. Obesity, type 2 diabetes, hyperlipidemia and other conditions associated with insulin resistance are generally present in patients with NAFLD[11,12]. A "two-hit" hypothesis has been proposed, involving the accumulation of fat in the liver ("first hit"), together with a "second hit" that produces oxidative stress. Hepatic steatosis has been recognised as the first of two hits in the pathogenesis of NASH, since the presence of oxidizable fat within the liver is enough to trigger lipid peroxidation[13]. However, many patients with fatty liver do not progress to steatohepatitis. Potential second hits for the evolution towards NASH include all mechanisms contributing to the development of inflammation and fibrosis. The presumed factors initiating second hits are oxidative stress and subsequent lipid peroxidation, proinflammatory cytokines (principally tumour necrosis factor alpha), and hormones derived from adipose tissue (adipocytokines)[12]. The progression of liver disease in CLDs presents with a common histopathological pathway which is the formation and accumulation of fibrosis leading to the development of progressive distortion of the hepatic architecture that is the hallmark of evolution to cirrhosis. Liver fibrosis is the result of chronic injury and it appears to play a direct role in the pathogenesis of hepatocellular dysfunction and portal hypertension[14,15]. Development of fibrosis is a progressive process starting from minimal fibrosis limited to the portal tracts, followed by more extensive fibrosis with septa expanding into the liver parenchyma, which can form bridges between two portal tracts or portal tracts and central veins, eventually ending in complete cirrhotic nodules. In patients with CLDs precise definition of the hepatic fibrosis stage is of paramount importance to evaluate the prognosis and the follow-up of the hepatic disease and to decide the need for antiviral therapy in HBV and HCV chronic infections. In CLDs liver biopsy has always been the gold standard for evaluating presence, type and stage of liver fibrosis and to characterize necroinflammation. This procedure, however, presents some limitations since it is invasive, costly and difficult to standardize. Recently, there has been increasing interest in non-invasive assessment of liver fibrosis by using surrogate markers measurable in the peripheral blood or by using instrumental devices, but some concerns about their large-scale clinical use have been raised, based on their performance and validation. This article aims to review the current status of the literature regarding non-invasive assessment of liver fibrosis in CLDs, considering its limitations and advantages. Finally, decisional algorithms to be applied to the most validated and reliable methods in clinical practice are here proposed.

Table 1 METAVIR and Ishak staging systems for liver fibrosis

Description	METAVIR (F)	Ishak (S)
No fibrosis	0	0
Portal fibrosis without septa	1	1-2
Portal fibrosis with few septa	2	3
Septal fibrosis without cirrhosis	3	4
Cirrhosis	4	5-6

Portal fibrosis is a stellate enlargement of portal tracts without any bridging fibrosis on the biopsy sample. Few septa means at least one fibrous septum on the core biopsy. Theoretically, a fibrous septum is a bridge of connective tissue between two portal tracts, a portal tract and a centrolobular vein, or between two centrolobular veins. Septal fibrosis means that the liver biopsy is crossed by several septa; the transition between F2 and F3 by METAVIR or S3 and S4 by Ishak begins when there are more fibrous septa than portal tracts without septa on the biopsy. Cirrhosis means that liver tissue is mutilated by nodular fibrosis that delineates hepatocytes nodules.

HISTOLOGICAL SYSTEMS TO STAGE LIVER FIBROSIS

Several semiquantitative scoring systems have been proposed to stage fibrosis and to grade necroinflammation in the liver. The Ishak's system is a revised version of the older histological activity index[16,17]. It describes grading and staging as two separate items and liver fibrosis is classified as absent (0), mild (1-2), moderate (3-4) and severe/cirrhosis (5-6). This classification system is mainly applied to hepatitis B and C. The METAVIR scoring system for staging has been frequently used in recent times particularly for chronic hepatitis C (Table 1)[18]. Brunt classification of fibrosis assessment is generally used for NASH and it includes five stages: stage 0, no fibrosis; stage 1, zone 3 perisinusoidal or pericellular fibrosis, focally or extensively present; stage 2, zone 3 perisinusoidal or pericellular fibrosis with focal or extensive periportal fibrosis; stage 3, zone 3 perisinusoidal or pericellular fibrosis and portal fibrosis with focal or extensive bridging fibrosis; stage 4, cirrhosis[19]. All these scoring systems have some limits, being semiquantitative, not linear and prone to intra- and inter-observer variation and to sampling variability.

LIVER BIOPSY: IS IT A GOLD OR A SILVER STANDARD?

Liver biopsy has long been the gold standard for staging of

Table 2 Pros and Cons of liver biopsy in staging of hepatic fibrosis

PROS	CONS
Staging of liver fibrosis	Invasiveness (pain, bleeding)
Grade of necroinflammation	Cost (hospitalization)
Steatosis (common in hepatitis C)	Sampling errors
Iron overload (common in hepatitis C)	Possibly refused by patient, concern of physician
Comorbidities (autoimmunity stigmates)	Static data, no information on fibrogenesis

liver fibrosis in CLDs. Liver biopsy has the advantage of obtaining direct information not only about fibrosis, but also about many useful parameters, such as inflammation, necrosis, steatosis, iron or copper deposits. Furthermore, it allows the identification of suspected or unexpected cofactors and comorbidities. However, biopsy is associated with potential morbidity and mortality and has several limitations (Table 2). A single liver biopsy provides static data but with no information on fibrogenesis and fibrolysis that characterise the dynamic processes related to extracellular matrix (ECM) metabolism. Moreover, many recent studies clearly indicate that liver biopsy is prone to sampling errors and may underestimate the amount of liver fibrosis. Cirrhosis could be missed on a single blind percutaneous liver biopsy in 10%-30% of cases[20,21]. When three different liver samples were analyzed, the percentage of correct diagnoses increased from 80% to 100%[22]. In more recent times, Regev *et al*[23] have shown that samples obtained from the right and left lobes of the liver during laparoscopy give different fibrosis staging in one third of cases, with a concordance rate of more than 90% between two experienced pathologists. Other studies have analyzed agreement/disagreement among pathologists. Although the use of more standardized scoring systems, such as those of the Ishak's, METAVIR's and Brunt's classifications, has improved the inter-observer and intra-observer variability, there are still several factors that may significantly influence the reliability of a liver biopsy. The size of the liver sample is very important, especially if we consider that a hepatic sample of 15 mm length represents 1/50000 of the whole parenchyma. Colloredo *et al*[24] have carefully analyzed the impact of the sample size on a correct staging of liver fibrosis in patients with hepatitis C. By reducing progressively the dimensions of the same liver biopsy, they reported that the smaller was the sample analyzed, the milder was the diagnosis made by the pathologist in relation to the stage of fibrosis. Other studies have reported that the type and the size of needle used are also important. The Tru-Cut needle was found to be superior to the Menghini needle, particularly for the diagnosis of more advanced fibrosis[25]. The use of a thicker needle ameliorates the accuracy of the diagnosis but also implies an increased risk of bleeding and perforation for the patient. Interestingly, Rousselet *et al*[26] reported that the degree of experience of the pathologist, as indicated by longer duration of practice or belonging to an academic setting, may have an outstanding impact on the diagnostic interpretation of liver biopsy, even higher than that determined by the one related to sample size. Another shortcoming of liver biopsy is its cost. A cost-benefit analysis showed that in the US the cost of a liver biopsy is 1032 USD and it could rise to 2745 USD when complications occur[27].

Table 3 Features of an adequate liver biopsy sample

Length (mm)	Portal tracts (n°)	Ref.
15	5	[28,29]
20	11	[30]
25	NA	[31]
Bigger is better	NA	[32]

NA: Not available.

LIVER BIOPSY: CONSENSUS AMONG PATHOLOGISTS?

Pathologists have tried to define the features (including length and number of complete portal tracts) of an adequate liver biopsy sample able to reduce the risk of misclassification of liver fibrosis (Table 3). Some authors would suggest that an adequate liver biopsy sample should contain more than 5 portal tracts and be at least 15 mm in length[28,29]. Other studies reported a higher threshold for optimized accuracy. Guido and Rugge have produced a critical review of the literature concerning the use of liver biopsy in chronic viral hepatitis[30]. They suggest that liver biopsy is very often flawed by unacceptable methodological limits and that a biopsy sample of 20 mm or more containing at least 11 complete portal tracts should be considered reliable for adequate grading and staging. Other authors have recommended even bigger samples, up to 25 mm in length[31]. Scheuer has recently concluded that "bigger is better"[32].

LIVER BIOPSY: CONSENSUS AMONG CLINICIANS?

The pathologist's need for obtaining a liver sample of adequate size is in contrast with the patient's need for a procedure causing limited pain and risks. Liver biopsy may in fact be a risky procedure for some patients, particularly for those with more advanced liver fibrosis. Indeed, one third of patients experience pain at the time of the procedure, and the proportion of 0.3%-0.6% of cases presents with serious adverse events like bleeding and even death in decompensated cirrhosis[33]. A French survey which interviewed 1177 general practitioners concluded that liver biopsy may be refused by up to 59% of patients with hepatitis C and that 22% of the physicians share the same concern for the invasiveness of the procedure[34]. On this topic, a survey assessing the consensus among Italian hepatologists on when and how to take a liver biopsy in chronic hepatitis C showed great divergence in the management of the same subgroup of patients[35]. A nationwide survey about assessment of liver fibrosis in hepatitis C among French hepatologist showed

that liver biopsy was still systematically performed by only 4% of respondents. Guidelines for the clinical use of non-invasive methods for assessment of liver fibrosis were required by 95% of the respondents[36].

THE IDEAL NON-INVASIVE METHOD FOR LIVER FIBROSIS

In view of all the shortcomings regarding liver biopsy, in the last decade clinical investigators have been searching for non-invasive methods for accurate information about liver fibrogenesis activity and fibrosis stage in patients with CLDs. Fibrosis is a structural change in the liver that accompanies chronic injury; fibrogenesis refers to the production of ECM. Fibrogenesis increases in response to injury and is essential to tissue repair. The key step in the pathophysiology of liver fibrogenesis is the balance between ECM deposition and removal. An excess of ECM produced after injury stimulates fibrolysis which is mediated by several specific matrix metalloproteinases (MMPs). The hepatic stellate cells (HSCs) are the major source of ECM[14]. Guidelines and Recommendations indicate that staging of liver fibrosis is the most important parameter for the definition of prognosis and for the subsequent management of the patient with CLD[37,38]. Natural history studies indicate that, if only an insignificant rate of patients without fibrosis will develop cirrhosis in the following 5 years, this percentage goes up to 20% for cases with portal fibrosis and to more than 40% for cases with septal fibrosis[39]. Moreover, the decision whether to start an antiviral therapy in cases of chronic viral hepatitis is highly influenced by the staging of liver fibrosis, since treatments are usually long, costly and cause side effects. Identification of patients with cirrhosis is essential to start screening for end-stage liver complications, including esophageal varices (OV) and HCC. International guidelines have defined two stages of liver fibrosis that significantly modify the management of the patients in clinical practice[37,38]: (1) Significant fibrosis, defined as a liver fibrosis stage (F) $\geqslant$ 2 according to METAVIR for hepatitis C or (S) $\geqslant$ 2 according to Ishak for hepatitis B. Significant fibrosis is a definitive indication to start antiviral therapy in chronic hepatitis B and in chronic hepatitis C due to difficult-to-treat genotypes (HCV-1 and HCV-4). For patients infected with HCV genotype 2 or 3 histological definition is not necessary except for those cases with relative contraindications, not motivated or elderly age. The recent Italian Guidelines on the management of chronic hepatitis B have underlined the importance of the stage of liver fibrosis not only in deciding who to treat, but also in deciding the first choice treatment: interferon for mild-moderate fibrosis and nucleoside/nucleotide analogues for cirrhosis, especially if decompensated[38]. (2) Hepatic cirrhosis, defined as liver fibrosis stage of (F) 4 by METAVIR and of (S) 6 by Ishak. Cirrhosis, even when fully compensated and still clinically occult, requires a different and more specific management than simple chronic hepatitis, including screening for OV with annual gastroscopy and for HCC with ultrasound and alpha-fetoprotein every 6 mo.

Table 4 Features of the ideal non-invasive method for liver fibrosis

Reliable (high diagnostic accuracy)
Widely available (simple, least expensive)
Providing information on both fibrosis stage and fibrogenesis activity
Validated by large-scale studies
Validated by independent studies (different authors from the proposing study)
Validated in various etiologies of CLDs (HCV, HBV, ALD, NAFLD)
Identifying clinically important fibrosis stages (significant fibrosis and cirrhosis)

CLDs: Chronic liver diseases; ALD: Alcoholic liver disease; NAFLD: Non-alcoholic fatty liver disease.

The ideal marker test would be able to accurately stage disease and also be sensitive to changes in fibrosis induced by the natural course of disease progression or by therapy (Table 4). Non-invasive methods for detecting liver fibrosis may be divided in two main groups: markers measured in peripheral blood, which could be single parameters or panels combining more parameters, and a technical device that measures the liver stiffness through transient elastography (fibroscan).

SERUM NON-INVASIVE MARKERS OF LIVER FIBROSIS

Among the proposed markers in the literature, some are directly linked to the modifications in ECM turnover occurring during fibrogenesis, the so-called "direct markers", while others reflect alterations in hepatic function but do not directly reflect ECM metabolism, the so-called "indirect markers"[14,15]. The direct markers of liver fibrosis include several glycoproteins (hyaluronan, laminin, human cartilage glycoprotein 39), the collagens family (procollagen Ⅲ, type Ⅳ collagen), the collagenases and their inhibitors and a number of cytokines connected with the fibrogenetic process (TGF-β1, TNF-α). These markers have a pathophysiologic rationale since they may be an expression of either deposition or removal of ECM, thus giving information on its metabolism. They may potentially be used not only to stage liver fibrosis, but also to assess the speed of liver fibrogenesis with the most relevant prognostic value, and also to estimate and monitor the efficacy of and the response to antifibrotic drugs. A limitation to the clinical use of direct markers of liver fibrosis is that they are not routinely available in all hospital settings. The indirect markers of liver fibrosis are biochemical parameters that are measurable in the peripheral blood. They are an indirect expression of liver damage and have a statistical association with liver fibrosis stage. While direct markers of liver fibrosis reflect the process of fibrogenesis, indirect markers satisfy the request for a simple and easy-to-perform marker. Both direct and indirect markers for liver fibrosis may be single or a combination of parameters (Tables 5 and 6). Most of them have been proposed and validated in chronic hepatitis C. Table 7 describes the accuracy of various

Table 5 Single serum non-invasive markers for liver fibrosis

Direct markers	Indirect markers
Hyaluronic acid	Platelet count
Laminin	AST, ALT
Procollagen Ⅲ	γGT
Type Ⅳ Collagen	γ-globulins
Metalloproteinases	Albumin
Inhibitors of metalloproteinases	Prothrombin time

serum non-invasive markers for liver fibrosis as reported in the literature. The performance of non-invasive markers is usually expressed as sensitivity, specificity, positive and negative predictive values (PPV, NPV), accuracy, and compared area under the receiving operating characteristic curve (AUROC).

Hyaluronic acid has been extensively studied in hepatitis C while few studies are available in other etiologies. Overall, a rather good accuracy of this marker in the different CLDs has been reported for detection of significant fibrosis, with an AUROC ranging from a minimum of 0.82 to a very good 0.92[40-46]. In a study conducted in 326 patients, the AUROC was 0.86 and the specificity was 95% for significant fibrosis while the AUROC was 0.92 and the specificity was 89.4% for cirrhosis when a cut off level of 110 μg/L was used[45]. However, another cohort study with more than 400 cases has reported an AUROC of only 0.73 for significant fibrosis[42]. In the same study, cirrhosis could be excluded with excellent NPV and sensitivity (100%) and with excellent AUROC (0.97) using a cut off level of 50 μg/L. Similar results were reported in another study of 486 patients in which hyaluronic acid levels < 60 μg/L excluded cirrhosis with 99% NPV[40]. In ALD the performance of hyaluronic acid for significant fibrosis varied significantly[43,46] while the marker showed very good performance for cirrhosis, with an AUROC of 0.93[46]. The results of a study conducted in 79 patients with NAFLD were also encouraging, since hyaluronic acid had a 0.92 AUROC value for cirrhosis[44]. On the basis of its good accuracy, especially for exclusion of cirrhosis, hyaluronic acid has also been used in panels combining other serum non-invasive markers for liver fibrosis. Recently it has been proposed in combination with AST-to-platelet ratio index (APRI) in hepatitis B. In this study, a combination of APRI > 1.5 and of hyaluronic acid > 300 ng/mL had 98.9% specificity and 93.7% PPV[47]. Laminin is another component of ECM that has been studied as a non-invasive marker. Serum levels of laminin have been used by several authors as a non-invasive parameter to assess liver fibrosis in ALD patients as well as in those presenting with viral hepatitis and hemochromatosis[48]. This determination, however, was progressively discontinued as it did not demonstrate superiority to those of other components of the ECM such as hyaluronic acid. It showed 77% accuracy for detection of significant fibrosis in hepatitis C in a detailed study on 243 patients with CLD[49]. With regard to NAFLD, however, the use of laminin serum levels could be further investigated since a single report, which investigated liver fibrosis in 30 overweight patients, showed a rather good accuracy (87%)[50]. Among the collagens, type Ⅳ collagen has been investigated as surrogate marker of liver fibrosis. Type Ⅳ collagen has been studied in hepatitis C and a good performance for significant fibrosis has been reported (AUC = 0.83)[51]. Murawaki *et al*[52] have compared the diagnostic performance of type Ⅳ collagen with that of hyaluronic acid in hepatitis C and reported the superiority of the latter marker. The role of type Ⅳ collagen has also been investigated in 112 patients with NAFLD and its performance has been compared with hyaluronic acid[53]. The results showed a better diagnostic accuracy for type Ⅳ collagen (0.828 *vs* 0.797 AUROC, respectively). Metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of metalloproteinases, TIMPs) have also been proposed as surrogate markers of liver fibrosis. Those reported to have some clinical impact include MMP-2 and TIMP-1[54]. Boeker *et al*[54] reported a very high performance of MMP-2 in detecting cirrhosis (0.97 AUROC). Unfortunately, it has been difficult to obtain good standardization of the method for routine clinical use. Some authors proposed panels of direct non-invasive markers with the aim of increasing the accuracy of the single parameters. Fibrometer combines age, platelets, prothrombin index, AST, α-2-macroglobulin, hyaluronan and urea. In a few studies, the AUROC for significant fibrosis has been reported as 0.89 in hepatitis C, raising to an excellent 0.943 in patients with NAFLD[55,56]. Patel *et al*[57] proposed fibrospect which combines hyaluronic acid, TIMP-1 and α-2-macroglobulin. It showed an AUC 0.832 for METAVIR stages F2-F4 fibrosis with PPV and NPV of 74.3% and 75.8%, respectively. Another model, named Hepascore, combines bilirubin, γGT, hyaluronan, α-2-macroglobulin, age, and sex, and showed in hepatitis C and ALD a quite good performance for diagnosis of significant fibrosis, ranging from 0.78 to 0.85, and excellent performance for cirrhosis, ranging from 0.89 to 0.92[58,59]. Unfortunately, for both these combination panels large-scale, independent validation studies are lacking. The European Liver Fibrosis (ELF) study group proposed a panel of markers combining age, hyaluronan, type Ⅲ collagen and TIMP-1. In a cohort study of more than one thousand patients with a variety of CLDs the panel detected moderate or advanced fibrosis (Scheuer stages 3, 4) with a 0.77 to 0.94 AUROC in hepatitis C and ALD, respectively[60]. The panel has also been recently validated in 196 patients with NAFLD, with 0.90 AUROC for detection of severe fibrosis, that could increase to 0.98 when the original panel was combined with simple markers[61]. Similar results in terms of accuracy have been recently obtained in 112 consecutive pediatric patients with NAFLD[62].

AST-to-ALT ratio (AAR) was one of the first non-invasive markers proposed. It is easily available and without any cost but it showed a highly variable performance in the studies conducted on HCV patients: sensitivity was between 31.5% and 81.3%, specificity was between 55.3% and 97% and accuracy ranged from 60%-83.6%[63,64]. Another concern about this test may be that it does not identify significant fibrosis but only cirrhosis. In a prospective study, we have also validated AAR in 110 patients with chronic hepatitis B and we obtained 78.9% accuracy for the diagnosis of cirrhosis[65]. AST-to-platelet ratio in-

Table 6 Combinations of serum parameters for non-invasive diagnosis of liver fibrosis

Marker	Description	Settings in which validation exists	Ref.
AST/ALT	AST to ALT ratio	HCV, HBV	[63-65,71,72]
APRI	AST to platelets ratio index	HCV, HBV, HIV/HCV	[64-67,72,75-80]
Forns' index	Age, BMI, γGT, cholesterol	HCV, HBV, HIV/HCV	[67,69,72,75]
Fibrotest	Age, gender, α-2-macroglobulin, γGT, haptoglobin, apolipoprotein A1, total bilirubin	HCV, HBV, ALD, NAFLD, HIV/HCV	[65,67,72,76-80]
ELF	Age, hyaluronic acid, type Ⅲ procollagen, TIMP1	HCV, ALD, NAFLD	[60-62]
Hepascore	Bilirubin, γGT, hyaluronic acid, α-2-macroglobulin, age, sex	HCV	[58,59]
Lok index	AST, ALT, platelets, INR	HCV	[68]
Fibroindex	AST, platelets, g-globulins	HCV	[71,72]
Fibrometer	Age, AST, platelets, hyaluronan, INR, α-2-macroglobulin, urea	HCV	[55,56]
Fibrospect	α-2-macroglobulin, hyaluronan, TIMP1	HCV	[57]
Fib-4	Age, AST, ALT, platelets	HCV, HBV, HIV/HCV	[73-75]

APRI: AST-to platelet ratio index; ELF: European liver fibrosis study group.

Table 7 Performance of several serum non-invasive markers for liver fibrosis (single or combination) as expressed as AUROC

Serum marker	Significant fibrosis	Cirrhosis	Ref.
Hyaluronic acid	0.73-0.92	0.85-0.97	[40-47]
Laminin	0.82	NA	[48,49]
Type Ⅳ collagen	0.83	NA	[51-53]
MMP-2	0.59	0.97	[54]
TIMP-1	0.71	0.90	[54]
ELF	0.77-0.94	NA	[60-62]
AAR	NA	0.51-0.83	[63-65,71,72]
Forns' index	0.75-0.86	NA	[67,68,70,72]
APRI	0.69-0.88	0.61-0.94	[15,64-67,72,76-80]
Fibrotest	0.74-0.87	0.71-0.87	[15,65,67,72,76-80]
Fibroindex	0.74-0.83	NA	[71,72]
Fibrometer	0.89-0.96	NA	[55,56]
Fibrospect	0.83	NA	[57]
Fib-4	0.79-0.85	0.80-0.91	[73-75]
Hepascore	0.82-0.85	0.90-0.94	[58,59]

AUROC: Area under the receiving operating characteristic curve; MMP-2: Metalloproteinase 2; TIMP-1: Tissue inhibitor of metalloproteinases 1; ELF: European liver fibrosis study group; AAR: AST-to-ALT ratio; APRI: AST-to-platelet ratio index.

dex (APRI) is a simple and cheap ratio between AST and platelets, easily available in the clinical practice. It classifies both significant fibrosis and cirrhosis but around 50% of the cases result as unclassified. APRI performance is variable among the studies on hepatitis C: sensitivity ranges between 41% and 91%, specificity between 47% and 95% and accuracy between 60% and 82.7% for significant fibrosis; for cirrhosis, sensitivity ranges between 38.4% and 65.8%, specificity between 86.7% and 93% and accuracy between 60% and 88.4%[15,66,67]. We have also validated APRI in hepatitis B, obtaining 76.1% accuracy for the diagnosis of significant fibrosis and 79.2% for the diagnosis of cirrhosis[65]. Most recently, APRI has been modified into Lok index by adding alanine aminotrasferase (ALT) and international normalized ratio (INR), with further improvement of the diagnostic accuracy, particularly for cirrhosis[68].

Forns' index is a simple panel resulting from the combination of age, γGT, cholesterol and platelets. It does not give any information about cirrhosis, but only about significant fibrosis. Around half of the cases cannot be classified. In hepatitis C, the accuracy reported in various studies was variable (between 50% and 85%)[67,69]. We have also validated Forns' index in hepatitis B, obtaining 64.8% accuracy for the diagnosis of significant fibrosis[65]. It has been suggested that Forns' index might be less accurate in patients with HCV genotype 3 which is associated with very low cholesterol levels[70]. However, this has not been confirmed by other data[67]. In a study performed on 3690 patients with chronic hepatitis C, a combination panel derived from platelets, AST, and γ-globulin named Fibroindex showed 0.83 AUROC in predicting significant fibrosis[71]. However, following validation studies it showed a lower performance[72]. Another combination of simple markers named Fib-4 was recently proposed and it uses platelets, ALT, AST and age. It showed good performance for detection of severe fibrosis (0.85 AUROC) and even better for the diagnosis of cirrhosis (0.91 AUROC) in chronic hepatitis C[73]. The performance of the panel was also evaluated in a cohort of patients with chronic hepatitis B, with similar accuracy for diagnosis of significant fibrosis (0.81 AUROC)[74]. The validity of Fib-4 as a non-invasive marker for liver fibrosis has also been investigated in patients with HCV/HIV coinfection and the reported accuracy was 0.79 for significant fibrosis and 0.80 for cirrhosis[75]. Fibrotest is a patented test that combines γGT, total bilirubin, haptoglobin, α-2-macroglobulin, apolipoprotein A1, age and gender[76]. To date, it is the most validated non-invasive method for liver fibrosis in various etiologies: HCV, HBV, ALD, NAFLD and HIV/HCV coinfected. Between 2001 and 2008 more than 60 scientific studies have investigated fibrotest and 20 of them are independent with respect to the group that have commercialized the test. Overall, independent studies have investigated fibrotest in more than 3000 patients with CLD, mostly hepatitis C. The accuracy reported ranges from 70%-85%[15,67,76]. Fibrotest has been applied to hepatitis B patients and the accuracy reported varies between 83.3% and 87.3% for significant fibrosis and between 86.1% and 94.4% for the diagnosis of cirrhosis[65,77]. In HIV/HCV coinfected patients AUROC was 0.85 for significant fibrosis and 0.87 for cirrhosis[78]. Fibrotest was also validated in ALD, with excellent results, especially for cirrhosis (0.84

AUROC for significant fibrosis and 0.95 AUROC for cirrhosis)[79]. Fibrotest was also applied in 170 patients with NAFLD and the AUROC for significant fibrosis was 0.86[80]. These results in HIV/HCV coinfected, ALD and NAFLD cases need, however, further confirmation from independent groups. Some conditions may alter the result of fibrotest, including Gilbert syndrome and hemolysis. In these cases the clinician should be cautious in the interpretation of the result and the test should be repeated. Overall, among the various serum markers proposed in the literature, APRI and fibrotest are the most validated in all etiologies, and also validated in many independent studies.

TRANSIENT ELASTOGRAPHY (FIBROSCAN)

Apart from serum markers, another method for non-invasive assessment of liver fibrosis is the measurement of liver stiffness[81]. Transient elastography is measured through a device that is called fibroscan (Echosens, Paris) which is composed of an ultrasound transducer probe mounted on the axis of a vibrator. Vibrations of mild amplitude and low frequency are transmitted by the transducer, inducing an elastic shear wave that propagates through the underlying tissues. Pulse-echo ultrasound acquisition is used to follow the propagation of the shear wave and to measure its velocity, which is directly related to tissue stiffness: the stiffer the tissue, the faster the shear wave propagates. Transient elastography measures liver stiffness in a volume that is approximately a cylinder 1 cm wide and 4 cm long, between 2.5 cm and 6.5 cm below the skin surface. This volume is at least 100 times bigger than a biopsy sample. Fibroscan examination is painless, rapid (less than 5 min) and easy to perform at the bedside or in the outpatient clinic. The examination is performed on a non-fasting patient lying flat on his/her back, with the right arm tucked behind the head. The probe transducer is placed on the skin, between the rib bones at the level of the right lobe of the liver where biopsy would be performed. The operator performs 10 valid acquisitions and then the software of fibroscan calculates the median value. The software itself determines whether each measurement is successful or not. Results are expressed in kilo-Pascals (kPa). Liver stiffness values range from 2.5-75 kPa. The results are immediately available and are operator-independent[82]. The exam can be done after a short learning curve (about 100 examinations). The validity of a fibroscan result should be based on two important parameters: (1) the interquartile range (IQR), which reflects the variability of the validated measures, and should not exceed 30% of the median value; (2) the success rate, that is the percentage of valid measurement, should be at least 60%. Despite the exam being relatively easy to perform, the clinical interpretation of results should always be in the hands of an expert clinician who should have at his disposal all clinical information regarding the patient. The result of the fibroscan is given according to cut-off values expressed in kPa: according to the various studies, presence of significant fibrosis is defined by a cutoff value of 7.1 to 8.7, and cirrhosis is diagnosed by a cutoff value of 12.5 to 14.5[83,84]. In various studies, the accuracy of fibroscan results were similar to that of serum non-invasive markers for the diagnosis of significant fibrosis, sometimes with inadequate figures (< 80%). On the other hand, fibroscan showed excellent performance for the diagnosis of cirrhosis (Table 8)[85]. Liver stiffness measurements can be difficult in obese patients or in those with narrow intercostal space and impossible in patients with ascites[81]. Failure rates range between 2.4% and 9.4% in the different studies[81-83,86]. Factors associated with inter- and intra-observator variability were BMI > 25, high grade hepatic steatosis and mild fibrosis (F0-F1 by METAVIR)[82]. A single report suggested that transaminase flares during chronic HBV infection may alter the result of fibroscan because of high flogosis and recruitment of inflammatory cells into the liver parenchyma[87]. Interestingly, a report suggested that acute viral hepatitis increases liver stiffness measured by fibroscan, thus the authors recommend that the extent of necroinflammatory activity needs to be carefully considered in future studies, particularly in patients with absent or low-stage liver fibrosis[90]. Non-invasive assessment of liver fibrosis with fibroscan has also been applied to ALD with 0.91 AUROC for significant fibrosis and 0.92 for cirrhosis[91]. Table 9 summarizes the main limitations of fibroscan. A recent meta-analysis concluded that for the diagnosis of significant fibrosis, transient elastography cannot be used sufficiently in clinical practice. Inclusion of transient elastography in an algorithm with a combination of non-invasive serum markers may be considered[92]. Transient elastography can be used in clinical practice as an excellent tool for the confirmation of cirrhosis when other clinical signs and examinations are non-decisive.

Table 8 Accuracy of fibroscan for the diagnosis of significant fibrosis and cirrhosis

Ref.	Etiology	Accuracy for ⩾ F2	Accuracy for F4
[81]	HCV	88	99
[83]	HCV	83	95
[84]	HCV	79	95
[86]	HCV	80	96
[87]	HCV	NA	95
[88]	HBV	87	88
[89]	HBV	90	94

COMBINATION ALGORITHMS AND IMPLEMENTATION OF NON-INVASIVE METHODS FOR LIVER FIBROSIS IN CLINICAL PRACTICE

The accuracy of most non-invasive methods for liver fibrosis showed variability among different studies and is still considered inadequate to substitute for liver biopsy and for implementation of non-invasive markers for liver fibrosis in clinical practice[15,29,93]. Some preliminary

Table 9 Limitations of fibroscan in clinical practice

Difficult to perform in obese patients (5% rate failure)
Inter-observer and intra-observer variability influenced by liver steatosis
Influence of ALT flares (HBV reactivation)
Lower performance for diagnosis of significant fibrosis

studies suggested that accuracy of non-invasive methods may improve when they are combined in diagnostic algorithms. We have recently proposed an approach that combines APRI and fibrotest sequentially with the aim of increasing the diagnostic accuracy[67]. This is a rational approach for the use of non-invasive markers for liver fibrosis in clinical practice. Indeed, these markers are used when they present with adequate accuracy, while liver biopsy is used only in those patients in which non-invasive markers showed inadequate accuracy. This approach has been named SAFE (Sequential Algorithms for Fibrosis Evaluation) biopsy and its aim is to reduce the number of liver biopsies that are necessary to correctly stage liver fibrosis and to minimize misclassified cases. Through stepwise modeling, two algorithms were developed with the aim of correctly classifying the two stages of liver fibrosis that are clinically significant: (1) significant fibrosis, (2) cirrhosis. The modeling of the algorithms was aimed at achieving > 90% accuracy and minimizing misclassified cases. In the model APRI has been used as first line test since it is cheap and simple, fibrotest has been used as second line test since it is costly and more complex. Liver biopsy has been used only as third line test in those cases in which the two non-invasive markers did not show adequate accuracy and/or in unclassified cases (only for APRI) (Figures 1 and 2). The modeling of the stepwise algorithms was based on the predictive values of the single markers. In the algorithm for significant fibrosis (Figure 1), 0.5 cut-off of APRI had low NPV to exclude significant fibrosis, while 1.5 cut-off showed high PPV to diagnose significant fibrosis. Similarly, 0.49 cut-off of fibrotest showed high PPV to diagnose significant fibrosis, whereas values less than 0.48 could not accurately exclude significant fibrosis. In the algorithm for cirrhosis (Figure 2), 1 cut-off for APRI showed high NPV to exclude cirrhosis, while 2 cut-off did not show sufficient PPV to diagnose cirrhosis. Similarly, 0.48 and 0.75 cut-offs of fibrotest showed good NPV and PPV, respectively, for cirrhosis, while intermediate values could not give accurate diagnosis.

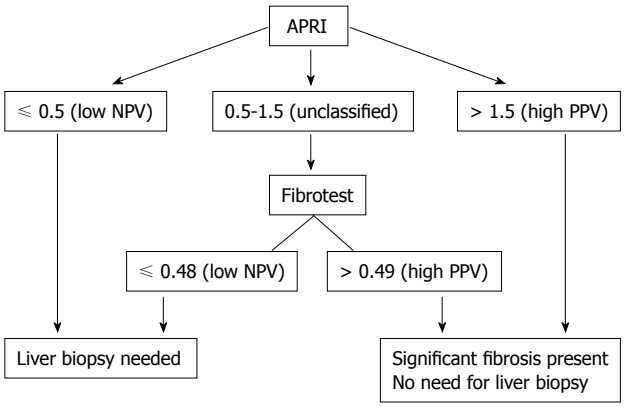

Figure 1 The SAFE-biopsy algorithm for significant fibrosis (≥ F2 by METAVIR). The figure reports the cut-offs used for APRI and Fibrotest in the decisional tree.

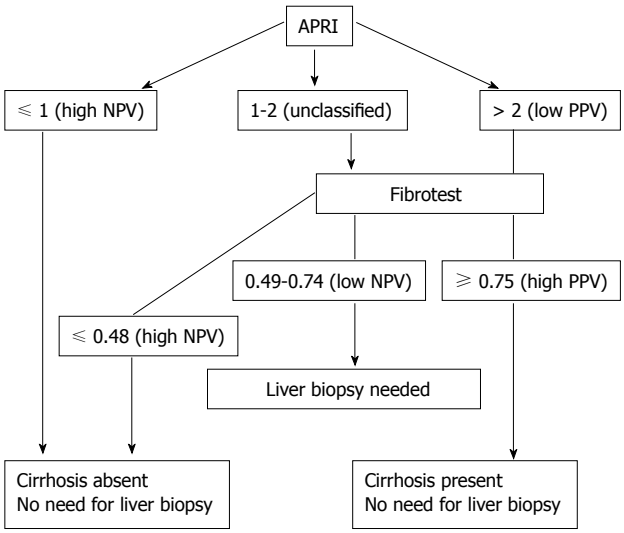

Figure 2 The SAFE-biopsy algorithm for cirrhosis (F4 by METAVIR). The figure reports the cut-offs used for APRI and Fibrotest in the decisional tree.

IMPLEMENTATION OF SAFE BIOPSY IN CLINICAL PRACTICE

In clinical practice, SAFE biopsy can provide the following responses: (1) Presence of significant fibrosis, then indication to administer antiviral therapy; (2) Presence of liver cirrhosis, then indication to specific follow-up with abdominal ultrasound, α-fetoprotein and gastroscopy; (3) absence of cirrhosis; (4) liver biopsy needed to correctly stage hepatic fibrosis.

The main concept of SAFE biopsy is that liver biopsy cannot be completely avoided but can be markedly reduced and limited to those cases in which serum markers for liver fibrosis do not show enough accuracy. Indeed, SAFE biopsy may avoid the diagnostic funnel represented by liver biopsy and it may stimulate general practitioners and patients to perform the initial screening for CLD. With this approach, liver biopsy and non-invasive markers for liver fibrosis are not antagonists, but they are agonists towards the common goal of correctly classifying liver fibrosis. SAFE biopsy has been recently validated in a multicentre, international study on serum non-invasive markers for liver fibrosis. This study, named SAFE protocol protocol, has enrolled more than 2500 cases of patients with CLD in whom APRI and fibrotest were available and liver histology was used as reference standard. The centers involved were from Italy, US, France and Romania. To date, this is the largest independent study on non-invasive methods for liver fibrosis. We have recently presented the results on 2035 cases with hepatitis C and they have confirmed high accuracy and high number of saved liver biopsies[94] (Table 10). The results of an interim analysis conducted on 210 HBV patients also showed high ac-

Table 10 Main features of SAFE biopsy[67,94] for significant fibrosis and cirrhosis in 2035 HCV cases

	Significant fibrosis	Cirrhosis
Sensitivity (%)	100	92.7
Specificity (%)	77	90.4
Accuracy (%)	90	93
AUROC	0.9	0.92
Saved biopsies (%)	47	82

SAFE: Sequential algorithms for fibrosis evaluation; AUROC: Area under the receiving operating characteristic curve.

curacy (> 90%) of SAFE biopsy algorithms for both significant fibrosis and cirrhosis, with a percentage of saved liver biopsies ranging from 45%-82%. We have also compared in 1013 HCV cases the performance of SAFE biopsy with another two algorithms combining non-invasive markers for liver fibrosis that were then proposed: Fibropaca algorithm, based on concordance of Forns' index, APRI and fibrotest; Leroy algorithm, based on concordance of APRI and fibrotest[95-97] (Table 11). Fibropaca algorithm and SAFE biopsy showed a similar accuracy but the latter saved more liver biopsies and allowed us to perform a minor number of non-invasive markers, with a consequent saving in terms of costs. The main advantages of SAFE biopsy include a larger first level screening of liver fibrosis, higher patient compliance and lower screening costs. In some specific settings, SAFE biopsy may show even more efficient results when compared with the diagnostic funnel represented by liver biopsy alone.

ALGORITHMS FOR IMPLEMENTATION IN CLINICAL PRACTICE

Castera *et al*[83] have recently proposed an algorithm which combines fibrotest and fibroscan with the aim of increasing the accuracy of the single non-invasive methods in hepatitis C. This algorithm results in an increased accuracy, especially for the diagnosis of significant fibrosis. A recent collaborative study was aimed at comparing the algorithm combining fibroscan and fibrotest (named Bordeaux algorithm) and SAFE biopsy in 302 patients with hepatitis C[98] (Table 12). The results showed that the Bordeaux algorithm saved more liver biopsies for diagnosis of significant fibrosis, although both algorithms saved a similar number of overall liver biopsies, and Bordeaux algorithm showed a higher overall accuracy for diagnosis of cirrhosis. On the other hand, Bordeaux algorithm uses fibrotest and fibroscan in all patients, while SAFE biopsy uses fibrotest in a subgroup of patients that are not well classified by APRI, which has virtually no cost. The two algorithms could be used for large scale screening of liver fibrosis and the choice of the algorithm may be based on the local availability of the non-invasive methods. Interestingly, the use of either fibroscan or fibrotest has been recently recommended in France by the Haute Autorité de Santé for the first line assessment of liver fibrosis in patients with hepatitis C without comorbidities[85]. Figure 3A and

Table 11 Comparison of the performance of SAFE biopsy[67,94], Fibropaca algorithm[96] and Leroy algorithm[97]. Results are expressed as percentages

	SAFE biopsy for diagnosis of		Fibropaca algorithm for diagnosis of		Leroy algorithm for diagnosis of
	⩾ F2	F4	⩾ F2	F4	⩾ F2
APRI needed	100	100	100	100	100
Forns needed	0	0	100	0	0
Fibrotest needed	41.7	57.6	100	100	100
Sensitivity	100	81.8	85.5	72.7	89.6
Specificity	78.2	92.4	89.9	96.7	97.8
Accuracy	90	91.2	87.6	94	93.5
Saved biopsies	43.8	79.1	51.7	76.2	29.2

⩾ F2: Significant fibrosis; F4: Cirrhosis; APRI: AST-to-platelet ratio index.

Table 12 Comparison of the performance of Bordeaux algorithm[98] and SAFE biopsy[67,94]. Values are expressed as percentages

	Bordeaux algorithm		SAFE biopsy	
	⩾ F2	F4	⩾ F2	F4
APRI needed	0	0	100	100
Fibrotest needed	100	100	43.7	61.9
Fibroscan needed	100	100	0	0
Accuracy	91	93	94	87
Biopsies saved	71.9	78.8	48.3	74.8

B show a rational proposal for the use of non-invasive methods for liver fibrosis in clinical practice, based on the local availability of the different methods and on their performances. A combination approach for clinical use has also been proposed by others[99]. Non-invasive methods for liver fibrosis and combination algorithms may be of paramount importance for the monitoring of progression of liver disease. Indeed, if it is acceptable to perform a liver biopsy at time 0, it is inconceivable however to perform a liver biopsy every year to monitor liver fibrosis progression, while this is feasible with non-invasive methods for liver fibrosis. According to local availability of the methods and attainment of non-invasive markers by the clinician, two different approaches may be used: (1) to fix the value with combined use of biopsy and non-invasive markers at time 0 and then monitoring with non-invasive markers; (2) to use non-invasive markers and then perform a liver biopsy when clinically necessary (Figure 4A and B).

MONITORING OF EFFICACY OF ANTIVIRAL THERAPIES

Apart from the diagnosis of liver fibrosis stage, few recent studies have focused on the possible use of non-invasive methods for liver fibrosis in the monitoring of antiviral therapies. Indeed, especially in hepatitis B, antiviral therapies may be long-term, such as treatments with nucleoside/nucleotide analogues, and the clinician may want to know not only the biochemical or virological response, but also and more appropriately the histological

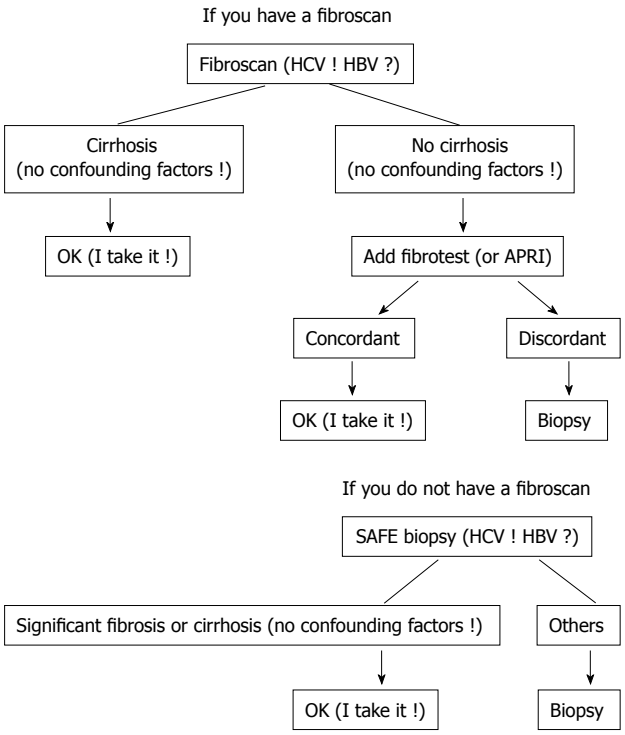

Figure 3 Diagnostic algorithms for implementation of non-invasive methods for liver fibrosis in clinical practice based on the local availability of the most validated methods.

response. Initial reports have shown that both fibrotest and fibroscan values change significantly during and after antiviral therapy in both hepatitis C and B[100-102]. Indeed, a significant improvement in fibrotest and fibroscan value has been reported in patients who achieve sustained virological response (SVR) *vs* those without SVR, and in some cases this was also maintained for 12 mo after therapy[101]. This may mean that there is a regression of liver fibrosis with antiviral treatment but further prospective, large-scale studies are needed.

MONITORING OF LIVER DISEASE COMPLICATIONS

A very attractive application of non-invasive methods for liver fibrosis may be the monitoring of liver disease complications to predict clinical events in compensated cirrhosis. Preliminary results suggest that liver stiffness values in cirrhotic patients may increase as liver disease is more advanced. In a retrospective study of 711 patients with CLD (95 with histologically-proven cirrhosis), liver stiffness values significantly correlated not only with the Child-Pugh score but also with clinical parameters (past history of bleeding varices or ascites, HCC), biochemical parameters (platelets, INR, factor V, albumin and bilirubin) and others (2-3 grade OV, splenomegaly on sonography, nodular surface, heterogeneous parenchyma) of liver disease severity[86]. Cut-off values of 27.5, 37.5, 49.1, 53.7 and 62.7 kPa had > 90% NPV for the presence of grade 2-3 OV, Child-Pugh scores B or C, past history of ascites, HCC and esophageal bleeding,

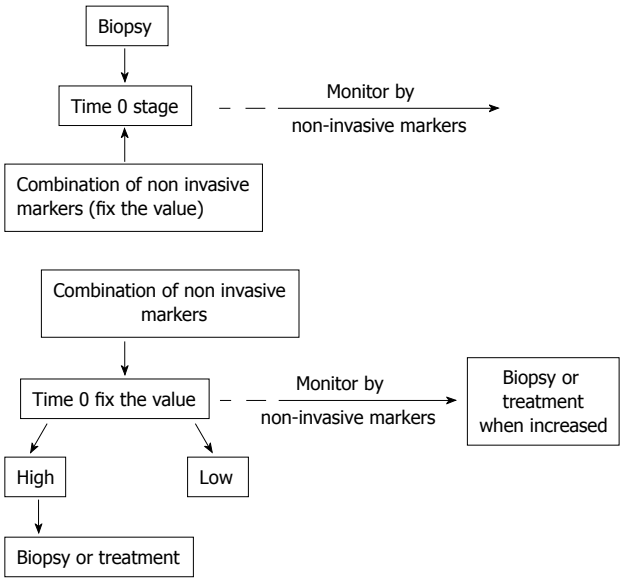

Figure 4 Perspectives for the use of non-invasive markers for long term monitoring of chronic liver diseases.

respectively. More recently, Vizzuti *et al*[103] reported a rather high sensitivity (90%) of fibroscan for prediction of OV with 17.6 kPa cut-off. Other preliminary studies have suggested that some non-invasive markers for liver fibrosis could predict the presence of OV. Sanyal *et al*[104] reported high NPV for excluding grade 2-3 varices when platelets were > 150 000/mm^3. Giannini *et al*[105] reported a good sensitivity (91.5%) with an overall accuracy of 86% for diagnosis of OV with a 909 cut-off of platelet count to spleen diameter ratio. A recent multicenter, international study was aimed at investigating in 510 consecutive cirrhotic patients the role of 7 simple non-invasive markers for liver fibrosis in predicting the presence of OV of any grade and of grade 2-3 OV[106]. The markers analyzed were platelets, AAR, Lok index, APRI, Forns' index, Fib-4, Fibroindex. Presence of grade 2-3 OV could be excluded with > 96% NPV by a specific cut-off of Lok index (1.5). None of the tests were able to predict the presence of grade 2-3 OV due to low PPV. A combination of Lok index (cutoff 0.9) and Forns' index (8.5) could predict the presence of OV of any grade with 88% PPV, 83% accuracy and 0.82 AUC. The conclusion was that, even if simple non-invasive markers for liver fibrosis cannot be a substitute for endoscopy for OV screening, they may be used to stratify cirrhotics by risk. In a recent prospective study of 298 patients with chronic hepatitis C, the performance of fibroscan, fibrotest and simple serum markers for detection of cirrhosis and its complications have been assessed[107]. The authors concluded that fibroscan is the most accurate method for diagnosis of cirrhosis but it cannot replace endoscopy for screening of OV. These preliminary findings are promising but need to be confirmed in long-term prospective follow-up studies.

Several recent studies have reported a correlation between liver stiffness values and portal hypertension,

assessed by measurement of hepatic venous pressure gradient (HVPG) which is considered the gold standard for the diagnosis and staging of portal hypertension[103,108-110]. Carrion *et al*[108] reported a close direct correlation between liver stiffness values and HVPG in 124 HCV-infected liver transplant recipients. More recently, Vizzutti *et al*[103] reported similar results in 61 patients with HCV-related severe CLD (METAVIR F3-F4). Other authors have failed to find similar results[111].

THE FUTURE: GENETICS FOR THE IDENTIFICATION OF PATIENTS WITH CHRONIC LIVER DISEASES AT RISK OF PROGRESSION

The identification of patients at high risk of developing progressive liver disease on the basis of genetic profile may be extremely useful in the future. A recent collaborative study used seven genetic variants to identify patients with hepatitis C at risk for developing cirrhosis, based on the analysis of paired liver biopsies. A cirrhosis risk score (CRS) was calculated on the basis of seven single nucleotide polymorphisms and the patient's gender[112]. In this case, increasing CRS was associated with fibrosis progression in HCV patients presenting with no liver fibrosis. CRS genetic signature could potentially be a useful prognostic indicator of those patients with HCV infection most likely to develop fibrosis progression and/or cirrhosis.

HIGHLIGHTS

Staging of liver fibrosis is essential in clinical practice for the management of patients with CLDs. Nowadays liver biopsy can no longer be considered the exclusive tool for the diagnosis of liver fibrosis since the available data support a rational use of the most validated non-invasive methods for liver fibrosis and especially of their combination algorithms. This is particularly true for chronic hepatitis C, where an adequate validation of some non-invasive methods for liver fibrosis exists. Non-invasive methods for liver fibrosis, when combined, may reduce by 50%-80% the number of liver biopsies needed for correctly classifying hepatic fibrosis. However, liver biopsy cannot be completely avoided but should be used in those cases in which non-invasive methods show poor accuracy. In clinical practice, the choice of the non-invasive method and, especially, of the combination algorithms may depend on their performance and on local availability. Further studies, especially in chronic hepatitis B, ALD and NAFLD, are needed to better assess performance of non-invasive markers in these settings and to develop rational algorithms for implementing non-invasive assessment of liver fibrosis. Future studies should also focus on non-invasive monitoring of antiviral treatment efficacy and cirrhosis complications, and genetic studies for precocious identification of patients who are at high risk of developing end-stage liver diseases.

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