Non-Invasive Biomarkers for Hepatic Fibrosis

Priyanka Lal$^{1,2}$, Aybike Birerdinc$^{1,2}$, Ancha Baranova$^{1,2}$, Zobair M. Younossi$^{1,3}$

$^1$ Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA

$^2$Center for the Study of Genomics in Liver Diseases, Molecular and Microbiology Department, George Mason University, Fairfax, VA

$^3$Center for Liver Diseases, Inova Fairfax Hospital, Falls Church, VA

Corresponding author and reprint requests:

Zobair M. Younossi, M.D., M.P.H.
Betty and Guy Beatty Center for Integrated Research
Claude Moore Health Education and Research Building
3300 Gallows Road, Falls Church, VA 22042
PHONE: (703) 776-2540 FAX: (703) 776-4386
E-mail: zobair.younossi@inova.org
ABSTRACT:

With great advancements in the therapeutic modalities used for the treatment of chronic liver diseases, the accurate assessment of liver fibrosis is a vital need for successful individualized management of disease activity in patients. The lack of accurate, reproducible and easily applied methods for fibrosis assessment has been the major limitation in both the clinical management and for research in liver diseases. However, the problem of the development of biomarkers capable of non-invasive staging of fibrosis in the liver is difficult due to the fact that the process of fibrogenesis is a component of the normal healing response to injury, invasion by pathogens, and many other etiologic factors. Current non-invasive methods range from serum biomarker assays to advanced imaging techniques such as transient elastography and magnetic resonance imaging (MRI). This review provides a systematic overview of these techniques, as well as both direct and indirect biomarkers based approaches used to stage fibrosis and covers recent developments in this rapidly advancing area.

INTRODUCTION

Liver fibrosis is defined as the building up of excessive amount of extracellular matrix, also known as scar tissue, in the liver parenchyma. While reviewing fibrosis as a component of the pathogenesis of a disease, it is important to remember that the process of fibrogenesis is also a component of the normal healing response to injury, invasion by pathogens, and many other etiologic factors. In the liver, this healing process normally involves the recruitment of immune and/or inflammatory cells to the site of injury, in order to counteract the effects of a damaging agent, secretion of extracellular matrix proteins, reorganization of the extracellular matrix and possible regeneration of new hepatic tissue. However, when the damage to the liver is chronic, excess fibrous connective tissue accumulates due to an imbalance between the production and dissolution of the matrix proteins. The process of excessive fibrogenesis taking place over an extensive period of time eventually distorts the normal parenchymal structure of the liver and
impairs its normal hepatic function, leading to different stages of fibrosis and cirrhosis. As chronic liver disease progress, hepatic fibrosis is accompanied by the formation of septae and nodules that intervene with the blood flow from the portal area of liver, producing portal hypertension and an abnormal angio-architecture which is a distinctive feature of cirrhosis.

As previously noted, liver fibrosis is generally accompanied by stress or injury to the liver parenchyma. This stress is exemplified by subsequent activation of the immune system accompanied by increased levels of certain cytokines and growth factors, which are the primary players in the development of fibrogenesis. It is important to realize that recent evidence suggest that liver fibrosis and even early cirrhosis can be reversible [1, 2], and this reversal is probably related to the suppression of the fibrotic response.

**THE BIOLOGY OF LIVER FIBROSIS:**

The most important cellular player in the production of the extracellular matrix is the myofibroblast. A wide array of cells of different origins can be converted into myofibroblasts and contribute to the production of fibrous tissue (Figure 1), including portal myofibroblasts and bone marrow-derived mesenchymal stem cells. Some epithelial cells including hepatocytes and biliary epithelial cells (cholangiocytes) can be activated to function as myofibroblasts through the process of Epithelial-Mesenchymal Transition (EMT) [3]. However, the predominant liver cell types that differentiate into activated myofibroblasts are quiescent hepatic stellate cells (HSC), also known as Ito cells or perisinusoidal cells. HSCs are located in the space of Disse and are the storage site for Vitamin A or retinoid [4].
The process of fibrogenesis is triggered as a response to damage to the liver by hepatotoxic substances and involve a variety of non-HSC types of cells. For example, hepatocytes can respond to this damage in multiple ways, including production of reactive oxygen species (ROS) and apoptosis, while the resident liver macrophages called Kupffer cells elicit a massive immune response resulting in the recruitment of other inflammatory cells to the site of injury [5]. The recruitment of additional inflammatory cells is usually achieved via the adhesive interaction between the selectin molecules in the endothelium of the blood vessels and the leukocytes. Attracted to the chemokines produced by the Kupffer cells, the leukocytes exit out of the vasculature towards the injury site and contribute to the release of additional pro-inflammatory and pro-fibrotic mediators, including cytokines such as tumor necrosis factor alpha (TNF-α) and various interleukins. Reactive oxygen and nitrogen species, proteases, and lipid metabolites such as prostaglandins and thromboxane are also released [6]. As a result of this response, quiescent HSCs lose their retinoid function and are converted to activated myofibroblasts [7]. These cells, in turn, contribute to the chemotaxis of leukocytes as well as their own chemotaxis through the production of chemokines and cytokines such as monocyte chemotactic protein-1 (MCP-1) [8]. As a result, activated HSCs start expressing the Platelet Derived Growth Factor (PDGF) receptor and Transforming Growth Factor (TGF) receptor. TGF-β is the central mediator of fibrogenesis, while PGDF stimulates proliferation of the HSCs.

Importantly, activation of HSCs is associated with a gradual replacement of the basement membrane-like extracellular matrix (ECM) within the space of Disse by the collagen rich fibers [7] and the production of fibrous bands [8]. In advanced stages of fibrosis, the liver contains approximately six times more ECM components than normal, including collagens (I, III, and
IV), fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycans [8]. The distribution of the fibrous material in the liver depends on the nature of the injury [8]. In chronic viral hepatitis and chronic cholestatic disorders, the fibrotic tissue is initially located around portal tracts, while in alcohol-induced liver disease (ALD) it first appears in the pericentral and perisinusoidal areas [9]. The portal area of the liver is centered at the hepatic artery, bile duct, and the portal vein embedded in the connective tissue matrix that normally contains a number of the fibroblasts surrounding the bile duct basal membrane. When liver injury is initiated at the portal field, the first layer of hepatocytes are liable to immediate destruction leading to an enlargement of the portal field and rapid activation of the portal fibroblasts [10]. Availability of the portal fibroblasts for myofibroblastic conversion makes the portal area especially susceptible to fibrotic changes.

**TYPES OF LIVER FIBROSIS:**

Liver Fibrosis can be subdivided into two categories: Congenital Liver Fibrosis and Acquired Liver Fibrosis.

Congenital Hepatic Fibrosis (CHF) is a rare type of liver disease, usually a developmental disorder of the porto-biliary system, specifically, malformation of the ductal plate with an excess number of immature embryonic duct structures, leading to periportal fibrosis as well as cholangitis, portal hypertension and associated complications [11]. Additionally, CHF is associated with ciliopathies or disorders of the primary cilia [12]. In these conditions, the degree of the hepatic fibrosis may vary, but the main cause of morbidity and mortality remains the failure of other organ systems [11].
Acquired fibrosis may result from the action of a number of pathogenic factors and toxic exposures such as long-term excessive alcohol consumption, cholestasis, autoimmune liver diseases, iron or copper overload, chronic viral hepatitis, the presence of non-alcoholic fatty liver disease (NAFLD) and other etiologic factors. These factors may work separately or in combination with each other to produce cumulative effects. This review will concentrate on acquired liver fibrosis and biomarkers that are being developed to quantify and stage it.

CAUSES OF ACQUIRED LIVER FIBROSIS:

The process of hepatic fibrogenesis is an example of the universal type of cellular response to a broad spectrum of chronic liver injury. The main etiologic factors responsible for liver fibrosis are chronic viral hepatitis, metabolic syndrome-related fatty liver disease, excessive alcohol consumption, and autoimmune liver diseases (including cholestatic liver disease) as well as iron and copper overload. All of these etiologic pathways can produce mediators eliciting the inflammatory response and, eventually, initiating fibrogenesis. In the following paragraphs, we will discuss a few of the most common causes of hepatic fibrosis.

Alcoholic Liver Disease: Excessive and chronic alcohol consumption is an important causal factor of liver fibrosis and cirrhosis. This is primarily due to the fact that the hepatocytes are the primary site for alcohol metabolism. The process of the breakdown of ethanol produces two pro-fibrotic agents, acetaldehyde and reactive oxygen species (ROS). Acetaldehyde can directly up-regulate the transcription of collagen I [13]. It can also indirectly contribute to the process of fibrogenesis by upregulating the synthesis of transforming growth factor-beta 1 (TGF-β1).
However, the hepatic stellate cells do not express alcohol dehydrogenase enzyme, therefore, it is likely that damaging alcohol derivative acetaldehyde originates in hepatocytes and enters collagen and TGF-β1-producing HSCs from an outside [14]. Similarly, ROS generated during alcohol metabolism in hepatocytes can also be taken up by HSCs to activate collagen production [15]. Chronic ethanol exposure sensitizes HSCs to various pro-inflammatory factors and elicits the production of inflammatory mediators that contribute to the fibrotic changes in the liver in fashion similar to that described for fibrogenesis in other types of chronic liver diseases.

**Non-alcoholic Fatty Liver Diseases (NAFLD):** From the spectrum of NAFLD, non-alcoholic steatohepatitis (NASH) can often be accompanied by liver fibrosis. NAFLD and its subtype of NASH are usually seen in individuals with metabolic syndrome (MS) or its components such as obesity, type-2 diabetes (DM), dyslipidemia, and insulin resistance. In general, development of NASH is augmented by the production of mediators such as free fatty acids, high glucose, and adipocytokines. Furthermore, these factors are also known to be involved in the development of hepatic fibrosis. To date, the pathogenesis of NASH-related liver fibrosis is not entirely well understood [8]. Evidence provided by numerous studies links obesity, insulin resistance and the progression of fibrosis together in one vicious circle [16]. For example, the well-known adipokine, leptin is produced proportionally to the mass of the visceral adipose compartment. It has previously been shown that leptin augments fibrogenesis by stimulating phagocytic activity and cytokine secretion by Kupffer cells and macrophages [17] as well as the proliferative and ROS generating activities of the endothelial cells [18]. Another adipokine, resistin, exerts pro-inflammatory actions in HSC by increasing the expression of both MCP-1 and interleukin-8 (IL-8) as well as activating the transcription factor, NFkB [19].
From examples mentioned above, one can derived that initial stages of the pathogenesis of liver fibrosis associated with NAFLD depends primarily on the soluble factors produced by excessive visceral adipose and on an skewed distribution of the soluble fat particles in the bloodstream.

**Cholestatic Liver Diseases:** Cholestasis (reduced bile duct excretion) is another well-known cause of liver fibrosis. Cholestasis triggers the proliferation of the cholangiocyte lining of the intrahepatic and extrahepatic bile duct systems through a complex regulatory milieu that involves both autocrine and paracrine factors [20]. The activation of biliary proliferation is known as ductular reaction. Proliferating bile duct epithelial cells produce the profibrogenic connective tissue growth factor (CTGF) that stimulates myofibroblast generation through EMT and collagen deposition [21]. The primary players in the fibrotic reaction to cholestasis are inflammatory response propagated by neutrophils and resulting from that oxidative stress. Furthermore, inhibition of ROS during cholestasis reduces fibrosis.

**Chronic Viral Hepatitis:** Chronic viral infections such as hepatitis B (HBV) or hepatitis C (HCV) viruses pose an important risk for the development of liver fibrosis. The process of fibrogenesis related to chronic viral infection follows the same path as fibrosis related to other etiologic factors. Nevertheless, this process is compounded by additional virus-specific factors. In chronic HCV infection, the viral core, NS5 and NS3 proteins have been demonstrated to initiate a cascade of molecular events that can eventually lead to fibrosis. HCV proteins appear to affect both lipid accumulation and degradation, with the consequent disruption of the normal process of lipid compartmentalization and metabolism, skewing towards ROS production.
In the case of HBV infection, studies have shown that the X protein of HBV (HBx) directly induces TGF-β secretion by hepatocytes and, thus, contributes to the paracrine activation of HSC's [22]. Interestingly, the superinfection of hepatitis delta virus (HDV) in patients who are chronic carriers of HBV, can also accelerate the progression of fibrosis [7]. The deleterious effect of this type of viral infection rests on the fact that, despite the massive inflammatory response in the liver, the viral particles cannot be cleared. However, the drastic rise in the release of cytokines, particularly, TNF-α, IL-1-α and -β, IL-2, IL-6, and IL-8, is strong enough to activate the myofibroblasts and induce fibrogenesis [23]. Both HIV-HBV and HIV-HCV co-infected patients are at increased risk for progression of their liver disease as compared to patients who are mono-infected with HCV or HBV [23]. In the case of HIV-HCV co-infection, the proposed mechanism involves enhanced induction of the production of ROS that occurs in an NFκB-dependent fashion [24].

The general mechanism of the fibrogenesis in chronic viral hepatitis is less clear than that in non-viral chronic diseases. Most likely, the pathogenesis is multifactorial as it involves a combination of both viral and host-specific factors, including oxidative stress, hepatic steatosis, increased iron stores, and increased rate of hepatocyte apoptosis, under the pressure of the viral proteins and viral replication.

With the advancements new regimens to treat patients with chronic liver diseases, the accurate assessment of liver fibrosis has become important for individualized management these patients. The lack of accurate, reproducible and easily applied methods for assessment of hepatic fibrosis
has been the major limitation for both the clinical management and research in liver diseases. The following paragraphs summarize the current modalities used for quantifying and staging hepatic fibrosis.

**Liver biopsy scoring techniques:**

For the past 50 years liver biopsy has been considered to be the gold standard for staging of liver fibrosis. Liver biopsy allows physicians to obtain information not only on fibrosis, but also on many other liver injuring processes, such as inflammation, necrosis, steatosis, hepatic deposits of iron or copper. A number of these pathologic factors are can help determine the diagnosis of chronic liver disease. However, many recent studies clearly highlight several crucial drawbacks of liver biopsy, including variable accessibility, high cost, sampling errors and inaccuracy due to inter- and intra-observer variability of pathologic interpretations [25]. In addition, there is small but important risk of liver biopsy-associated morbidity and mortality, with pain and hypotension as the most frequent complications and intraperitoneal bleeding and injury to the biliary system as the most serious complications. Studies reveal that the risk for hospitalization after liver biopsy is 1-5%, the risk for severe complications is 0.57%, and mortality rates vary from 0.009% to 0.12% [26, 27]. Because of these reasons, some patients may opt to forgo liver biopsy and may not know the stage of their liver disease with important prognostic implications.

The history of the fibrosis scoring systems dates back to 1981 when the histological features of chronic hepatitis were evaluated for potential importance in determining its prognosis by Knodell and colleagues [28]. The Ishak score, or revised Knodell system, has primarily been applied to chronic hepatitis B and C. It considers grading and staging as two separate items; liver fibrosis is
classified as: 0 = absent, 1-2 = mild, 3-4 = moderate and 5-6 = severe/cirrhosis. The first three axes of Knodell HAI (Histologic Activity Index) relate to the necroinflammatory grade of the disease while the fourth feature assesses the stage of the disease by evaluating the degree of fibrous portal tract expansion, fibrous portal-portal linking, portal-central fibrous bridges, and the formation of fibrous septa and parenchymal nodules [29]. This grading system has been subsequently modified by other pathologists [30, 31]. There are some limitations of HAI index, in particular, related to the interobserver variation [32]. Another limitation of the Ishak/Knodell fibrosis score is its nonlinearity as it includes scores 0, 1, 3, and 4.

The Metavir scoring system was designed specifically for patients with hepatitis C using a sum of experience-based opinions of 10 pathologists augmented by subsequent stepwise discriminant analysis [33]. The scoring uses both grading and staging systems as it includes two separate scores, one for necroinflammatory grade (A for activity) and another for the stage of fibrosis (F). The grade is a number based on the degree of inflammation, which is usually scored from 0-4, with A0 being no activity and A3 to A4 considered severe activity. Determining the amount of inflammation is important because it can correlate with hepatic fibrosis. The degree of activity is assessed by the integration of the severity of both (periportal) necrosis and lobular necrosis as described in a simple algorithm [34]. The fibrosis score (F) is defined as: F0 = no scarring, F1 = portal fibrosis without septa, F2 = portal fibrosis with rare septa, F3 = numerous septa without cirrhosis and F4 = cirrhosis or advanced scarring of the liver [35]. The intra- and inter-observer variability of Metavir seems to be improved [36]. The main advantage of the Metavir score for hepatitis C is its relative simplicity, its focus on necroinflammatory lesions, and its increased sensitivity in the fibrosis score due to the addition of one extra fibrosis evaluating level.
However, the limitations of the Knodell score also apply to the Metavir score as it retains the semi quantitative and categorical nature of fibrosis staging. Use of the liver biopsy scoring systems often varies among different pathology laboratories, which makes score comparisons among patients from different centers rather difficult. Built-in sampling error problems associated with accepted scoring systems requires the need to design studies with extremely large sample sizes \[37\].

In addition to staging hepatic fibrosis for viral hepatitis, three pathologic criteria have been used for patients with NAFLD. Of these, the original classification of NAFLD subtypes was developed to histologically categorize NAFLD into 4 subtypes): type 1 NAFLD = steatosis alone; type 2 NAFLD = steatosis with lobular inflammation only; type 3 NAFLD = steatosis with hepatocellular ballooning; or type 4 NAFLD = steatosis with Malloy-Denk bodies or fibrosis. According to these criteria, types 3 and 4 NAFLD were considered to be NASH. Subsequently, Brunt’s criteria was developed to grade NASH and used for clinical research in patients with NAFLD. According to Brunt’s criteria, liver biopsy with at least fat and lobular inflammation is graded as mild (grade 1), moderate (grade 2) or marked (grade 3) NASH. More recently, the NAFLD Activity Score (NAS) was developed to provide a pathologic numerical score for patients who most likely have NASH. Elements of NAS and the stage of fibrosis provides separate scores for steatosis (0-3), hepatocellular ballooning (0-2), lobular inflammation (0-3) and fibrosis (0-4). Accordingly, NAS is the sum of the first three features with most patients with NASH having a NAS score of \( \geq 5 \). Fibrosis, according to Brunt and NAS is scored from 0 to 4 (grade 0 = none; 1 = centrilobular/perisinusoidal; 2 = centrilobular plus periportal; 3
These pathologic protocols for NAFLD suffer from a lack of data assessing their inter-observer variability as well as their inability to predict liver-related mortality.

To overcome the previously mentioned complications posed by liver biopsy, alternative non-invasive methods for quantifying and staging liver fibrosis have been developed. These methods range from serum biomarker assays to advanced imaging techniques such as transient elastography and magnetic resonance imaging (MRI) (Figure 2).

**Imaging Techniques:**

The activation of HSCs and deposition of the ECM leads to alterations in liver microstructure and abnormalities in its microvasculature that are reflected by an increase in the liver stiffness and changes in the blood flow. Recent radiological advances allow the bedside assessment of liver stiffness with techniques like Fibroscan and MRI.

**Transient Ultrasound Elastography (Fibroscan):**

An ultrasound-based technology for quantitatively assessing hepatic stiffness has been introduced in the last several years both in Europe and in other parts of the world. Fibroscan measures the stiffness (or elasticity) of the hepatic parenchyma using both ultrasound (5 MHz) and low-frequency (50 Hz) elastic waves produced by a specialized ultrasound vibrator applied to the body wall and coupled with 1D ultrasound imaging that measures the propagation speed of a wave using a pulse-echo ultrasound. Since fibrotic tissue is harder than healthy liver tissue, the shear wave measurement provides immediate quantitative assessment of the “degree of stiffness”
which takes less than 5 minutes to collect. FibroScan was reported to be of value in the diagnosis of the fibrosis accompanying various liver diseases including hepatitis B and C, alcoholic liver disease, and non alcoholic fatty liver disease (NAFLD) [39]. The inter equipment, intra observer (96-98%) and inter observer agreement (89–98%) of FibroScan has been shown to be good for patients with chronic viral hepatitis. However, the success rate of the procedure depends on observer expertise as well as on the width of the inter-costal space, the presence of ascites, the body mass index of patient and presence of visceral adiposity [40, 41].

In patients with chronic liver disease, FibroScan is considered to be a reliable method for the diagnosis of extensive fibrosis (F3) and has shown to have good sensitivity (>85%) and specificity (>90%) for detecting cirrhosis [42]. Meta-analysis of the existing literature, however, indicates that the diagnostic performance of transient ultrasound elastography is much lower in patients with early-stage hepatic fibrosis, increased fatty infiltration of the liver on biopsy, or high body mass index (≥28 kg/m²) [42].

**Magnetic Resonance Imaging (MRI):**

MRI technology can be used to measure both liver stiffness and characteristic water-diffusion abnormalities associated with cirrhosis [43]. It should not be confused with magnetic resonance spectroscopy (MRS), which provides images of the metabolic abnormalities in subjects with liver disease.

Several types of enhanced MRI have been developed to evaluate the degree of liver fibrosis. One such modification, Magnetic Resonance Elastography (MRE) directly visualizes and
quantitatively measures acoustic shear waves progressing through the liver tissue. MRE involves a three-step process: 1) generating mechanical waves within the tissues of interest; 2) imaging the micron level displacements caused by propagating waves using a special MRI technique with oscillating motion-sensitizing gradients; and 3) processing the wave images using an inversion algorithm to generate quantitative maps of the physical properties of the liver [44].

Over the last few years, MRE has made its transition from the laboratory to clinical research and, in very selected centers, to clinical practice. The current evidence support the observation that a normal mean liver stiffness value by MRE in the setting of chronic liver disease is consistent with stage 0 fibrosis on liver biopsy [44], while fibrosis of stages 1-4 are also diagnosed accurately [44-46]. In a study encompassing 50 patients with biopsy-proven liver disease and 35 healthy volunteers, receiver operating characteristic (ROC) analysis showed that, with a shear stiffness cutoff value of 2.93 kPa, the predicted sensitivity and specificity for detecting liver fibrosis were 98% and 99%; respectively [45]. Notably, stage I fibrosis detection was possible; while the detection of hepatic fibrosis with stages 2 or more, the area under the ROC curve (AUC) was 0.96 [46]. It was also noted that the technical success rate for MRE is significantly higher than that of transient elastography (94% vs. 84%). The only practical limitation of performing MR elastography in very obese patients is that the patient has to fit within the magnetic bore, the diameter of which is also further reduced by the presence of the transducer. Furthermore, MRE use can be limited for claustrophobic patients or those with severe hemochromatosis. Additionally, MR elastography is associated with substantially higher costs than FibroScan. These limitations preclude MRE from widespread clinical acceptance.
Serum Biomarkers of Fibrosis:

In recent years, interest in identifying and describing liver fibrosis by using non invasive surrogate markers has been on the rise. Serum markers of liver fibrosis offer an attractive, cost effective alternative to liver biopsy for both patients and clinicians. In addition to being substantially less invasive, there are practically no complications, little or no sampling and observer related variability. Moreover, measurements may be performed repeatedly, thus, allowing for a dynamic monitoring of fibrosis [47].

The Ideal Liver Fibrosis Marker:

The diagnostic value of serum markers of liver fibrosis has been investigated in numerous studies. Based on clinical and research needs, the ideal marker for liver fibrosis would have the following characteristics:

- Be highly sensitive and specific to identify different stages of fibrosis
- Be readily available, safe, inexpensive and reproducible
- Be applicable to the monitoring of disease progression or regression as apart of natural history of liver disease or treatment regimens
- Not be susceptible to false positive results, for example, in individuals with inflammation related to other diseases

Although no single ideal marker exists, several markers have been identified as possible useful indicators of fibrosis when used in conjunction with each other.
Biomarkers of fibrosis are commonly divided into Direct and Indirect markers. Direct markers are fragments of the liver matrix components produced by hepatic stellate cells (HSC) during the fibrotic process and are involved in regulating the progression and regression of fibrosis. Indirect markers include molecules released into the blood due to liver inflammation, molecules synthesized/regulated or excreted by the liver, and markers of processes commonly disrupted due to liver function impairment, such as insulin resistance (Table 1; [48]). Direct and indirect markers may be used alone or - more commonly - in combination with each other, to produce composite scores. The calculation of such scores can be relatively simple or can be based on complicated formulas (e.g. those underlying Fibrotest/Fibrosure). The most commonly used markers are discussed in detail below.

**Direct Biomarkers:**

*Procollagen type I carboxy terminal peptide (PICP):* In the healthy human liver the most abundant collagens are the fibril-forming types I and III. These collagens are synthesized as precursor molecules with large propeptide extensions at both the N- and C-termini cleaved from procollagen by proteinases, then the mature form of the collagen is integrated into the ECM [49, 50]. During fibrogenesis, type I collagen levels increase up to eightfold. Notably, type I collagen levels increase significantly more than that of type III, changing the type I/III ratio from 1:1 in the healthy liver to 1:2 in the cirrhotic liver [51]. Measuring serum levels of PINP released during collagen formation may be useful as a marker of fibrogenesis, either alone, in ratios or in combination with other techniques [52]. However, there is no correlation between PICP and type IV collagen or Procollagen type III amino-terminal peptide (PIIINP). PICP levels are normal in
patience with mild chronic hepatitis C and elevated in 50% of patients with moderately advanced or advanced chronic hepatitis C, including patients with liver cirrhosis of this etiology [53].

**Procollagen type III amino-terminal peptide (PIIINP):** PIIINP is a major constituent of most dense and loose connective tissue in the body. Its relative concentration is higher in the early phase of the fibroproliferative response, such as in wound healing or in hepatic fibrogenesis [54]. Importantly, the increase of PIIINP in the basement membrane during fibrosis is reflected by an increase in its serum level. In acute hepatitis, levels of serum PIIINP correlate with aminotransferase levels. In chronic liver disease, serum PIIINP reflects the stage of liver fibrosis [41] and is useful for respective diagnostics in alcoholic liver disease (ALD), viral hepatitis and primary biliary cirrhosis [48]. In ALD, reduction or normalization of PIIINP levels has been observed in those who abstain from alcohol [55]. Interestingly, in chronic HCV, PIIINP levels also correlate with the scores for necrosis. The sensitivity and specificity for PIIINP based liver fibrosis detection were 76–78% and 71–81%; respectively, although the latter could be increased as high as 88% with the use of fragments of other profibrotic collagens. Unfortunately, PIIINP is not specific for the fibrosis of the liver as it is also elevated in acromegally, lung fibrosis, chronic pancreatitis, and rheumatologic disease [51].

**Metalloproteinases (MMPs):** MMPs belong to a family of structurally related proteolytic enzymes that mediate the degradation of the ECM and the basement of membranes [56]. The MMP family is divided into secreted and membrane-anchored enzymes. The three most commonly studied human metalloproteinases are MMP-2 (gelatinase-A), MMP-3 (stromelysin), and MMP-9 (gelatinase-B). MMPs are synthesized in a latent form and then converted into the
extracellular active form via cleavage of specific conserved sequences [56]. During hepatic fibrogenesis, MMPs involved in fibrillar collagen degradation are down-regulated, whereas the expression of MMP-2 is markedly increased. MMP-2 is secreted by activated HSC's and elevated levels of MMP-2 and its proenzyme have been observed in various diseases involving the liver [57]. The potential for MMP-2 for predicting liver fibrosis remains unclear as some contradictory data have been reported by studies performed so far [58, 59]. In contrast to MMP-2, MMP-9 levels are not elevated in chronic hepatitis or cirrhosis but could prove valuable in the diagnosis of hepatocellular carcinoma [60].

Tissue inhibitors of matrix metalloproteinases (TIMPs): TIMPs are secreted proteins that complex with MMPs to modulate activity and activation. Hepatic fibrosis arises when TIMPs inhibit ECM degradation excessively. TIMP-1 controls most MMP activity, whereas TIMP-2 inhibits MMP-2 by both inhibiting activity and preventing the formation of active enzymes [61]. Elevated levels of TIMPs have been observed in chronic liver disease. For example, chronic hepatitis C causes the elevation of both TIMP-1 and TIMP-2 in corollary with fibrosis progression [58]. Furthermore, the altered balance between circulating MMP-9 and TIMP-1 may play an important role in aggravating liver injury progression in chronic liver diseases [62]. Recently a study was performed to determine the relationship between serum MMP-9, TIMP-1 and fibrosis in chronic liver disease in 50 patients divided into three groups: chronic hepatitis, liver cirrhosis and hepatocellular carcinoma [62]. Results showed that the lowest MMP-9 serum levels in chronic hepatitis patients compared to the controls ($P<0.05$). Serum MMP-9 decreased during the progression of chronic hepatitis to cirrhosis showing the lowest level in the cirrhotic group. Serum TIMP-1 was significantly higher in the cirrhotic group as compared to chronic
hepatitis ($P<0.05$) and controls ($P<0.05$). MMP-9 was negatively correlated to both TIMP-1 and the histological severity in chronic hepatitis. There was a positive correlation between TIMP-1 and the degree of fibrosis ($r=0.73$, $P<0.001$). Lastly, there was a statistically significant increase of MMP-9 ($P<0.001$) and TIMP-1 ($P<0.05$) in HCC patients as compared with the other groups. These findings raised the possibility of using serum TIMP-1 as a non-invasive assay in liver fibrosis [62].

Transforming growth factor-β1 (TGF-β1) is a pleiotropic cytokine involved in tissue growth, differentiation, ECM production and the immune response. Three isoforms (β1, β2 and β3) of this cytokine have been identified, but only TGF-β1 is linked to liver fibrogenesis [63]. TGF-β1 is also commonly accepted as a central component of fibrogenic response to wounding and is up-regulated in a variety of different diseases such as those involving the skin, pancreas, liver, and others [64]. In the liver, TGF-β1, stimulates activation of HSCs into myofibroblast-like cells, producing type I, III and IV collagen, proteoglycans (e.g. biglycan and decorin), and glycoproteins (e.g. laminin, fibronectin, tenascin and glycosaminoglycan; [65]). Such changes in the composition of the ECM further stimulate fibrogenesis and HSC activation, which also strongly induce TGF-β1 to maintain an elevated level. Finally, TGF-β1 promotes the activation of portal fibroblasts, and the recruitment of circulating myofibroblasts to the injured liver [8]. A small study of 38 patients with chronic HCV found a close correlation between TGF-β1 levels and the rate of fibrosis progression [41].

Hyaluronic acid (HA) is a glycosaminoglycan, a component of the ECM synthesized by the HSC. In a recent investigation of cirrhosis due to non-alcoholic fatty liver disease and other
etiologies, HA was found to be the best class I biomarker of fibrosis, having an area under curve (AUC) of 0.97, sensitivity of 86–100% and specificity of about 88% [66]. Since the negative predictive value of hyaluronan, at a cut-off value of 60 lg/L, is much higher (98–100%) than the positive predictive value (61%), the main utility of serum hyaluronan lies in its ability to exclude advanced fibrosis and cirrhosis [65].

**YKL-40 (chondrex)** is a mammalian member of a protein family that includes bacterial chitinases. The pattern of its expression in certain tissues such as human liver or cartilage suggests a function in remodeling or degradation of the extracellular matrix [67]. In liver diseases, serum levels of YKL-40 are closely related to the degree of histologically documented fibrosis, the highest values being found in severe fibrosis [49]. Sensitivities and specificities around 80% and an AUC of 0.81 for fibrosis are reported for fibrosis in HCV patients, however, for those with alcoholic liver disease, a specificity of 88% and a low sensitivity of 51% were calculated [68].

**Laminin** is a major non-collagenous glycoprotein synthetized by the HSC. Laminin is deposited in the basement membrane of the liver. Its levels increase during fibrosis around the vessels, in the perisinusoidal spaces and the portal tract [69]. Elevated levels of laminin and pepsin resistant laminin (laminin P1) were found in patients with both chronic viral hepatitis and alcoholic liver disease in correlation with perisinusoidal liver fibrosis and with the incidence of cirrhosis complications [70].
Connective tissue growth factor (CCN2) is synthesized by both activated HSC and hepatocytes, depending on the levels of TGF-β [71]. Expression of this growth factor in the liver tissue is up-regulated as is its serum levels, in the occurrence of fibrogenesis. There is a negative correlation between fibrosis progression and CTGF, as serum CTGF levels decrease in the end stage of cirrhosis. The AUCs for fibrosis vs. control and cirrhosis vs. control were calculated to be 0.955 and 0.887, respectively, the sensitivities 100% and 84%, respectively, the specificities 89% and 85%, respectively [71].

Paraoxonase 1 (PON-1) is an enzyme that hydrolyzes lipid peroxides, has antioxidant properties and influences hepatic cell apoptosis. In circulation it binds to high-density lipoproteins. Measurement of serum PON1 activity has been proposed as a potential test for the evaluation of liver function. However, this measurement has not been extensively applied in routine clinical practice since the substrate commonly used for PON1 measurement, paraoxon, is toxic and unstable [72]. Ferre N et al., 2002 studied 68 patients with liver cirrhosis, 107 patients with chronic hepatitis, and 368 apparently healthy volunteers [73]. Baseline and salt-stimulated PON1 activities were measured by the hydrolysis of paraoxon. Results showed that baseline and stimulated PON1 activities were decreased ($P < 0.001$) in chronic hepatitis and in liver cirrhosis. PON1 activity was significantly correlated with serum total proteins, albumin, and bilirubin in patients but not in controls. The combination of baseline serum PON1 with five standard biochemical tests had higher classification accuracy (94% of patients; 96% of controls) than the five standard tests alone (75% of patients; 96% of controls). ROC analysis showed that AUROC for chronic hepatitis was 0.89 and was 0.96 for cirrhosis, both compared to controls. Combining
PON-1 with a panel of standard liver tests, a 96% sensitivity and specificity for the detection of liver fibrosis and a 97% sensitivity for the detection of liver cirrhosis was achieved [72].

*Microfibril-associated glycoprotein 4 (MFAP-4)* is a ubiquitous protein playing a potential role in ECM turnover during fibrogenesis. It contains fibrinogen-like domains and an Arg-Gly-Asp sequence in the N-terminus that serves as the ligand motif for the surface receptor integrin. In a recent study, quantitative analysis of MFAP-4 serum levels in a large number of patients showed high diagnostic accuracy for the prediction of non diseased liver versus cirrhosis (AUROC = 0.97, \( P < 0.0001 \)) as well as stage 0 versus stage 4 fibrosis (AUROC = 0.84, \( P < 0.0001 \)), and stages 0 to 3 versus stage 4 fibrosis (AUROC = 0.76, \( P < 0.0001 \)) [74].

**Limitations of Direct serum biomarkers of fibrosis:**

- They reflect the rate of matrix turnover (not only deposition) and have a tendency to be more elevated when associated with high inflammatory activity. As a consequence, extensive matrix deposition might not be detected in the presence of minimal inflammation;
- They are not liver–specific and their serum levels may be elevated in the presence of concomitant sites of inflammation;
- Serum levels of markers depend on clearance rates, which are influenced by the dysfunction of endothelial cells, impaired biliary excretion or renal function [48].

**Indirect Biomarkers of Fibrosis**

*AST/ALT ratio*: Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are hepatic enzymes that are released into the bloodstream from hepatocytes damaged in the process
of hepatocellular injury or death. The predictive value of the AST/ALT ratio has been validated in non-alcoholic liver disease, chronic viral hepatitis, primary sclerosing cholangitis, and primary biliary cirrhosis [75]. However, the ratio of AST to ALT has important limitations. In many forms of acute and chronic liver injury or steatosis (fatty infiltration of the liver), this ratio is less than or equal to 1. This is particularly true in patients with steatosis accompanying hepatitis C, while in alcoholic hepatitis, an AST/ALT ratio greater than 2 is commonly observed. While these ratios are suggestive of certain etiology of liver conditions, there is too much overlap between groups to rely on AST/ALT exclusively when making a diagnosis – for example, in patients with hepatitis C and a history of alcohol abuse [76].

The **PGA index** combines the measurement of the Prothrombin Index, γ glutamyl transferase levels and apolipoprotein A1. It was subsequently modified to the PGAA index by the addition of α2-macroglobulin, which resulted in marginal if any improvement in its performance. In chronic liver diseases, the PGA index has a relationship to both the inflammation and the fibrosis ($P<0.01$, $P<0.05$ respectively). In a recent study, its accuracy was observed between 66% and 72% for predicting cirrhosis in alcoholic patients [77]. However, other studies showed that its sensitivity and specificity for the diagnosis of early liver cirrhosis is even lower [78].

The **AST-to-Platelet Ratio Index (APRI)** is calculated as AST (× upper limit of normal range) × 100 / platelet count ($10^9$/L). This index has previously been validated as a surrogate marker of significant hepatic fibrosis in HIV/HCV-coinfected patients, and has recently been used to determine advanced fibrosis in HIV-monoinfected patients [79]. APRI was accurate in estimating fibrosis in patients with HCV (AUROC 0.87–0.89, sensitivity 94-100%, specificity
95-100%) and with HCV/HIV co-infection, although some studies have reported that it fails in accurately staging fibrosis in patients with hepatitis C, as one study noted APRI was unable to classify 40–65% of patients with chronic HCV or HBeAg negative chronic hepatitis B [80].

The Forns index is based on 4 routine clinical variables: age, platelet count, cholesterol levels, and γ glutamyl transferase. This method can be used to differentiate patients with mild (F0-F1) fibrosis from those with severe (F2-F4) fibrosis (AUROC 0.81), but it is less accurate in distinguishing patients with grades F2 versus F4. The Forns index has been validated in other cohorts (positive predictive values 94% for significant fibrosis and negative predictive values 100% for cirrhosis) as a predictive tool for response to anti-HCV therapy [80].

The HepaScore combines age, gender, bilirubin, γ glutamyl transferase, hyaluronic acid, and γ2-macroglobulin into a score from 0.00 to 1.00 [81]. In 512 chronic HCV patients, automated HepaScores showed good predictive performances for significant fibrosis (AUROC=0.81), severe fibrosis (AUROC=0.82), and cirrhosis (AUROC=0.88). For significant fibrosis, HepaScore (cut-off=0.5) had a sensitivity of 0.77, a specificity of 0.70, a positive predictive value of 0.71 and a negative predictive value (NPV) of 0.77. HepaScore <0.25 excludes significant fibrosis with a sensitivity of 0.95 and a NPV of 0.90 and HepaScore <0.75 excludes cirrhosis with a sensitivity of 0.86 and a NPV of 0.97 [82].

The FIB-4 score which combines platelet count, ALT, AST and age, was originally developed for use in HIV-HCV co-infection. Use of this index correctly classified 87% of patients with FIB-4 values outside 1.45–3.25 and avoided biopsy in 71% of the validation set with an AUROC
of 0.765, sensitivity of 70% and a specificity of 97% for differentiating Ishak 0–3 from 4–6 [83]. This model was subsequently validated by Vallet-Pichard in a large cohort of HCV mono-infected patients, with the finding that using these ranges, 78% of 847 biopsies were correctly classified [AUROC 0.85 for severe fibrosis and 0.91 for cirrhosis] [84].

The **SHASTA Index** which consists of serum hyaluronic acid (HA), AST, and albumin was evaluated in a cohort of 95 patients with HIV/HCV co-infection. Using a cut off of 0.8 resulted in a specificity of 100% and a positive predictive value of 100%, but this applied to less than 5% of patients. At the other end of the spectrum, a cutoff of less than 0.30 was associated with a sensitivity of more than 88% and a negative predictive value of more than 94%. Overall 42% of patients could be correctly classified at either extreme; however 58% were classifiable with scores between 0.3 and 0.8. However, the SHASTA index in HIV/HCV has performed significantly better than the APRI test [85].

The **$^{13}$C-methacetin breath test (MBT)** is amongst several $^{13}$C breath tests used for the quantitative non-invasive assessment of cytochrome P450-dependent hepatocellular function [86]. MBT is rapidly metabolized by healthy liver cells into acetaminophen and $^{13}$CO$_2$ by a single dealkylation event, so the increase of $^{13}$CO$_2$ in breath samples can be quantified by isotope ratio mass spectrometry or non dispersive isotope-selective infrared spectroscopy [86]. MBT offers several advantages: it assesses both liver inflammation and fibrosis, it does not involve a blood test and can provide an immediate result at the point-of-care [87]. MBT has been shown to have high sensitivity (92.6%) and specificity (84.1%) in predicting liver cirrhosis. The areas
under the curve were found to be 0.958 for predicting cirrhosis and 0.827 for identifying patients with advanced fibrosis [88].

The FIBROSpect II test uses a combination of components in the fibrogenic cascade, such as hyaluronic acid, TIMP-1 (tissue inhibitor of metalloproteinase), and α-2-macroglobulin to calculate a composite score. The test is intended to differentiate mild fibrosis (Metavir stages F0 to F1) from more severe disease (Metavir stages F2 to F4), and had been shown to do so with an accuracy of 75% in a retrospective study of 696 CHC patients [89]. In a later study of 252 CHC patients, an AUROCs for FIBROSpect II and morphometry for stages F2 through F4 for concordant biopsy specimens were observed to be 0.823 and 0.728, respectively. Sensitivity and specificity were 83.5% and 66.7%, respectively, with an accuracy of 80.2% [90].

The FibroTest and FibroSure are identical tests marketed under different names in Europe and America for the assessment of fibrosis and necroinflammatory activity. The FibroTest score is computed by accessing a proprietary website and entering the patient’s age, sex, and results for serum haptoglobin, α2-macroglobulin, apolipoprotein A1, γ-glutamyltransferase, and bilirubin analyses [91]. It generates a score that is correlated with the degree of liver damage in people with a variety of liver diseases. Due to the variability of component of assays and analyzers, FibroTest assays can only be performed in validated laboratories [92]. A recent study showed an AUROC of 0.69 and 0.91 for the diagnosis of significant fibrosis (F≥2) and liver cirrhosis in 74 patients comprising of 36 with HCV, 10 with HBV, and 28 with primary biliary cirrhosis [93]. The sensitivity and specificity values for FibroTest based detection of primary severe fibrosis were found to be 75% and 85%, respectively [78].
The *FibroIndex* was developed by Koda M and co-authors [94] for liver fibrosis in chronic hepatitis C. This test relies on platelet count, AST and serum IgG. FibroIndex showed high predictive values for significant fibrosis, including in a subgroup of HCV cases with normal alanine aminotransferase (NALT), as indicated by an AUROC of 0.77 and 73.5% accuracy [95]. The sensitivity and specificity of FibroIndex for detecting fibrosis in patients with HCV were 78% and 74% [96]. In a comparative study, the validated AUROC of the FibroIndex for predicting significant fibrosis was found to be 0.83 and 0.82, which is better than those of the Forns index and APRI in patients with chronic hepatitis C. The authors suggested FibroIndex as a simple and reliable measure for predicting significant fibrosis in chronic hepatitis C that could also be useful as a surrogate marker during antifibrotic treatment for chronic hepatitis C [94].

The *FibroMeter* is a combination of the platelet count, prothrombin index, AST, γ2 macroglobulin, hyaluronate, blood urea nitrogen and age. The good performance and applicability of FibroMeter was validated in a number of chronic liver diseases, including chronic viral hepatitis B or C, alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD), which makes this test clinically relevant for everyday clinical practice. FibroMeter has two main diagnostic targets - fibrosis stage corresponding to the histological staging system Metavir and the amount of fibrosis which corresponds to morphometric determinations of the fibrotic area [97]. An important feature of the FibroMeter is that it presents the amount of liver fibrosis as a percentage of fibrous tissue within the liver. Another significant feature of FibroMeter is that it validates the results through an expert system that detects erroneous results
FibroMeter displays a high overall diagnostic accuracy and is the only test capable of the correct classification of 100% of HCV patients without fibrosis or with cirrhosis, while 90% correct classification was achieved for any HCV patient with the following reliable diagnostic intervals: F0/1, F1/2, F2±1, F3±1 [98]. In a study of 272 HIV-HCV co-infected patients, FibroMeter was able to stage liver fibrosis in all patients with AUROCs of 0.78 with a correlation coefficient index of 0.37 [99].

The ActiTest is a modification of the FibroTest, which in addition to the other variables includes ALT. This test reflects both hepatic fibrosis and necro-inflammatory activity. A meta-analysis that included 1570 patients concluded that at a cut-off of 0.31, the negative predictive value (NPV) of the FibroTest for excluding significant fibrosis was 91%. Additionally, the AUROC of this test was independent of the genotype or viral load (similarly to that of the FibroTest) [41].

Two additional panels have been developed to assess hepatic fibrosis specifically in NAFLD. First, the so called “Simple Test” for fibrosis in NAFLD is a relatively easy to use panel that includes age, hyperglycemia, body mass index, platelet count, albumin, and AST/ALT [100]. When the purpose of performing a liver biopsy in NAFLD is to determine the extent of hepatic fibrosis, using the Simple Test can correctly stage 90% of patients, obviating the need for liver biopsy in approximately 75% of patients. In addition to the “Simple Test”, another panel for hepatic fibrosis is the Original European Liver Fibrosis (OELF) panel [101], which includes age, hyaluronic acid, amino-terminal propeptide of type III collagen, and tissue inhibitor of matrix metalloproteinase 1. A simplified version OELF is ELF, which does not include age, and seems to perform well in patients with NAFLD [102]. The performances of ELF and OELF were found to be almost identical. In a recent study, when the ELF panel was used for “ruling in” severe
fibrosis, only 14% of NAFLD patients in this cohort required a liver biopsy. The combination of Simple/ELF panel reached an AUROC of 0.98 for distinguishing severe fibrosis from initial stages of the fibrotic disease in patients with NAFLD [102].

The *Proteomics based tests* assess patterns of protein or glycoprotein by mass spectroscopy using serum samples. Importantly, while a series of ‘peaks’ generated, the precise identities of these peaks remain unknown. For example, Callewaert N et al., 2004 developed tests based on the altered N-glycosylation of total serum protein (GlycoCirrhoTest and GlycoFibroTest) [103], which could be both cost-effective and could rapidly determine a signature profile for n-glycans. At first, it was reported that the combination of GlycoCirrhoTest with the FibroTest produced a sensitivity of 79% and specificity of 86% in distinguishing cirrhosis from non cirrhotic disease. However, later tests showed limited applicability of the test to discern the etiology of liver diseases. Specifically, under galactosylation did not show a significantly different quantitative alteration in the cirrhotic and noncirrhotic population of all etiologies [104]. Moreover, the same modifications seem to continuously reappear in all liver diseases: hyperfucosylation, increased branching and a bisecting N-acetylglucosamine [104]. Larger prospective studies are necessary to determine the clinical application of these new technologies.

The recently developed *Phosphoproteomics* tests serve the goal of improving and understanding the pathogenesis of liver fibrosis to more than actually contributing to the practicality of clinical diagnostics. For example, phosphoproteomics based tests predict the fibrosis of the liver have been used to profile the phosphorylated (i.e. activated) forms of the major signaling proteins in visceral adipose samples of patients with NAFLD [105].
CONCLUSION

Successful individualized management of chronic liver disease depends on the correct staging of liver fibrosis. In order to provide the means of monitoring the course of liver disease and its response to therapy, staging should be performed in a non-invasive and reproducible manner. The fact that the process of fibrogenesis is a component of the normal healing response hampers the development of disease-specific biomarkers. A number of non-invasive techniques ranging from serum biomarker assays to advanced imaging techniques such as transient and MR elastography are being developed. However, most of these tests are not widely validated and fail to differentiate between early stages of fibrosis. In fact, most of these tests can primarily distinguish cirrhosis from no or minimal fibrosis. Major validation efforts to enroll large cohorts of patients with chronic liver diseases controlled for important confounders such as ethnicity, body mass index and etiology of liver disease, must be undertaken.
FIGURES

Figure 1. A wide array of cells of different origins can be converted into myofibroblasts.

Figure 2. A scheme depicting various means of liver fibrosis diagnostics. Liver biopsy is an invasive method that remains an imperfect golden standard. Proteomics based profiles are unlikely to be introduced to routine clinical care anytime soon, but are valuable from the research point of view. Imaging techniques, serum biomarkers and biomarker panels are advancing along the route to the clinic stage, but require extensive validation.
<table>
<thead>
<tr>
<th>Indices</th>
<th>Individual components</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST/ALT ratio</td>
<td>Aspartate aminotransferase, Alanine aminotransferase</td>
<td>53</td>
<td>100</td>
<td>[57, 80, 81]</td>
</tr>
<tr>
<td>PGA</td>
<td>Protrombin index, GGT, apolipoprotein A1</td>
<td>91</td>
<td>81</td>
<td>[57, 82-84]</td>
</tr>
<tr>
<td>APRI</td>
<td>AST/platelet count</td>
<td>89</td>
<td>75</td>
<td>[57, 85, 86]</td>
</tr>
<tr>
<td>FibroSpect II</td>
<td>HA, TIMP-1, α2-macroglobulin</td>
<td>83.5</td>
<td>66.7</td>
<td>[57, 95, 96]</td>
</tr>
<tr>
<td>FibroTest/FibroSure</td>
<td>γ2 macroglobulin, γ2 globulin, γ globulin, apolipoprotein A1, GGT, total bilirubin</td>
<td>75</td>
<td>85</td>
<td>[57, 84, 97-99]</td>
</tr>
<tr>
<td>Fibroindex</td>
<td>Platelet count, AST, GGT</td>
<td>78</td>
<td>74</td>
<td>[57, 100-102]</td>
</tr>
<tr>
<td>Fibrometer</td>
<td>Platelet count, γ2 macroglobulin, AST, age, prothrombin index, HA, blood urea nitrogen</td>
<td>81</td>
<td>84</td>
<td>[57, 103-105]</td>
</tr>
<tr>
<td>ActiTest</td>
<td>Age, gender, GGT, ALT, haptoglobin, apolipoprotein A1, haptoglobin, α2-macroglobulin, total bilirubin</td>
<td>96</td>
<td>44</td>
<td>[45, 57]</td>
</tr>
<tr>
<td>Forns</td>
<td>Age, platelet count, GGT, choles tered levels</td>
<td>94</td>
<td>51</td>
<td>[86, 57]</td>
</tr>
<tr>
<td>Hepascore</td>
<td>Age, gender, bilirubin, GGT, HA, γ2-macroglobulin</td>
<td>63</td>
<td>89</td>
<td>[87, 88, 57]</td>
</tr>
<tr>
<td>FIB-4</td>
<td>Platelet count, ALT, AST, platelet count, age</td>
<td>70</td>
<td>74</td>
<td>[89, 90, 57]</td>
</tr>
<tr>
<td>SHASTA Index</td>
<td>HA, AST, albumin</td>
<td>100</td>
<td>52</td>
<td>[91, 112]</td>
</tr>
<tr>
<td>Simple test</td>
<td>age, hyperglycemia, BMI, platelet count, albumin, AST/ALT</td>
<td>78</td>
<td>58</td>
<td>[106, 113]</td>
</tr>
<tr>
<td>OELF/ELF</td>
<td>age, HA, N-terminal propeptide of type III collagen, TIMP-1</td>
<td>90</td>
<td>41</td>
<td>[107, 108]</td>
</tr>
</tbody>
</table>

GGT: γ glutamyl transferase, HA: hyaluronic acid, TIMP-1: Tissue inhibitors of matrix metalloproteinase-1, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase
References


35. Franciscus A. HCV Diagnostic tools: Grading and staging a liver biopsy. HCSP; March 2010; Version 2.4.


Figure 1. A wide array of cells of different origins can be converted into myofibroblasts.
Figure 2

Staging of fibrosis

- Direct Biomarkers
- Indirect Biomarkers
- Combinational panels
- Liver Biopsy
- Liver imaging
- Proteomics profiles