

Abstract

In this study, we have first described the normal liver structure including the hepatic acinus that is characterized by its structural and functional heterogeneity. Second, we have addressed the pathogenesis of liver fibrosis: the major source of excess extracellular matrix appears to be perisinusoidal stellate cells. The concept of reversibility of liver fibrosis opens the way for new therapeutic perspectives.

We have then analyzed the different methods of assessment of liver fibrosis. Liver biopsy is the current reference standard. However, it is invasive and subject to sampling error.

Consequently, many attempts are made to develop non-invasive tests: biochemical tests and imaging methods, including functional MR imaging with perfusion, diffusion or spectroscopy, have been proposed. Among the imaging methods, elastography by measuring directly the liver stiffness appears as one of the most promising techniques.

Lastly, we have described our research that was focused on MR elastography. Our results show that MR elastography is a feasible, accurate and reproducible method to stage liver fibrosis, and that it is superior to biochemical testing with aspartate-to-platelets ratio index and ultrasound elastography to stage liver fibrosis. Further studies remain to be done to decrease the long examination time of MR elastography and, consequently, to integrate it into a comprehensive hepatic MR protocol.

I. INTRODUCTION

II. NORMAL LIVER STRUCTURE

1. **The classic lobule**
2. **The hepatic acinus**
3. **Hepatic sinusoid and sinusoidal cells**
4. **Extracellular matrix in the liver**
 - 4.1. **Collagens**
 - 4.2. **Glycoproteins**
 - 4.3. **Proteoglycans**
5. **Functional heterogeneity**

III. LIVER FIBROSIS

1. **Definition, etiology, natural history and risk factors**
2. **Pathogenesis of hepatic fibrosis and cirrhosis**
3. **Cirrhosis**
 - 3.1. **Microcirculatory changes**
 - 3.2. **Regeneration**
 - 3.3. **Morphology of cirrhosis**
 - a. **Micronodular cirrhosis**
 - b. **Macronodular cirrhosis**
 - c. **Incomplete septal cirrhosis**
 - 3.4. **Clinical status**
4. **Reversibility of fibrosis and cirrhosis**

IV. METHODS FOR THE ASSESSMENT OF LIVER FIBROSIS

1. **Histological analysis**
 - 1.1. **Histological methods of assessment of liver fibrosis**

- a. Semi-quantitative scoring systems
 - b. Computed morphometry image analysis
- 1.2. Limitations of liver biopsy
 - a. An invasive procedure
 - b. Sampling error
 - c. Inter- and intraobserver variability
- 2. Biochemical tools
 - 2.1. Biochemical tests
 - 2.2. Serum extracellular matrix markers of fibrosis
- 3. Imaging tests
 - 3.1. Anatomical imaging
 - 3.2. Functional imaging
 - a. Perfusion
 - b. Diffusion
 - c. Spectroscopy
 - i. Hydrogen-1 (^1H) MR spectroscopy
 - ii. Phosphorus-31 (^{31}P) MR spectroscopy
 - iii. Limitations
 - 3.3. Elastography
 - a. Ultrasound elastography
 - i. Apparatus, elasticity estimation and acquisition procedure
 - ii. Performance
 - iii. Reproducibility
 - iv. Technical success rate
- 4. Comparisons and combinations of non-invasive tests

5. The limitations of the assessment of the non-invasive tests

V. MR ELASTOGRAPHY

1. Theory

2. Experimental setup

3. First study: preliminary study

3.1. Phantom

3.2. Subjects

a. Healthy volunteers

b. Patients

i. Histological analysis

ii. Measurements with MR elastography

4. Second study: clinical study

4.1. Patients

4.2. Histological analysis

4.3. Measurements with MR elastography

5. Third study: comparison with APRI and ultrasound elastography

5.1. Patients

5.2. Measurements with MR and US elastography

a. MR elastography

b. US elastography

5.3. Histological analysis

5.4. Influence of inflammatory activity and steatosis

5.5. Reproducibility

5.6. Comparison with APRI and US elastography

VI. CONCLUSIONS AND PERSPECTIVES

VII. REFERENCES

VIII. MANUSCRIPTS

I. INTRODUCTION

Liver fibrosis and its end-stage, liver cirrhosis are among the ten top causes of death in the Western World. Cirrhosis is also an important cause of morbidity. The societal costs of cirrhosis are immense, because of the medical expenses to treat the many complications, perform liver transplantation and ensure long-term postoperative care [1].

Liver fibrosis can be caused by many chronic liver diseases. In the Western countries, the most frequent causes of liver fibrosis are chronic HCV infection, alcohol abuse, and nonalcoholic steatohepatitis [2]. While each insult may cause progressive injury, the enormous functional reserve of the liver masks to some extent the clinical impact. In the majority of patients, progression to cirrhosis occurs after an interval of 15–20 years. Cirrhosis leads to liver dysfunction, portal hypertension and hepatocellular carcinoma.

Diagnosis and staging of liver fibrosis is important, with liver biopsy being the currently accepted method [3, 4]. However, it is a costly procedure that carries a small risk of severe complications and is not well accepted by the patients. In addition, its accuracy remains debated because of sampling variability caused by the small size of the hepatic biopsies and the heterogeneity of liver fibrosis [5-11].

The development of reliable non-invasive markers of liver fibrosis is essential to assess the prognosis of the disease, to select the patients needing treatment and to assess the response to therapy [2, 12-14]. An abundance of data now emphasize that fibrosis is dynamic and, with effective intervention, reversible [13]. Successful treatment of viral hepatitis, autoimmune liver disease, alcohol-related disease, schistosomiasis, and others results not only in clinical improvement but also in decreased histological fibrosis [15-19]. Although experimental studies have revealed targets to prevent fibrosis progression in rodents, the efficacy of most treatments has not been proven in humans. As additional therapies are developed, whether for

specific diseases or fibrosis per se, fibrosis reduction may emerge as the standard for demonstrating efficacy.

Several non-invasive methods have been proposed to stage liver fibrosis, including biochemical tests and imaging methods. The biochemical tests are composite scores (aspartate aminotransferase to platelets ratio index (APRI), FibroTest, ...) or serum markers of fibrosis such as hyaluronic acid [20-25]. However, the value of these diagnostic methods remains debated, especially for the diagnosis of intermediate stages of fibrosis [14, 20].

Among the imaging methods, elastography is emerging as a reliable method to stage liver fibrosis. It is based on the observation that fibrosis leads to increased tissue stiffness.

Elastography can be performed with ultrasound or magnetic resonance (MR) imaging. Most clinical studies have been performed with ultrasound elastography [26-30]. The purpose of our research was to assess the value of MR elastography for the non-invasive staging of liver fibrosis in patients who had liver biopsy for suspicion of chronic liver disease. First, we evaluated the feasibility of the method in phantoms, healthy volunteers and patients. Second, we assessed the value of MR elastography for the staging of liver fibrosis in patients during a time period of more than two years. Third, the assessment was completed by a comparison between MR and ultrasound elastography.

II. NORMAL LIVER STRUCTURE

1. The classic lobule

Kiernan's concept of classic lobule [31] is traditionally represented as hexagonal in outline with, at its centre, a central vein, a terminal tributary of the hepatic vein. The blood from the terminal afferent vessels perfuses sinusoids which pass into segments of adjacent hexagonal lobules. It seems therefore improbable that the classic lobule could subserve the role of a functional unit.

2. The hepatic acinus

The hepatic acinus [32-35] has been defined as a small parenchymal mass arranged around a small portal tract containing a terminal portal vein and its accompanying hepatic arteriole and bile duct: the acinus lies between two (or more) terminal hepatic venules into which it drains (*Fig. 1*).

The simple acinus has been further subdivided into zones 1, 2 and 3 which are related to the zonal heterogeneity of liver tissue. These zones represent areas which receive blood progressively poorer in nutrients and oxygen; zone 3 thus represents the microcirculatory periphery, and the most peripheral portions of zone 3 from adjacent acini form the perivenular area.

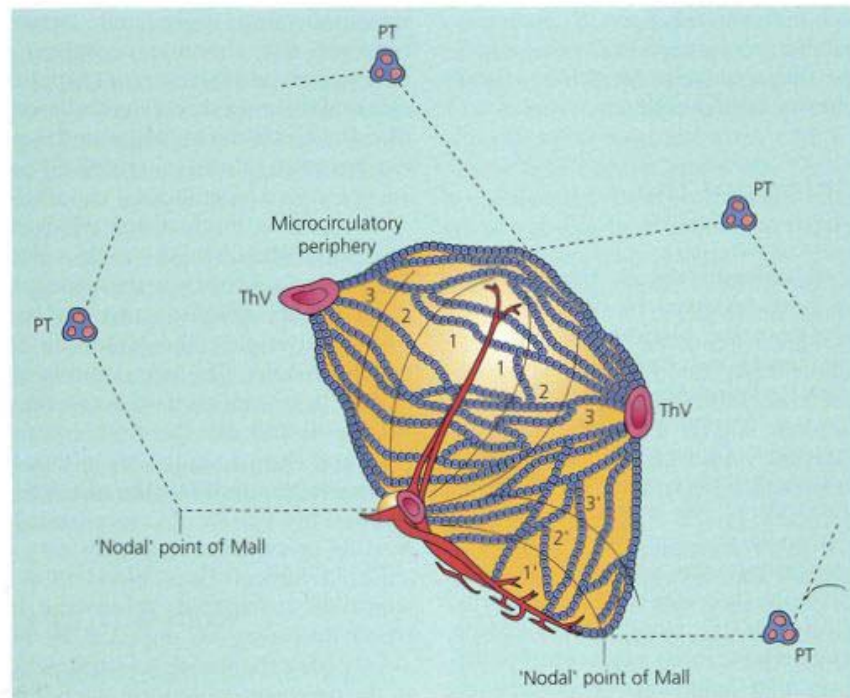


Figure 1. *Diagrammatic representation of the simple acinus and the zonal arrangement of hepatocytes. Two neighbouring classique lobules are outlined by the discontinuous lines, and the acinus occupies adjacent sectors of these. Although only one channel is shown as forming the central core of the acinus, the acinus is arranged around the terminal branches of the portal vein, hepatic artery and bile ductule. Zones 1, 2 and 3 represent areas which receive blood progressively poorer in nutrients and oxygen; zone 3 thus represents the microcirculatory periphery, and the most peripheral portions of zone 3 from adjacent acini form the perivenular area. The nodal points of Mall represent vascular watershed areas where the terminal afferent vessels from neighbouring acini meet. PT = portal tract; ThV = terminal hepatic vein (central vein of « classic lobule »); 1, 2, 3 = microcirculatory zones; 1'; 2'; 3' = microcirculatory zones of neighbouring acinus; ----- = outline of « classic lobule ». Adapted from Rappaport (From: MacSween R, Desmet VJ, Roskams T, Scothorne RJ. *Developmental anatomy and normal structure*. Churchill Livingstone, 2002).*

3. Hepatic sinusoid and sinusoidal cells

Blood flowing into a group of sinusoids can be arterial, venous or mixed, depending upon sphincteric and contractile activity. Approximately two-thirds of the blood supply comes from the portal venules. Within the liver, the portal veins divide into successive generations of veins to the terminal portal venules which are found in portal tracts. From the terminal portal venules arise very short side branches i.e. the inlet venules, which pass through the periportal limiting plate to open into the sinusoids. These inlets are reported to be guarded by sphincters composed of sinusoidal lining cells – the afferent or inlet sphincters [36].

Four distinct types of sinusoidal cells can be identified [37]:

- the endothelial cells,
- the stellate cells,
- the Kupffer cells,
- the liver-associated lymphocytes.

The lining of the sinusoids is formed by endothelial cells. They form an attenuated cytoplasmic sheet perforated by numerous holes (fenestrae) and, unlike endothelial cells elsewhere, they apparently do not form junctions with adjacent endothelial cells. Since a basement membrane is absent on the deep surface of the sinusoidal endothelium, there is continuity between the sinusoidal lumen and the perisinusoidal space of Disse. This unique structure allows the endothelial cells to filter the sinusoidal blood: solutes pass freely through the fenestrae from the lumen into the space of Disse and come into contact with the hepatocytes; large particles such as chylomicrons are excluded.

The space of Disse lies primarily between the sinusoidal wall and the outer sinusoidal surface of the hepatocyte. This extravascular space constitutes the immediate medium of exchange between blood and hepatocytes. The extracellular matrix that is present within the space of Disse influences hepatocyte, sinusoidal endothelial cell and stellate cell function and interaction between this matrix and these cells is of fundamental importance in maintaining their differentiation, growth and function [38-40].

The space of Disse contains the hepatic stellate cells [41], which have four main functions:

- They produce the extracellular matrix proteins both in the normal and fibrotic liver [42-47].
- They act in a pericyte-like manner around the sinusoids and may have a contractile function [47-50].
- They are a major site of storage for vitamin A [51].
- They play a role in hepatic regeneration both in the normal liver and in response to liver injury [52, 53].

Kupffer cells are hepatic macrophages and are located in the lumen of hepatic sinusoids. They are of considerable importance in host defence mechanisms. Their primary function includes the removal by ingestion and degradation of particulate and soluble material from the portal blood flow.

The liver-associated lymphocytes which lie on the luminal aspect of the endothelium were first described by Wisse et al. [54] as so-called pit cells and were shown to correspond to natural killer lymphocytes.

4. Extracellular matrix in the liver

The extracellular matrix in the liver contains several components: collagens (type I and III comprise more than 95% of the collagen in the normal liver) [55], glycoproteins (laminin, fibronectin) and proteoglycans (heparan sulfate).

4.1. Collagens

Types I, III, IV, V and VI of collagen have been identified in the liver:

- Type I collagen corresponds to the collagen in portal tracts and around the walls of hepatic veins.
- Type III collagen corresponds to the reticulin framework of the sinusoids.
- Type IV collagen forms the basement membranes around bile ducts, arteries and veins.
- Type V collagen is closely associated with basement membranes and with the matrix in the space of Disse.
- Type VI collagen is found in the interstitial matrix of the portal tract.

4.2. Glycoproteins

The collagens are intimately complexed and interwoven with glycoproteins and proteoglycans to form the total supporting structure of the liver. The non-collagenous glycoproteins include laminin, entactin and elastin. Laminin is a large glycoprotein produced by perisinusoidal stellate cells and endothelial cells in the normal liver. Laminin promotes cell adhesion, migration, differentiation and growth and is an important mediator of capillary formation by

endothelial cells. The fibronectins represent a class of large molecular weighted glycoproteins that, in extracellular matrix, exists as thin filaments associated with collagen fibres. Entactin is a glycoprotein restricted to basement membranes, and hence is generally absent from the space of Disse. Elastin fibres are normally scattered throughout portal tracts.

4.3. Proteoglycans

The proteoglycans include heparan sulphate, chondroitin sulphate, dermatan sulphate and hyaluronic acid. The strong anionic charge on the proteoglycans contributes to their binding to the other constituents of the extracellular matrix. Heparan sulphate particularly modulates the proliferative and secretory characteristics of mesenchymal cells and is an essential extracellular component of basement membranes. The proteoglycans can function as adhesion molecules and can act as receptor molecules on cell surfaces. As such, they have been identified as an important reservoir for cytokines and growth factors, by binding up these diffusible substances within the matrix. Remodelling of the extracellular matrix, as during regeneration, can release substantial quantities of cytokines and growth factors.

5. Functional heterogeneity

The functional heterogeneity in the liver that has been most studied is the zonality [56]. Zonality refers to in-homogeneous distribution patterns of structural and functional aspects along the portal-central axis, with differences between periportal and centrolobular zones [57].

Several types of zonation have been recognized [58]. In the gradient type of zonation, all hepatocytes are able to express a particular gene, but the level of expression depends on the

position of the hepatocyte along the portocentral radius. In the compartment type of zonation, the expression of genes is restricted to either the periportal or the pericentral compartment.

The dynamic type of zonation is characterized by adaptive changes in expression in response to changes in the metabolic or hormonal state. The stable type of zonation, on the other hand, is characterized by the virtual absence of such adaptive changes.

Zonal heterogeneity concerns not only hepatocytes, but other components (sinusoids, hepatic stellate cells, Kupffer cells and extracellular matrix) of liver tissue as well [59]. For example, there is heterogeneity in the blood flow through the sinusoids. In the peripheral zones the sinusoids form an interconnecting polygonal network. Downstream, however, they become organized as parallel vessels which open into the terminal hepatic venule.

III. LIVER FIBROSIS

1. Definition, etiology, natural history and risk factors

Fibrosis is a wound-healing response of the liver to repeated injury [12]. The natural history of liver fibrosis is influenced by both genetic and environmental factors. Epidemiological studies have identified polymorphisms in a number of candidate genes that may influence the progression of liver fibrosis in humans [4]. These genetic factors may explain the broad spectrum of responses to the same etiological agent found in patients with chronic liver diseases.

Fibrosis leading to cirrhosis can accompany virtually any chronic liver disease. The vast majority of patients with liver fibrosis have chronic viral hepatitis C and B, or steatohepatitis associated with either alcohol or obesity, but other etiologies include parasitic diseases (e.g. schistosomiasis), autoimmune attacks on hepatocytes or biliary epithelium, neonatal liver diseases, metabolic disorders including Wilson's, hemochromatosis and a variety of storage diseases, chronic inflammatory conditions (e.g. sarcoidosis), drug toxicity (e.g. methotrexate or hypervitaminosis A), and vascular derangements, either congenital or acquired.

The development of cirrhosis typically requires decades, with two notable exceptions:

- neonatal liver disease – infants with biliary atresia may present at birth with severe fibrosis and marked parenchymal distortion,
- a subset of patients who undergo liver transplantation for cirrhosis caused by HCV [60] or HBV [61] develop rapidly progressive cholestasis and recurrent cirrhosis within months, requiring re-transplantation.

There is no clear explanation for these instances of « fulminant fibrosis », but they underscore the possibility that fibrosis is not always a slowly progressive event.

Our understanding of the natural history of fibrosis is most complete in chronic hepatitis C [62, 63]. The disease can run a remarkably variable course, from decades of viremia with little fibrosis, to rapid onset of cirrhosis in 10–15 years. Remarkably, it is host, not viral factors that correlate with fibrosis progression in HCV based on the following evidence:

- there is no relationship between viral load or genotype and fibrosis even though these factors greatly impact response to antiviral therapy,
- human promoter polymorphisms may correlate with fibrosis risk [64],
- host immune phenotype may be critical since there is more rapid progression in immunosuppressed patients [65].

In a study of 2235 French patients with chronic hepatitis C, Poynard et al. [62] identified three independent factors associated with an increased rate of hepatic fibrosis: age at infection older than 40 years; concurrent liver disease due to HBV or alcohol; and male gender. In addition to these well characterized cofactors, identified risk factors for more rapid progression of HCV also include: increased body mass index, associated with hepatic steatosis; HIV infection or immunosuppression following liver transplantation; and iron overload [12].

Moreover, Poynard et al. [62] defined fibrosis progression per year as the ratio between the fibrosis stage in METAVIR units and the estimated duration of infection in years. If the time of infection cannot be estimated, the same information can be determined if two biopsies are obtained several years apart, since this too will provide an estimate of progression rate over time. In the study of Poynard et al. [62], the median rate of fibrosis progression per year was 0.133 fibrosis unit. The rate of fibrosis progression was not normally distributed (median 0.133 lower than the mean 0.252). This finding suggests the presence of at least three populations: rapid fibrosers, intermediate fibrosers, and slow fibrosers. Based on the median rate of fibrosis progression without treatment, the median expected time to cirrhosis was 30

years; 33% of the patients had an expected median time to cirrhosis of less than 20 years and 31% did not progress to cirrhosis.

The ability to assess the rate of progression of fibrosis can be useful for at least two reasons:

- if little fibrosis progression has occurred over a long interval, then treatment with antiviral therapy may be deemed as less urgent,
- the approximate time to the development of cirrhosis can be estimated.

However, it is important to note that risk estimates described by Poynard et al. are not universally accepted. Indeed, it appears increasingly likely that fibrosis progression may not be entirely linear, with more advanced stages associated with accelerating, non-linear progression [66].

2. Pathogenesis of hepatic fibrosis and cirrhosis

Accumulation of extracellular matrix in liver fibrosis results from both increased synthesis (fibrogenesis) and decreased degradation (fibrolysis) [67]. Decreased activity of extracellular matrix metalloproteinases (MMPs) is mainly due to an overexpression of their specific inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). The distribution of the fibrous material depends on the origin of the liver injury. In chronic viral hepatitis, chronic biliary disorders and autoimmune hepatitis, the fibrotic tissue is initially located around portal tracts, while in alcoholic and nonalcoholic steatohepatitis, or chronic vascular liver disease, it locates in pericentral and perisinusoidal areas [68]. The major source of excess extracellular matrix in the injured liver appears to be perisinusoidal stellate cells (*Fig. 2*) [69]. Following chronic injury, stellate cells activate and transdifferentiate into myofibroblast-like cells, acquiring proliferative, contractile, and fibrogenic properties [46, 70]. Activated stellate cells migrate and accumulate at the sites of tissue repair, secreting large amounts of extracellular matrix

and regulating extracellular matrix degradation. The cytokines secreted by Kupffer cells and other inflammatory cells predominantly stimulate the stellate cells.

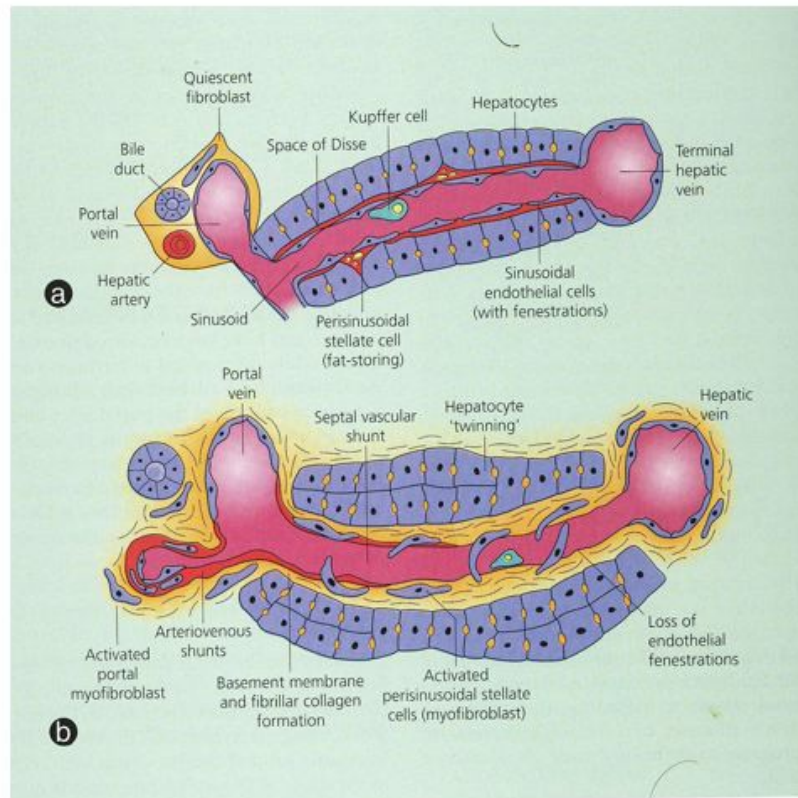


Figure 2. Key events in the evolution of cirrhosis. (a) The normal microanatomy of the liver is depicted, showing the channels for flow of portal venous blood through the sinusoids of the parenchyma, and normal sinusoidal architecture. (b) With evolution to cirrhosis, the following events occur. Abnormal arteriovenous shunts and vascular shunts from portal to hepatic veins develop. Portal tract fibroblasts proliferate and become myofibroblasts. Perisinusoidal stellate cells lose their fat stores, proliferate and develop a myofibroblast phenotype. Portal and perisinusoidal myofibroblasts deposit extracellular matrix, expanding the portal tracts and the space of Disse, respectively. Hepatocyte regeneration, leading to « twinning » of hepatocytes plates, is also shown (From: Crawford J. liver cirrhosis. In: MacSween RN, Burt AD, Portmann BC, Ishak KG, Scheuer PJ, Anthony PP, eds. Pathology of the liver. 4th ed. London, England: Churchill Livingstone, 2002; 575-620).

These cytokines include:

- Platelet derived growth factor (PDGF), mainly produced by Kupffer cells, the predominant mitogen for activated stellate cells [71].
- Transforming growth factor beta 1 (TGFβ1) which stimulates the matrix production by activated stellate cells [72-74].
- Endothelin-1, the key contractile stimulus towards stellate cells, which leads to increased portal resistance by constricting the sinusoids [75, 76].

Among cytokines, it is interesting to note that adipokines, which are cytokines mainly derived from the adipose tissue, regulate liver fibrogenesis. Leptin is required for stellate cell activation and fibrosis development [77, 78]. In contrast, adiponectin markedly inhibits liver fibrogenesis [79]. The actions of these cytokines may explain why obesity influences fibrosis development in patients with chronic hepatitis C [80].

In liver fibrosis, the amounts of all types of collagens, glycoproteins and proteoglycans can increase markedly. Deposition of the extra-cellular matrix during fibrogenesis can increase the reservoir of stored cytokines and growth factors within the liver.

Aberrant deposition of extracellular matrix within the hepatic parenchyma produces an environment in which:

- Scarring with type I collagen develops. Over a period of many months, the collagen fibrils cross-link. The process confers resistance to degradative enzymes [14].
- The proteoglycans can function as adhesion molecules. As such, they have been identified as an important reservoir for cytokines and growth factors, by binding up these diffusible substances within the matrix, and they can release substantial quantities of cytokines and growth factors during remodelling of the extracellular matrix [81].

In liver fibrosis, extracellular matrix proteins normally present in basement membranes are deposited within the space of Disse, creating a major barrier for solute exchange between hepatocytes and sinusoidal blood. In combination with the loss of fenestrations in the sinusoidal endothelium, this process is called « capillarization » of the sinusoids.

Moreover, as the normal matrix in the space of Disse provides signals that maintain the differentiated function of surrounding cells, the transformed matrix in liver fibrosis may result in loss of differentiated hepatocellular function. This has been demonstrated in clinical studies in which the process of capillarization of sinusoids correlated well with impairment of liver function [82].

3. Cirrhosis

3.1. Microcirculatory changes

Vascular modifications are central to the development of cirrhosis [83].

First, capillarization of the sinusoids transform the fenestrated sinusoids into continuous capillaries. [84]. Moreover, the acquisition of a myofibroblast phenotype by the perisinusoidal stellate cells increases intraparenchymal vascular resistance since tonic contraction of these cells constricts the sinusoids.

Second, sclerosis of the portal tracts and their vascular branches increases presinusoidal vascular resistance.

Third, fast vascular channels in the bridging fibrous septa connect the vascular structures in the portal region (hepatic arteries and portal veins) and terminal hepatic veins, shunting blood around the parenchyma [85]. These alterations explain that mean transit time for labelled

erythrocytes decreases from 19.9 ± 3.7 s in patients without cirrhosis, to 12.2 ± 4.4 s in patients with cirrhosis [86].

Finally, Wanless et al. [87] have specifically examined the potential role of portal and hepatic veins thrombosis in the development of cirrhosis. Hepatic vein lesions were associated with regions of confluent fibrosis and parenchymal extinction, whereas portal vein lesions were associated with prominent regional variation in the size of cirrhotic nodules.

3.2. Regeneration

Depending on the type of injurious agent, the nature of the liver disease and the extent of hepatic destruction, liver regeneration may occur by at least two mechanisms [88-90]. First, adult differentiated hepatocytes may undergo division and replication, responding quickly to liver damage associated with mild to moderate hepatocellular loss. Second, more extensive or massive hepatic necrosis stimulates the proliferation of progenitor cells.

Regeneration is recognized by twinning of the liver cell plates, evident as a double line of hepatocytes with nuclei apparently running in parallel. With thickening of liver cell-plates, the parenchyma expands against the constraining fibrous septa, and tends to take up a spherical shape. The ultimate size of the nodule is determined in part by the anatomical location of the fibrous septa (*Fig. 3*). If matrix deposition is occurring at the acinar level, the nodules will grow out of monoacinar units and will be small. If matrix deposition encompasses many acinar units (« multiacinar »), the growing nodules may be much larger.

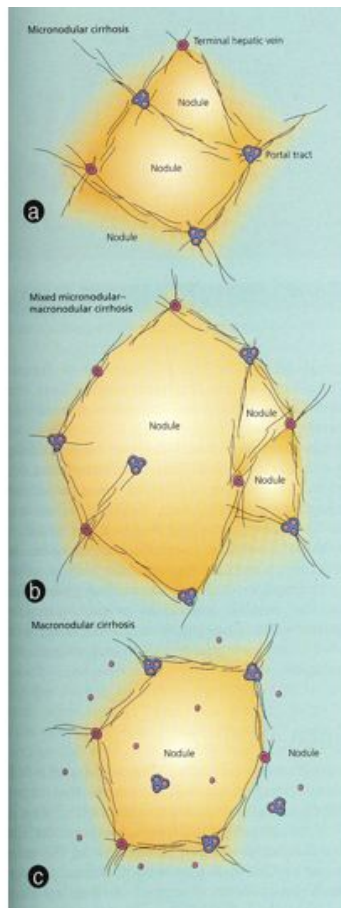


Figure 3. Nodule size in cirrhosis. (a) *Micronodular cirrhosis.* Fibrous septa bridge essentially all portal tracts and terminal hepatic veins, subdividing the liver at the level of individual acini. (b) *Mixed micronodular-macronodular cirrhosis.* Fibrosis septa encircling larger areas of parenchyma with incomplete septal subdivision, are interspersed with regions of liver with complete septal subdivision at the acinus level. (c) *Macronodular cirrhosis.* Fibrous septa encircle large tracts of parenchyma, leaving residual intact portal tracts and terminal hepatic veins. There usually is an excess of terminal hepatic veins draining each parenchymal nodule (From: Crawford J. *liver cirrhosis*. In: MacSween RN, Burt AD, Portmann BC, Ishak KG, Scheuer PJ, Anthony PP, eds. *Pathology of the liver*. 4th ed. London, England: Churchill Livingstone, 2002; 575-620).

3.3. Morphology of cirrhosis

Cirrhosis has been classified into « micronodular », « macronodular », and « mixed nodularity » [91]. In this formulation, micronodular cirrhosis is defined as a liver having uniform nodules of less than 3 mm in diameter, with delicate bands of fibrous tissue subdividing the hepatic parenchyma. In macronodular cirrhosis, the majority of nodules are greater than 3 mm in diameter, and are separated by coarse bands of scar tissue.

a. Micronodular cirrhosis

When hepatic injury and fibrosis are confined predominantly to the parenchyma, with lesser involvement of the portal tracts, the liver is subdivided at the acinar level, leading to micronodular cirrhosis. The overall shape and external appearance of the liver in micronodular cirrhosis may not be greatly altered. The nodules are small in size and uniform in appearance. The ratio of fibrous matrix to parenchyma is greater than in macronodular cirrhosis and consequently the liver is uniformly firm or even hard [81].

Normal liver acini are approximately 1 mm in diameter. Since micronodular cirrhosis arises from subdivision of parenchymal acini, the nodules may be less than 1 mm in diameter.

Nodules up to 3 mm in diameter may contain complete acinar units within them. Fibrosis may be dominant in one zone (e.g., around the terminal hepatic venules in alcoholic liver disease) but cirrhosis has not developed if the fibrosis is restricted only to one zone. Rather, a characteristic of micronodular cirrhosis is deposition of fibrous septa along the sinusoidal channels that connects the smallest portal tracts to their adjacent terminal hepatic venules. As the fibrous septa progressively link portal tracts to terminal hepatic veins, small islands of hepatic parenchyma are isolated.

Alcohol abuse is the most frequent association with micronodular cirrhosis in Europe and North America. Less common aetiologies for this pattern include hereditary haemochromatosis, chronic biliary diseases and hepatic venous outflow obstruction.

b. Macronodular cirrhosis

The size of the liver in macronodular cirrhosis is much more variable. The parenchyma exhibits large bulging nodules which are separated by fibrous bands that become broader and denser as the severity of liver injury and parenchymal damage progresses [81].

Multiple hepatic acini are incorporated into single nodules. The multiacinar pattern of nodule formation is most easily created when zone 3 of a simple acinus is in close proximity to one of the large conducting portal tracts that support multiple acinar regions.

This latter pattern of « macronodular » cirrhosis represents the end stage of almost any form of chronic liver disease [92]. Diseases which tend to produce a macronodular pattern at earlier stages include chronic viral hepatitis and autoimmune hepatitis.

c. Incomplete septal cirrhosis

Cirrhosis which does not feature full transformation of the liver may exhibit ill-defined large bulging nodules with only slender fibrous septa; this has been termed « incomplete septal cirrhosis » [81].

The etiology of an individual case of incomplete septal cirrhosis is usually not known, and « burn out » alcoholic or chronic viral or drug-induced hepatitis have been implicated.

3.4. Clinical status

The natural history of cirrhosis is characterized by an asymptomatic phase, termed « compensated » cirrhosis followed by a rapidly progressive phase with development of portal hypertension and/or liver dysfunction, termed « decompensated cirrhosis ». As the disease progresses, portal pressure increases and liver function decreases, resulting in the development of ascites, variceal bleeding, encephalopathy and jaundice. The development of any of these complications marks the transition from a compensated to a decompensated phase. Most frequently, ascites is the first of these signs to appear [8]. Therefore, it is usually considered a landmark sign of decompensated cirrhosis. Survival of patients with compensated cirrhosis is significantly longer than that of decompensated patients with median survival times of 12 and 2 years, respectively.

Four clinical stages of cirrhosis have been defined in the recent Baveno IV consensus conference [11]:

- Stage 1 is characterized by the absence of esophageal varices and ascites (mortality rate: 1% per year).
- Stage 2 is characterized by the presence of esophageal varices without ascites and without bleeding (mortality rate: 3.4% per year).
- Stage 3 is characterized by ascites with or without esophageal varices in a patient that has never bled (mortality rate: 20% per year).
- Stage 4 is characterized by gastro-intestinal bleeding with or without ascites (mortality rate: 57% per year).

Stages 1 and 2 correspond to patients with compensated cirrhosis while stages 3 and 4 refer to decompensated cirrhosis. Hepatocellular carcinoma develops at a fairly constant rate of 3% per year and is associated with a worse outcome at whatever stage it develops.

The most robust predictor of death in cirrhosis is the Child–Pugh score (with its components: albumin, bilirubin, ascites, encephalopathy and prothrombin time) and age. In addition to these markers of liver insufficiency, significant predictors that come to light in the compensated stage, are those related to portal hypertension, such as varices, splenomegaly and platelet count. This probably indicates that, in the compensated stage, measurements of portal pressure will have an important prognostic value. In the group of patients with decompensated cirrhosis, in addition to the Child-Pugh score, parameters of renal dysfunction (creatinine) arise as powerful prognostic indicators and, therefore, it is not surprising that the MELD score (which incorporates creatinine in addition to markers of liver dysfunction) has become a valuable method.

From a clinical point of view, it is important to assess prognostic variables separately in compensated and decompensated cirrhosis. Better still, and as recently concluded in the Baveno IV consensus conference, prognostic indicators should be targeted at the four specific subgroups of patients with cirrhosis that have a different risk of dying.

4. Reversibility of fibrosis and cirrhosis

There is now mounting evidence that hepatic fibrosis can be reversible. In humans, resolution of liver fibrosis can occur after successful treatment of the underlying cause of liver disease. This observation has been made in patients with chronic hepatitis C and B, alcohol-induced liver injury, NASH, hemochromatosis, secondary biliary cirrhosis and autoimmune hepatitis [17, 19, 93-96]. It may take years for significant regression to be achieved.

The dogmatic nature of the tenet of irreversibility of cirrhosis was voiced most explicitly by Perez Tamayo [97]. However, attentive reading of Perez Tamayo's notes reveals that this pathologist was writing a plea in favour of reversibility of fibrosis in cirrhosis, not on

reversibility as such. A study has shown that liver fibrosis even in patients with cirrhosis can regress following HCV eradication with alpha-interferon/ribavirin therapy [19]. Among a large cohort of patients successfully treated with this combination, there were 150 patients with cirrhosis, half of whom had a reduction in their fibrosis score according to METAVIR staging, with several patients regressing by two or more stages [98].

Liver fibrosis is thus reversible, including liver fibrosis in cirrhosis. However, cirrhosis implies more than just diffuse septal fibrosis of the liver. Further essential components of cirrhosis are architectural distortion (nodular parenchymal regeneration) and vascular derangements, including intrahepatic porto-systemic shunts [99]. Especially the vascular component of intrahepatic portosystemic anastomoses is considered as a determinant of no return, the major factor causing “irreversibility”, thus explaining why the cirrhotic state is reported as “for all practical purposes irreversible” in most textbooks and papers on liver pathology. “For all practical purposes” implies a reference to the present state of affairs, awaiting new developments, and hence not really dogmatic. It remains for the future to find out whether, or to what extent, also established abnormal vascular shunts in cirrhosis are subject to remodulation or breakdown, in view of the emerging concepts of plasticity and remodulation of vascular structures.

There are three major problems with most published reports on cirrhosis reversal [100, 101]. The first is an ambiguous terminology on two levels: confusion between fibrosis and cirrhosis, and ambiguity of the term reversal (or regression) itself, since minimal, partial or total reversal may be covered by the term reversal (regression) if no further specification is given. Therefore, the terms reversal and regression should be specified, and the terms fibrosis and cirrhosis should not be used interchangeably. A second problem in several reports on cirrhosis is the definition used for reversal: a conversion of a METAVIR fibrosis stage 4 (F4) to a posttreatment score of F2 or less. This clearly is a misunderstanding in the mind of some

about the meaning of semiquantitative scoring of disease progression (staging). All staging systems in use intend to quantitate progression (rather than regression) of disease, based not only on progression of fibrosis but also on architectural (nodular) parenchymal changes. Thus, the endstage in all scoring systems for staging corresponds to cirrhosis that is clearly and unmistakably identifiable on needle liver biopsy, that is, cirrhosis of the (predominantly) micronodular type. Because of sampling error in needle liver biopsies, a macronodular cirrhosis, and even more an incomplete septal type of cirrhosis, will be scored as fibrosis stage METAVIR F1, F2 or F3. In other words, a posttreatment conversion from METAVIR F4 to F2 or less is no proof of disappearance of cirrhosis, since it may still correspond to cirrhosis of a macronodular variety. A third problem is that cirrhosis comprises a full spectrum of stages itself: precirrhosis (incipient cirrhosis), early cirrhosis, fully developed cirrhosis, advanced cirrhosis and decompensated cirrhosis. The conclusion is that all studies until now have demonstrated variable reversal of fibrosis in cirrhosis, and not complete reversal of cirrhosis.

There are broadly two kinds of matrix degradation in liver, one that disrupts the low density matrix of normal liver (« pathologic matrix degradation ») and may therefore worsen liver disease, the other, the degradation of excess scar that may help restore the architecture of the injured liver to normal (« restorative matrix degradation ») [12, 102]. The liver contains abundant MMPs and collagenases capable of degrading extracellular matrix. Increased collagenolytic activity is a major mechanism of fibrosis resolution [98]. Fibrillar collagens (I and III) are degraded by MMPs. During fibrosis resolution, MMP activity increases due to a rapid decrease in the expression of TIMPs. Partial degradation of fibrillar collagen occurs, and the altered interaction between activated stellate cells and extracellular matrix favors apoptosis of stellate cells [102]. Another possibility for the decrease of activated stellate cells during resolution of hepatic fibrosis is the reversion of stellate cell activation. It is, however,

unknown whether an activated stellate cell can revert to a quiescent state in vivo, although it has been observed in culture.

Currently, the most effective way to treat liver fibrosis is to clear the primary cause of liver disease. This strategy has been shown effective in most chronic liver diseases [93, 95, 96, 98]. In patients with chronic HCV infection, current antiviral treatments (interferon plus ribavirin) clear viral infection in more than half of the patients. Sustained virological response is associated with improvement in liver fibrosis [98]. Patients with no sustained response may also experience improvement of liver fibrosis, which suggests that interferon has an intrinsic antifibrotic effect.

However, the improved understanding of mechanisms underlying hepatic fibrosis offers a new framework for developing antifibrotic therapies and makes effective antifibrotic therapy an imminent reality. In particular, the pathway of stellate cell activation provides an important framework to define sites of antifibrotic therapy. The inhibition of the renin-angiotensin system is probably one of the most promising strategy in treating liver fibrosis [2]. Among vasoactive cytokines, angiotensin II seems to play a major role in liver fibrogenesis. Key components of the renin-angiotensin system are locally expressed in chronically injured livers, and activated stellate cells de novo generate angiotensin II that induces hepatic inflammation and stimulates an array of fibrogenic actions. Importantly, pharmacological and/or genetic ablation of the renin-angiotensin system markedly attenuates experimental liver fibrosis. Moreover, renin-angiotensin inhibitors are widely used as antifibrotic agents in patients with chronic renal and cardiac diseases and appear to be safe when administered for prolonged periods of time. Little information is available on the use of this approach in patients with chronic liver diseases. Preliminary pilot studies in patients with chronic hepatitis C and NASH suggest that renin-angiotensin blocking agents may have beneficial effects on fibrosis progression [103, 104].

A limitation of the current antifibrotic approaches is that antifibrotic drugs are not efficiently taken up by activated stellate cells and may produce unwanted side effects. Cell-specific delivery to stellate cells could provide a solution to these problems. Promising preliminary results have been recently obtained using different carriers (cyclic peptides coupled to albumin and recognizing collagen type VI receptor and/or PDGFR) [105]. Second, there is currently no standard treatment for liver fibrosis. Although experimental studies have revealed targets to prevent fibrosis progression in rodents [106], the efficacy of most treatments has not been proven in humans. This is due to the need to perform serial liver biopsies to accurately assess changes in liver fibrosis and the necessity of long-term follow-up. The development of reliable noninvasive markers of liver fibrosis should have a positive impact on the design of clinical trials [2].

IV. METHODS FOR THE ASSESSMENT OF LIVER FIBROSIS

1. Histological analysis

Liver biopsy is the reference examination for the assessment of liver fibrosis [3]. However, liver biopsy is an invasive procedure, that is subject to sampling error.

1.1. Histological methods of assessment of liver fibrosis

Liver fibrosis can be assessed by using semi-quantitative scoring systems or morphometry. Whereas semi-quantitative scores are in widespread clinical use, morphometry is mainly used for research.

a. Semi-quantitative scoring systems

In the last half of the 20th century, the use of liver biopsy has grown to serve multiple purposes: confirmation of clinical diagnosis, assessment of severity of necro-inflammation and fibrosis and assessment of therapeutic intervention. Semi-quantitative scoring systems have been proposed to improve this evaluation. These are based on a limited number of well-characterized morphologic patterns. The purpose of these scores are 2-fold: first, to provide a systematic methodology and terminology to replace the traditional qualitative one, and second, to develop a means of evaluating serial biopsies in asymptomatic patients for tracking disease progression or the response to therapeutic intervention [107].

The Knodell histology activity index [108] was the first semi-quantitative system and is widely regarded as the benchmark for objective and reproducible description of the various

morphological lesions of chronic hepatitis. The histology activity index comprises 3 categories for necro-inflammation and 1 for fibrosis, with points for the severity of the lesion in each category. The first category, periportal and bridging necrosis, includes 7 levels of increasing severity, whereas the other 3 categories have just 4 levels. This gives greater weight to the first category on the rationale that periportal (piecemeal) and bridging necrosis are indicative of progression to cirrhosis. The total sum of points constitutes the final score, or histology activity index. The most frequently cited criticism of the Knodell histology activity index is that it is the sum of necroinflammatory and fibrosis scores and, therefore, does not distinguish ongoing hepatitis from parenchymal remodeling with fibrosis.

The explosion of scientific information on viral and nonviral hepatitis in the last decades of the 20th century led pathologists to question the conventional nomenclature of chronic persistent and chronic active hepatitis because of a growing understanding that etiology may be more significant than morphological classification in predicting the natural history of liver disease [109-112]. Ishak's 1994 review [109] popularized the use of the more inclusive term « chronic hepatitis », de-emphasizing the distinction between chronic persistent and chronic active hepatitis. The recommendation to include etiology in the classification of chronic hepatitis was one of the major contributions of a consensus conference held in 1994 [113]. Ludwig [110] and Batts and Ludwig [114] proposed systems of classification that subdivide chronic liver disease into nonbiliary or biliary categories.

Newer systems for grading and staging incorporate the view that necro-inflammation is not only a measure of severity but also of ongoing disease activity and the parameter most potentially responsive to therapy. This is referred to as « grade ». The lesions of fibrosis are referred to as « stage » and indicate long-term disease progression.

Among the various scoring systems giving rise to what has been referred to as « the scoring jungle » [115], we may quote the following ones:

- The Scheuer system [112] is less complex than the histology activity index, gives the portal and lobular components of activity equal weight, and groups the periportal and portal lesions into a single category.
- Ludwig's proposed system [110, 114] is similar to Scheuer's.
- The French METAVIR Cooperative Study Group proposes a comprehensive but complex system for the histological evaluation of hepatitis C. In contrast to the fibrosis score that appears quite simple including only five stages, the grading reflects the combined ratings for focal lobular necrosis, portal inflammation, piecemeal necrosis and bridging necrosis [116].
- A recent modification of the Knodell histology activity index, commonly referred to as the Ishak system [117], provides consecutive scores for well-defined lesions within 4 separate categories that are added together for the grade.

Most of these scoring systems that are now in widespread use were described originally for chronic viral hepatitis but are now applied to nonviral hepatitis as well.

b. Computed morphometry image analysis

Despite the fact that the scoring systems for fibrosis are categories rather than measurements, several studies have reported fibrosis progression rates in terms of « units per year » calculated on the basis of sequential biopsies performed for clinical indications at varying intervals [66, 118-121] or on the basis of single biopsies with rates calculated from the presumed duration of infection [62, 122]. This approach necessarily assumes that the categories represented by the stages are at equal distance in terms of severity, amount of fibrous tissue, or time of development. This is not supported by strong evidence. Indeed, several studies have shown that there is a great overlap between the various stages of the

scoring systems in the amount of fibrous tissue measured in the biopsies [123, 124]. Thus, the increase in fibrosis is not linearly related to a progression through histologic stages.

Therefore, morphometry is an interesting alternative to semi-quantitative scoring systems.

Morphometry consists in quantifying the extent of fibrosis as a ratio of the area of fibrosis relative to the liver area. In contrast to semiquantitative assessment, which is based on the pathologist's analysis, morphometry is a partly automated technique based on computerized pattern recognition that bypasses the subjectivity of the pathologist's judgment. This technique is analyzed with connective tissue stains (especially picrosirius red). This quantitative method has been used recently in several therapeutic trials of antifibrotic agents in experimental models or in humans [125-129]. Goodman et al. [123] used morphometry to quantify the amount of fibrous tissue in liver biopsies performed at baseline and after 48 weeks in 245 patients with chronic hepatitis C. The mean morphometrically determined collagen content increased by 58% between baseline and 48 weeks, whereas significant progression of the Ishak score was not detected. Morphometry appears thus as a much more sensitive tool than semi-quantitative scoring systems to demonstrate fibrosis progression. Morphometric analysis has several limitations. First, morphometry requires good quality, unfragmented liver specimens [123]. In advanced fibrosis or cirrhosis, needle biopsies performed with suction techniques are often fragmented, consisting of parenchymal nodules that leave most of the fibrous tissue behind. Image analysis of such biopsies will markedly underestimate hepatic fibrosis. Second, sampling error is the major drawback of morphometry [5, 130]. There can be significant differences in the amount of fibrous tissue between closely adjacent parts of the liver. In the study by Bedossa et al. [5], the coefficient of variation of fibrosis measurement was 55% by using image analysis on 15-mm long biopsy specimens and 45% on 25-mm biopsy specimens. It clearly appeared that image analysis was limited by the heterogeneity of fibrosis within the liver. Such a magnitude in the coefficient of variation

cancels the potential advantage provided by the high accuracy of the morphometry except for biopsy specimens of 40 mm or longer, a condition rarely obtained within routine practice. However, even though a single needle biopsy may overestimate or underestimate the amount of fibrosis, image analysis of the statistical sample provided by needle biopsies from many patients can be used to estimate the average amount of fibrosis in the cohort with a high degree of accuracy. Thus, a study using morphometry to measure fibrosis progression should include unfragmented, preferably large biopsies from a sufficiently large number of patients. Third, in patients with advanced liver disease, no relationship was found between the increase in fibrous tissue and portal pressure or synthetic capacity of liver in the study by Goodman et al. [123]. This suggests that factors other than the absolute amount of fibrous tissue play an important role in the clinical and laboratory changes of advanced liver disease. The architectural distortion that accompanies nodular parenchymal regeneration as well as shunting of blood through fibrous septa may be of equal or greater importance than the amount of scar tissue. Therefore, in the evaluation of an individual patient, the histologic diagnosis, based on the combination of architectural changes and amount of fibrosis, may be more important than the amount of fibrosis alone. Lastly, morphometry is time-consuming compared with simple histologic scoring, and the necessary equipment and expertise are not widely available.

3.2. Limitations of liver biopsy

a. Invasive procedure

Liver biopsy is performed percutaneously or less often through a transjugular approach, respectively in 91% and 9% in a nationwide study of Cadranet et al. [131]. In the same study,

Menghini-type needles were used in 80% of the percutaneous liver biopsies and Trucut needles in 20%.

Minor complications after percutaneous liver biopsy include pain (in the right upper quadrant or right shoulder) and hypotension (due to a vasovagal reaction). Moderate pain occurs in 20-40% of cases [131]. Severe pain in the abdomen can be caused by a more serious complication. In the study of Cadranet et al. [131] analyzing 2084 biopsies that were performed for diffuse parenchymal liver diseases, severe pain requiring the use of morphine and resulting in hospitalization was observed in 3% of cases. Vaso-vagal episodes occurred in 2%. Liver biopsy is thus a procedure that is not well accepted by patients.

Severe complications occurred in 0.3-0.5% in the study of Cadranet et al. [131]. These complications were:

- Hemorrhage including intraperitoneal hemorrhage (free intraperitoneal blood may result from laceration caused by deep inspiration during biopsy or may be related to a injury of a branch of the hepatic artery or portal vein), intrahepatic or subcapsular hematomas, and hemobilia.
- Peritonitis and septicemia that developed rarely in patients with biliary obstruction and cholangitis despite the fact that transient bacteremia occurred in 5-13% of the patients.
- Puncture of other viscera and pneumothorax.

The frequency of complications increased with the number of passes and decreased with experience of operator and ultrasound-guidance. The mortality rate after percutaneous liver biopsy is approximately 1 in 10,000 to 1 in 12,000.

With transjugular biopsy, the risk of bleeding is minimized. However, the samples obtained are small and fragmented, a disadvantage of the technique.

b. Sampling error

A liver biopsy is usually considered adequate by the pathologists when it has a length of at least 25 mm [5]. Additionally, the number of portal triads in the specimen is important to consider. Most hepatopathologists are satisfied with a biopsy specimen containing at least six to eight portal triads. However, the liver core biopsy specimen represents a very limited part of the liver (1/50000) [3] and fibrosis is heterogeneous. Therefore, the information in the sample may not fully reflect the true pathological picture.

In a study of 124 patients with chronic HCV infection who underwent laparoscopic-guided biopsy of both the right and left hepatic lobes, the results were discordant by one histological stage (modified Scheuer system) in 33% of the cases, but differences greater than one stage were observed in only 2.4% [9]. These findings introduce uncertainty into longitudinal observations involving sequential liver biopsy. Whereas a two-stage change in fibrosis stage in longitudinal studies is likely significant, a one-stage change may not be. Liver biopsy appears adequate for a 3-level staging of fibrosis but not for a finer delineation. This limits its value, particularly for diseases such as chronic hepatitis C, which typically progresses slowly, on average 0.15 stage per year [62, 64, 132, 133].

Siddique et al. showed a difference of at least one fibrosis stage between two specimens (at least 15-mm long) obtained at the same puncture site in 45% of the patients analyzed [10].

Colloredo et al. reported that fibrosis tended to be underestimated as the biopsy specimen length diminished [6]. Sampling error may also lead to underdiagnosis of cirrhosis. Maharaj et al. by performing three transcutaneous biopsies in the same patients using different entry points, reported that in proven cirrhotic patients, a histopathologic feature of cirrhosis was present in all three biopsy specimens of only 50% of the patients [7]. Similarly, Abdi et al. performed several post-mortem biopsies and showed that the diagnosis of cirrhosis could be

obtained from one biopsy specimen in only 16 of 20 cases, but that the performance increased to 100% with three biopsy specimens [134]. As shown by these studies, the problem of heterogeneity can be partially resolved by taking several biopsy specimens from the same patient, a procedure that raises ethical concerns because of the increased risk of morbidity and mortality.

c. **Inter- and intraobserver variability**

Several studies have addressed the problem of inter- and intraobserver variability in fibrosis evaluation: they generally concluded that the reproducibility in scoring fibrosis was good whatever the scoring system used by the pathologists, and that intraobserver agreement was better than interobserver agreement [135, 136]. Moreover, there is an inverse relationship between the number of fibrosis classes and agreement [135, 136]. The use of only five stages with the METAVIR scoring system led to greater concordance among pathologists than the Ishak system that includes seven stages [135, 137, 138]. It was also found that agreement varied widely according to the pathological feature examined and was better for fibrosis than for activity. In a therapeutic trial for viral hepatitis, the 10 pathologists of the METAVIR group working in pairs and using either the METAVIR or the Knodell systems agreed substantially in their fibrosis scores for 30 hepatitis C biopsies. There was less concordance in scoring necro-inflammatory lesions. The results were similar with other systems such as the Knodell and Scheuer scoring systems [107, 139].

In a study including 254 liver specimens and 15 pathologists using the METAVIR score, Rousselet et al. [136] showed that the agreement was better for senior (0.60 ± 0.24) than junior pathologists (0.52 ± 0.30 , $P < .05$). Moreover, in the same study, agreement of the METAVIR scoring system did not depend on the length of the specimen. With some of the

pathologists, the agreement decreased when the length of the specimen increased. Moreover, activity and fibrosis scores increased when the length of the specimens increased, a finding also reported in another study [6, 136]. These data suggest that the probability of finding various types of lesions increases when the specimen size increases. This explains the decrease in agreement and the increase in grade and stage with increased biopsy length. Finally, the degree of experience (specialization in liver pathology) rather than the specimen characteristics (e.g., length) influences the agreement.

4. Biochemical tools

The ideal noninvasive diagnostic test for hepatic fibrosis should be simple, readily available, inexpensive, and accurate [14]. No test meets this definition, although a number of approaches have been proposed and are being evaluated.

Non-invasive tests contain two categories, namely the biochemical and the liver imaging tests [140, 141].

4.1. Biochemical tests

The biochemical tests comprise routinely available tests such as Forns' index (age, gamma glutamyl transpeptidase (γ GT), cholesterol, and platelet count) [21], Fibroindex (platelet count, aspartate aminotransferase and γ GT) [142] and aspartate aminotransferase to platelet ratio index (APRI) [25]. More complex and expensive indirect non-invasive tests are the PGAA (prothrombin time, γ GT, apolipoprotein A1, α_2 -macroglobulin) index [143], the Hepascore (age, α_2 -macroglobulin, hyaluronate, bilirubin and γ GT) [144] and the Fibrotest (α_2 -macroglobulin, haptoglobin, γ GT, apolipoprotein A1, γ -globulin and total bilirubin) [23,

145]. Indexes are associations of blood markers selected on the basis of purely statistical grounds. These indexes can be considered as surrogate markers as most of the items are not directly caused by liver fibrosis.

Wai et al. [25] found that APRI predicted significant fibrosis (Ishak score ≥ 3) and cirrhosis (Ishak score ≥ 5) with areas under the receiver operating characteristic (ROC) curves of 0.88 and 0.94, respectively. For the Forns' test [21], the area under the ROC curve was 0.81 for patients with significant fibrosis (Scheuer's classification ≥ 2). For the more widely validated Fibrotest index, the area under the ROC curve for detecting patients with significant fibrosis (METAVIR $\geq F2$) varied from 0.73 to 0.84 [23, 145, 146].

However, there are limitations to the use of these markers:

- Some of the parameters tested such as γ GT or total bilirubin have a genetic heterogeneity explaining elevated values in some normal subjects.
- Almost all markers can be influenced by extrahepatic diseases or conditions such as hemolysis.
- To what extent variations of indirect markers with time reflect variations in liver fibrosis is unknown but a close relationship seems improbable as there is no direct link between these markers and liver fibrosis.

Fibrotest has false-positive results caused by increases in bilirubin or decreases in haptoglobin. This is particularly relevant for hepatitis C patients who are treated with ribavirin, which commonly causes hemolysis. False-positive results may be caused by Gilbert's syndrome and cholestasis. Moreover, acute inflammation causes increases in α_2 -macroglobulin and haptoglobin, also affecting the test. Because of these limitations, the value of these indirect tests is debated, especially for the diagnosis of intermediate fibrosis [147].

4.2. Serum extracellular matrix markers of fibrosis

As already noted, fibrosis is dynamic, with increased fibrogenesis and fibrolysis, both of which may yield an increased level of circulating extracellular matrix components. Several assays are thus directed at measuring the breakdown products of extracellular matrix constituents and the enzymes that regulate their production or modification [148], including: (a) glycosaminoglycans such as hyaluronic acid and fibrous proteins such as laminin or undulin (type XIV collagen); (b) propeptides of types I, III and IV collagen; (c) enzymes such as MMPs and TIMPs. Recently a test associating hyaluronic acid, amino-terminal propeptide of type III collagen and TIMP 1 has been proposed by the European liver fibrosis study group [149].

Although these tests are useful in detecting advanced fibrosis (cirrhosis) in patients, as well as minimal or no fibrosis, they are less effective for differentiating intermediate stages of fibrosis. Additionally, it should be stressed that there is no specific type of collagen or extracellular matrix component in the liver. Therefore, fibrosis-specific markers may reflect fibrogenesis in other organs (i.e., pancreatic fibrosis in alcoholic patients). Moreover, the serum concentration of the matrix components depends on their degradation rate which can be impaired in renal failure or cholestasis. Finally, the serum concentration reflects the metabolism of the matrix components rather than their static amount in the liver. In other words, serum concentration reflects fibrogenesis and fibrolysis rather than fibrosis itself.

Other investigators have used new highthroughput technologies in a global screening approach. Techniques based on proteomics, glycomics and genomics are now considered of potential interest [150-152]. In a study using the proteomic technique and including 46 patients with chronic hepatitis B, Poon et al. [151] showed that the predictive artificial neural

network fibrosis index derived from the proteomic fingerprint strongly correlated with Ishak scores ($r = 0.831$) and was significantly different among stages of fibrosis: the areas under the ROC curve for this analysis were 0.906 and 0.921, for advanced fibrosis (Ishak stage ≥ 3) and cirrhosis, respectively.

Callewaert et al. [150] used a technique of glycomics based on DNA sequencer/fragment analysis technology, for the diagnosis of liver cirrhosis. They studied a group of 106 patients with chronic liver disorders at various stages of severity, regardless of the underlying etiology. Analysis of the serum N-glycome profile showed significant modifications of five peaks in cirrhosis. This technology yielded a biomarker that distinguished compensated cirrhotic from noncirrhotic patients, with 79% sensitivity and 86% specificity, by measuring two easily detectable peaks (resulting in the glycocirrhorest biomarker). However, this index was not relevant for the diagnosis of less advanced stages (from F0/F1 to F3 according to the METAVIR system).

Most of the studies dealing with surrogate markers of liver fibrosis in chronic hepatitis C infection did not check the reliability of their algorithms in other diseases associated or not with liver injury. Diseases outside the liver may influence the results. For example, Callewaert et al. studied a group of patients with non-hepatic autoimmune diseases, including rheumatoid arthritis, ankylosing spondylitis or Crohn's disease, and showed that at least 15% of these patients displayed a glycocirrhorest suggestive of cirrhosis. Other limitations of glycomics and proteomics include their variability and cost [153].

Proteomics and glycomics will give further insights into the molecular and cellular biology of chronic liver injury. As indicated by Callewaert et al. [150], specific modification of serum glycomics observed in liver cirrhosis may be related to the presence of regenerative nodules. They detected an increase in the modification of serum N-glycans with a bisecting N-acetylglucosamine (GlcNAc) residue. In normal liver, the enzyme responsible for this

modification, N-acetylglucosaminyltransferase III (GnT-III), is expressed in nonparenchymal cells, but in regenerating liver, GnT-III is also expressed in hepatocytes. Consequently, it is conceivable that modification of serum glycoproteins induced by GnT-III expression is a hallmark of the presence of regenerative nodules that histologically define liver cirrhosis.

5. Imaging tests

5.1. Anatomical imaging

Advanced fibrosis can be detected with imaging modalities, including ultrasound, computed tomography, and magnetic resonance imaging. For example, ultrasound correctly diagnosed cirrhosis in up to 90% of cases in some studies [154, 155]. However, anatomical imaging is insensitive to mild or moderate fibrosis.

At CT, MR imaging and ultrasonography [156], the cirrhotic liver usually appears to be small and diffusely heterogeneous. Moreover, architectural distortion is often observed with:

- Nodularity of the liver surface: Surface nodularity has been mostly studied with sonography. It is more easily observed when ascites is present or when high-frequency ultrasound beams are used.
- Atrophy of the right lobe and hypertrophy of the left and caudate lobes: These morphological changes can be assessed by calculating the ratio of transverse caudate lobe width to right lobe width or by measuring the segment 4. In the study of Harbin et al. [157], a caudate-to-right lobe ratio greater than 0.65 provided 96% confidence for diagnosing cirrhosis. Lafortune et al. [158] have proposed to measure segment 4 between the left wall of the gallbladder and the ascending portion of the left portal vein. A segment-4 diameter of less than 30 mm is highly specific sign for cirrhosis.

- Widened fissures between segments/lobes. The porta hepatitis and interlobar fissures frequently appear widened because of atrophy of the right lobe and hypertrophy of the left lobe.

Cirrhosis is also characterized by the development of nodules that are best characterized at MR imaging and that include: regenerative nodules (on T1-weighted images: hypointense relative to liver parenchyma; T2: hypointense), dysplastic nodules (T1: hyperintense; T2: hypointense) and hepatocellular carcinoma nodules (T1: hypo/iso/hyperintense; T2: hyperintense; after contrast material injection, hypervascular) [159, 160].

Signs of portal hypertension include splenomegaly, ascites, and portosystemic collateral vessels [159]. Recanalized paraumbilical vein is the collateral vessel most easily detected with color Doppler sonography. Decrease of portal venous velocity ending with hepatofugal portal venous flow can also be detected in patients with portal hypertension. Additionally, the phasic oscillations in hepatic venous flow are dampened in cirrhosis: this has been termed « portalization » of the hepatic waveform because it resembles the flow pattern seen in the normal portal vein. Finally, the hepatic artery is frequently enlarged in cirrhosis.

In the study by Aube et al. [155], cirrhosis could be correctly diagnosed in 82-88% of the patients with chronic liver disease using a few ultrasonographic signs (liver surface, spleen length...). However, in this study, the diagnostic accuracy of ultrasound was decreased by 7% because of the anatomical limitations of this technique. Adding Doppler signs such as the hepatic vein spectrum or the maximum portal vein velocity, diagnostic accuracy could be improved up to approximately 90%.

Several studies evaluated the ability of superparamagnetic iron oxide (SPIO)-enhanced MR imaging to detect liver fibrosis in patients with chronic liver disease [161, 162]. SPIO particles are reticuloendothelial-specific particulate contrast agents for MR imaging which markedly shorten T2 relaxation rate. Consequently, the signal intensity of the liver

parenchyma decreases on SPIO-enhanced T2-weighted images, except in the fibrotic areas.

This increases the contrast between the regenerating nodules and the fibrotic septa and improves the detection of liver fibrosis. Fibrosis can also be detected because it enhances on delayed phase gadolinium-enhanced images.

Recently, Aguirre et al. [163] showed that advanced hepatic fibrosis can be detected by using double-enhanced MR imaging, using SPIO and gadolinium chelates. However, less advanced fibrosis (F2) cannot be detected with this method.

5.2. Functional imaging

Functional MR imaging methods have been proposed to assess liver fibrosis. These methods include perfusion MR imaging, diffusion MR imaging, and spectroscopy.

a. Perfusion

Perfusion MR imaging can detect the early microcirculatory alterations in fibrosis and cirrhosis [164, 165]. Perfusion assessment with MR imaging is more complicated than with CT [166]. This is explained by the fact that the linear relationship between signal intensity and contrast agent concentration that is observed at CT, holds true only for low contrast agent concentrations at MR imaging. However, the relationship between relaxation rate ($1/T_1$) and contrast agent concentration is linear, as predicted by the Solomon-Bloembergen equation [167]:

$$1/T_1 = 1/T_{10} + r_1[\text{Gd}]$$

where r_1 is relaxivity and T_{10} is the relaxation time in the absence of contrast material.

This means that the pharmacokinetic models that analyse concentration-time curves can be applied to MR data only if the acquired time-signal curves are first transformed into $R_1(=1/T_1)$ -time curves using external phantoms or if R_1 -time curves are obtained directly by using more elaborated MR acquisition schemes.

Several studies of perfusion MR imaging in fibrosis and cirrhosis have been reported [164, 165]. In the study of Annet et al. [164], MR hepatic flow parameters were significantly modified in cirrhosis (decrease of the portal and total hepatic flow, increase of the arterial flow and the mean transit time), and were correlated with the severity of cirrhosis (Child-Pugh classification) and portal hypertension. In contrast, flow parameters measured with Doppler ultrasonography did not differ significantly between patients with and without cirrhosis. Only the right hepatic arterial resistance index and the portal flow were weakly ($r < 0.7$) correlated with portal pressure. No correlations were observed between Doppler parameters and Child-Pugh classification. Several authors confirmed that Doppler indexes had no relationship to the severity of the histological liver injury. In the study by Lim et al. [168], no significant differences in the Doppler indexes (hepatic artery velocity, resistive index, portal vein velocity, ratio of the hepatic artery velocity to the portal vein velocity and phasicity of the hepatic veins) were observed with increasing severity of liver disease. The superiority of MR imaging relative to Doppler ultrasonography may be explained by several reasons. Assessment of the portal venous flow and flow velocity at Doppler ultrasonography can provide misleading results if a paraumbilical vein is dilated: patients with dilated paraumbilical vein have higher portal pressure than patients without dilatation, but they do not have decreased portal flow or flow velocity at Doppler ultrasonography. In contrast, the perfusion parameters measured at MR imaging are not influenced by a patent paraumbilical vein, because they are measured in the right liver rather than the portal vein. Moreover,

Doppler measurements are operator dependent and therefore have a limited clinical role in the non-invasive assessment of liver fibrosis.

Annet et al. [164] hypothesized in their MR imaging study that the increase of mean transit time could be explained by the capillarization of fenestrated sinusoids and the deposition of collagen in the extravascular spaces that occur in cirrhosis. This explanation was confirmed by the study of Van Beers et al. [169] in which dynamic MR imaging after injection of a low-molecular-weight contrast agent of 0.56 kDa (Gd-DOTA), and two high-molecular-weight contrast agents of 6.47 kDa and 52 kDa (P792 and P717) was performed in rabbits with liver fibrosis induced by cholesterol and diethylstilbestrol. Because of the capillarization of the sinusoids, the hepatic mean transit time of the low-molecular-weight agent was increased in the rabbits with liver fibrosis, whereas the hepatic distribution volume accessible to the high-molecular-weight agents decreased. The authors also observed a significant correlation between the collagen content of the liver and the distribution volume of P717. No correlation, however, was found between the extent of fibrosis and the distribution volume of P792 or Gd-DOTA. This indicates that agents with higher molecular weight (> 50 kDa) are better suited to detect vascular permeability changes in liver fibrosis.

b. Diffusion

Diffusion-weighted MR imaging (DWMRI) measures the apparent diffusion coefficient (ADC) of water, a parameter that is dependent on the tissue structure. Diffusion corresponds to the motion of water molecules in biological tissues, so-called Brownian motion [170]. The protons contained in the fibrotic liver are less abundant than those in water and are tightly bound. Several reports on the use of DWMRI in the liver have shown a decrease of ADC in

cirrhosis, which is thought to reflect a restriction of water diffusion in the fibrotic tissue [170-172]. However, overlap has been reported in the results between normal and fibrotic liver [170, 173].

The results of DWMRI measurements are influenced by the choice of an acquisition parameter called the b value. The b value is a function of the amplitude and duration of the diffusion gradients and of the time allowed for the proton to diffuse between two successive gradient pulses. When small b values are used, ADC is overestimated because perfusion largely influences the measurements. In contrast, when large b values are used, ADC tends to be underestimated because of the diminished image quality caused by the low signal intensity of the liver. Moreover, the ADC measurements are influenced by the number of b values used. According to the signal attenuation equation, $S_b = S_0 \exp(-b \times \text{ADC})$, only two measurements are required to evaluate the ADC: one in the absence (S_0) and one in the presence of a diffusion gradient with a given b value (S_b). However, the ADC evaluation is more precise when several b values are used, allowing a linear regression analysis to be performed from the derived equation, $\ln S_b = \ln S_0 - b \times \text{ADC}$ [171, 174, 175].

To obtain high signal intensity during DWMRI of the liver, Lewin et al. [176] used a parallel imaging technique [177] and combined multiple b values (0, 200, 400 and 800 seconds/mm²) into a single acquisition. In this study [176], patients with substantial fibrosis ($\geq F2$) had significantly lower hepatic ADC values than patients without or with mild fibrosis (F0 - F1) (mean: 1.10 ± 0.11 versus $1.30 \pm 0.12 \times 10^{-3} \text{ mm}^2/\text{s}$) and healthy volunteers (mean: $1.44 \pm 0.02 \times 10^{-3} \text{ mm}^2/\text{s}$). The area under the ROC curve was 0.79 for staging patients with $F \geq 2$. However, the influence of perfusion on the ADC measurements remains debated as shown by an animal study by Annet et al. [178]. In rats with liver fibrosis induced by intraperitoneal injections of carbon tetrachloride (CCl_4), these authors indeed found that the ADC decreased in living rats according to the severity of liver fibrosis (controls: $1535 \pm 294 \text{ mm}^2/\text{second}$;

CCl₄ (for 9 weeks): $943 \pm 132 \text{ mm}^2/\text{second}$; $P = 0.002$). However, immediately after killing the rats, no significant difference in ADC was observed between animals with and without fibrosis (controls: $599 \pm 59 \text{ mm}^2/\text{second}$; and CCl₄ (9 weeks): $668 \pm 65 \text{ mm}^2/\text{second}$; $P > 0.05$). The decrease of ADC in the living rats is thus not explained by a restricted diffusion due to hepatic fibrosis, because this restricted diffusion should persist after death. Other factors, such as a decrease of perfusion, could explain the decrease of the in vivo ADC observed in rats with liver fibrosis. This explanation is supported by several observations. First, the ADC was significantly higher in the living rats than in the dead ones, which suggest that including the perfusion fraction led to overestimation of the ADC measurements in the living rats. Second, the decrease of ADC after death was not equivalent in each group of rats (there was a significantly smaller decrease in the fibrotic groups compared to the control group). This difference might be explained by decreased liver perfusion in fibrosis and cirrhosis. These observations strongly suggest that the measurement of ADC does not merely reflect restricted water diffusion caused by extracellular matrix deposition characterizing fibrosis in the liver, but is heavily influenced by changes in perfusion associated with hepatic fibrosis.

c. Spectroscopy

Liver fibrosis can be detected with hydrogen-1 (^1H) or phosphorus-31 (^{31}P) MR spectroscopy.

i. Hydrogen-1 (^1H) MR spectroscopy

In the study by Cho et al. [179], the metabolite-to-lipid ratios of glutamine and glutamate complex, phosphomonoesters, glycogen and glucose complex increased as the stage of chronic hepatitis became higher at hydrogen-1 (^1H) MR spectroscopy of the liver.

ii. Phosphorus-31 (^{31}P) MR spectroscopy

A typical ^{31}P MR spectrum of the human liver *in vivo* contains resonances that can be assigned to phosphomonoesters (PME), containing information from sugar phosphates in the glycolytic pathway and from cell membrane precursors such as phosphoethanolamine and phosphocholine; to phosphodiester (PDE), containing information from endoplasmic reticulum and from cell membrane degradation products such as glycerophosphorylcholine and glycerophosphorylethanolamine, in addition to signals from inorganic phosphate (P_i) and nucleotide triphosphates (NTP), including adenosine triphosphate (ATP) [180-182].

Resonances from phosphomonoesters and phosphodiester probably reflect the cell turnover of the hepatocytes whereas ATP reflects the energy state in the cells. The liver attempts to regenerate itself with increasing injury, thus giving rise to increased turnover of cell membrane products. Several studies have thus reported good correlation between liver fibrosis and either increased PME resonance or decreased PDE resonance [180, 182-191]. Moreover, the PME/PDE ratio has been viewed traditionally as an index of cell membrane

turnover and thus provides an indirect measure of disease severity within the liver: an elevated PME/PDE ratio is associated with more severe liver damage [23, 192, 193]. Lim et al. [194] showed that the ratio of PME to PDE resonance in ^{31}P MR spectroscopy was a sensitive marker of cirrhosis and could differentiate mild from moderate chronic hepatitis, and separate these 2 groups from cirrhosis.

In a study using ^{31}P MR spectroscopy, Noren et al. [195] have proposed a new parameter, the anabolic charge, $AC = [\text{PME}] / ([\text{PME}] + [\text{PDE}])$ as a possible tool for evaluating metabolic events in the liver. Both phosphodiesterases and anabolic charge were significantly correlated to the stage of fibrosis.

iii. Limitations of spectroscopy

First, respiratory movement can prevent adequate signal acquisition owing to the inconstant position of a voxel. Second, overlapping the peaks makes it difficult to discriminate all metabolite peaks. This is a common limitation of current commercial machines with low magnetic field strength. Third, the lack of consensus concerning data acquisition and analysis makes comparisons between studies difficult. Absolute quantification should be obtained but is difficult to perform. Finally, only few studies of MR spectroscopy of the liver have been reported and these studies included a limited number of patients. Much more experience is needed before eventually including MR spectroscopy of chronic liver disease into routine clinical practice.

5.3. Elastography

The stiffness of tissue can be assessed with palpation. Palpation is restricted to parts of the body that are accessible to the physician's hand. Elastography prolongs manual palpation within the body and can thus be defined as « palpation by imaging ».

The classic approach for measuring the elasticity of a tissue is to apply a known stress and to measure the resulting strain (static elastography). The strain distribution is observed or measured using a imaging method such as ultrasound [196]. More often, elastography measures the propagation of mechanically applied strain waves in tissues with ultrasound or MRI (transient or dynamic elastography) [28, 197, 198].

a. Ultrasound elastography

i. Apparatus, elasticity estimation and acquisition procedure

The method most commonly used is one-dimensional (1-D) transient elastography, a technique that analyzes the propagation of low-frequency shear waves, whose velocity is directly related to elasticity [28].

The use of transient vibrations presents two advantages. First, the transmitted elastic wave can be temporally separated from reflected waves. Thus, the method is less sensitive to boundary conditions than other elastographic methods. Second, the acquisition time is short (typically less than 100 ms), which enables measurements to be made on moving organs. The 1-D transient elastography technique is not intended to produce elasticity images because data are collected on the axis of a single-element transducer.

Elasticity measurement apparatus

The Fibroscan (*Fig. 4*) is composed of a probe that contains a low-frequency vibrator (typically 50 Hz). An ultrasonic single-element transducer operating at 5 MHz is built on the axis of the vibrator. This transducer is focused at 35 mm, which corresponds to the middle of the region of interest (ROI).



Figure 4. *The FibroScan is a dedicated machine that is composed of a probe and a control unit. The probe includes an ultrasonic transducer mounted on the axis of a vibrator (From: EchoSens, Paris, France).*

Elasticity estimation

Elasticity is derived from the velocity of the low frequency elastic wave in a ROI located from 25 to 65 mm below the skin surface (*Fig. 5*). This ROI is chosen to avoid the subcutaneous tissue and the liver fibrous capsule in most of the patients.

The low-frequency elastic wave is mainly a shear wave. Assuming that liver is an isotropic, purely elastic and incompressible medium, the Young's modulus E corresponds to the stiffness of the liver and is expressed as [199]:

$$E = 3\rho(V_s)^2$$

where V_s is the shear velocity and ρ is the mass density.

Several measurements are performed in each patient. An algorithm automatically rejects shear velocity estimates obtained with a linear regression coefficient of determination (r^2) inferior to 0.85. The elasticity estimation is fully automated. The median value of the validated ($r^2 \geq 0.85$) estimates is kept as the elasticity value of liver for a given patient.

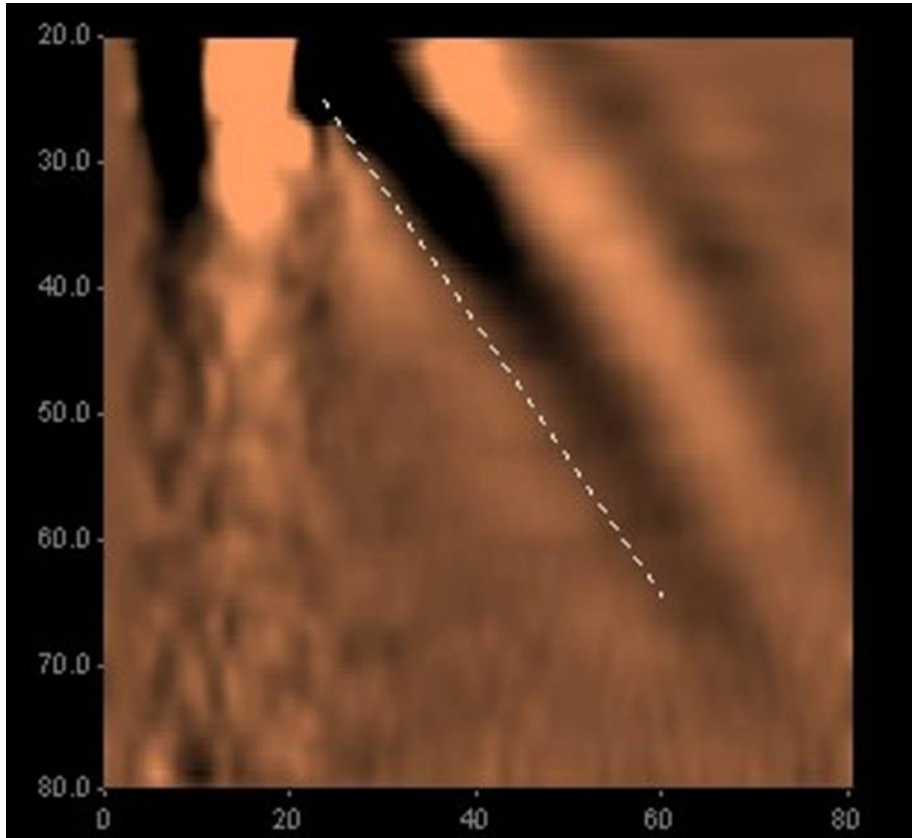


Figure 5. *Amplitude of the strains induced in a healthy patient liver as a function of depth and time at ultrasound elastography. The elastic wave velocity, V_s , is the slope of wave pattern (From: EchoSens, Paris, France).*

Acquisition procedure

Acquisitions are performed in the right lobe of the liver through an intercostal approach (the diameter of the transducer is only 9 mm and fits between the costal bones of most patients). The chest wall prevents the liver from being directly compressed by the probe itself. The second contribution of the chest wall is that it gives a static, almost plane surface for probe positioning. In fact, the surface on which the low frequency vibration is sent should be as plane as possible. During the acquisition, patients are lying on their back with their right arm behind their head. Ten successful acquisitions are performed in each patient. The success rate is calculated as the ratio of the number of successful acquisitions over the total number of acquisitions. The entire examination lasts less than 5 minutes [28].

ii. Performance

Staging of liver fibrosis

The slope of the wave pattern and, thus, the shear velocity increases as the fibrosis grade increases. A significant positive correlation between stiffness measurements and fibrosis stages is found. This observation is consistent because stiffness of tissues largely depends on their molecular building blocks (collagen) and on the microscopic structural organization of these blocks (septa). The main advantage of liver elastography compared with biochemical markers is that it measures a quantitative parameter directly in the liver. Moreover, the elasticity estimate is averaged over a volume that can be approximated by a cylinder of 40 mm-length (between 25 mm and 65 mm below the skin surface) and 10 mm-diameter. This

volume represents 1% of the liver total volume, which is much more relevant than the biopsy sample size, which corresponds to only 1/50,000 of the liver volume [3].

Regarding the accuracy of the FibroScan, the areas under the ROC curve that were found were usually above 0.80 for $F \geq 2$ and $F \geq 3$, and above 0.90 for $F = 4$ [27, 28, 30, 200-206] (*Table 1*).

Table 1. *The areas under the ROC curve of the stiffness measurements obtained with transient ultrasound elastography in different studies.*

	Aetiology	N	AUROC			Cut-off values		
			$F \geq 2$	$F \geq 3$	$F = 4$	$F \geq 2$	$F \geq 3$	$F = 4$
Sandrin et al.	HCV	67	0.88	0.91	0.99	X	X	X
Ziol et al.	HCV	251	0.79 (0.73 – 0.84)	0.91 (0.87 – 0.96)	0.97 (0.93 – 1.00)	8.8 (0.56; 0.91)	9.6 (0.86; 0.85)	14.6 (0.86; 0.96)
Castera et al.	HCV	183	0.83 (0.76 – 0.88)	0.90 (0.85 – 0.94)	0.95 (0.91 – 0.98)	7.1 (0.67; 0.89)	9.5 (0.73; 0.91)	12.5 (0.87; 0.91)
Kettaneh et al.	HCV	935	0.79 (0.75 – 0.82)	0.89 (0.86 – 0.92)	0.91 (0.87 – 0.94)	X	X	X
Corpechot et al.	PBC/PSC	99	0.88 (0.81 – 0.95)	0.91 (0.85 – 0.97)	0.96 (0.93 – 1.00)	7.3 (0.84; 0.87)	9.8 (0.91; 0.90)	17.3 (0.93; 0.95)
Foucher et al.	Various	711	0.80 (0.75 – 0.84)	0.90 (0.86 – 0.93)	0.96 (0.94 – 0.98)	7.2 (0.64; 0.85)	12.5 (0.65; 0.95)	17.6 (0.77; 0.97)
Gomez-Dominguez et al.	Various	94	0.74 (0.63 – 0.83)	0.72 (0.58 – 0.74)	0.94 (0.87 – 0.98)	4 (0.94 – 0.33)	11 (0.58 – 0.89)	16 (0.89; 0.96)
Fraquelli et al.	Various	200	0.86 (0.81 – 0.89)	0.87 (0.83 – 0.93)	0.90 (0.85 – 0.98)	7.9 (0.72; 0.84)	10.3 (0.76; 0.90)	11.9 (0.91; 0.89)
Coco et al.	Various	228	0.93 (0.90 – 0.96)	X	0.96 (0.93 – 0.98)	8,3 (0.85; 0.91)	X	14 (0.78; 0.98)
Ganne-Carrie et al.	Various	1007	X	X	0.95 (0.93 – 0.96)	X	X	11.7 (0.88; 0.89)

Influence of inflammatory activity and steatosis

Activity could have been expected to increase liver stiffness because of inflammatory infiltrate and oedema, whereas steatosis could have been expected to soften the liver. In the study by Ziol et al. [30], a multivariate analysis including fibrosis, activity, and steatosis showed that fibrosis was the only parameter significantly correlated to liver stiffness.

However, no patient had massive steatosis without advanced fibrosis. Therefore, the potential effect of steatosis on liver stiffness may have been hidden by the strong effect of fibrosis.

Similarly, no influence of steatosis on elasticity measurements was found in the study by Kettaneh et al. [205]. However, only patients with chronic hepatitis C were included, and steatosis was absent or mild in more than 80% of the patients.

These findings support the study by Yeh et al. [29] on elasticity measurements of *ex vivo* human liver samples that reported a correlation between liver stiffness and fibrosis but did not show any obvious correlation between steatosis and elastic modulus. Further studies are required to investigate the effect of pure steatosis (without fibrosis) on liver stiffness, more particularly in NASH patients.

Regarding activity, Coco et al. [201] showed that in patients with biochemical remission, liver stiffness was lower than in patients with identical fibrosis stage, but elevated ALT ($P < 0.001$). The liver stiffness profiles paralleled those of ALT, increasing 1.3- to 3- fold during ALT flares in 10 patients with hepatitis exacerbations. Liver stiffness remained unchanged in 21 patients with stable biochemical activity. These findings suggest that both inflammatory infiltrates and oedema might have significant impacts on elasticity. Here again, further studies are needed to clarify the influence of inflammation on liver stiffness.

Severity of cirrhosis

In cirrhotic patients, liver stiffness measurements show a large range (e.g., from 12.5 to 75.5 kPa in the study by Foucher et al. [207]). Cirrhosis is clearly not a homogeneous condition and it is still difficult to classify patients according to the severity of their disease. Moreover, several authors have shown that a significant percentage of patients with cirrhosis can be misclassified as F3 at histological analysis.

In a study of Ganne-Carrie et al. [204] including 1257 patients with chronic liver diseases of various causes, the cut-off value for the diagnosis of cirrhosis was 14.6 kPa (positive and negative predictive values, 74% and 96%). Among the false-negatives results ($n = 35$), the main histological findings were macronodular cirrhosis and absence of significant inflammation reflecting inactive diseases in patients with chronic hepatitis C previously treated by interferon or with sustained virological response. The authors also suggested that cutoff values could be optimized for each cause, mainly chronic viral hepatitis versus alcoholic or nonalcoholic steatohepatitis. This is presumably attributable to the variation in the amount of fibrosis between various conditions.

In a study including 711 patients with chronic liver diseases of various causes, Foucher et al. [27] established cut-off values for complications of cirrhosis. The suggested cut-off values for the presence of oesophageal varices stage 2/3, cirrhosis Child-Pugh B or C, past history of ascites, hepatocellular carcinoma, and oesophageal bleeding were 27.5, 37.5, 49.1, 53.7, and 62.7 kPa, respectively. In clinical practice, such findings could be relevant for the follow-up of patients with cirrhosis. Moreover, the risk of cirrhosis decompensation could be excluded in patients with liver stiffness measurements < 27 kPa (cut-off value for Child A stage). Liver stiffness measurement may be useful for assessing the severity of cirrhosis, irrespective of the cause of liver disease.

Variceal rupture is the second cause of death in cirrhosis, justifying early screening for oesophageal varices. The risk of variceal haemorrhage is clearly related to the size of oesophageal varices [208, 209]. Therefore primary prevention of variceal bleeding applies to patients with previously diagnosed large oesophageal varices (grade II or III). Periodical upper tract endoscopy in these patients might result in a heavy economical burden [210]. Furthermore repeated examinations, when not performed under general anaesthesia or profound sedation, are often poorly accepted by patients who may refuse further follow-up [211]. For these reasons selection of patients with a high probability of bearing oesophageal varices and especially large varices at risk of rupture has been proposed using various non-invasive criteria [212-216]. There is still a need for a simple reliable parameter that would allow limiting the indications of upper tract endoscopy to a subgroup of patients especially at risk. In a study including 165 patients with cirrhosis, Kazemi et al. [217] found that the areas under the ROC values of liver stiffness measurement were 0.84 (95% CI: 0.78–0.90) for the presence of oesophageal varices and 0.83 (0.76–0.89) for varices grade P II. Liver stiffness measurement value < 19 kPa was highly predictive of the absence of oesophageal varices grade P II (Se: 84%, PPV: 47%, NPV: 93%). Consequently, the authors suggested that stiffness measurements were able to identify a large group of patients having a low probability of bearing varices, limiting therefore the indications of endoscopic screening.

iii. Reproducibility

The reproducibility of FibroScan appears excellent in the literature [28, 201, 203], but should be further assessed in routine clinical practice. In the study by Sandrin et al. [28], the reproducibility was investigated in 15 patients: the intraoperator standardized coefficient of variation was 3.2% (range, 2 – 18%), and the interoperator variation was 3.3%. The

interoperator variation was thus similar to the intraoperator variation. The intra-patient coefficient of variation was 18%. This may be attributed to a heterogeneous distribution of fibrosis in the liver.

In the study by Fraquelli et al. [203] including 200 patients with chronic liver disease, the FibroScan was performed twice by two different operators: the interobserver agreement was 0.98 (95% CI, 0.977 - 0.987) and the intraobserver agreement was 0.98 for both raters.

iv. Technical success rate

Ultrasound elastography with the FibroScan is easy to perform: a short period of training (limited to 50 exams) is sufficient [28, 205]. However, elasticity measurement can be difficult or even impossible to obtain in patients who are obese, have narrow intercostal spaces or have ascites. In a study including 935 patients, Kettaneh et al. [205] showed that the success rate was lower in obese than in lean patients. Elasticity measurements are impossible in patients with ascites (even if clinically undetected) because shear waves do not propagate through liquids [28].

7. Comparisons and combinations of non-invasive tests

Various studies have proposed to use combinations of indirect and direct non-invasive tests. In the study by Castera et al. [200], the combined use of FibroScan and Fibrotest offered the best diagnostic performance both for significant fibrosis ($F \geq 2$) and for severe fibrosis-cirrhosis ($F3 - F4$). The areas under the ROC curves of FibroScan, Fibrotest and the combination of FibroScan and Fibrotest were respectively: for $F \geq 2$, 0.83, 0.85 and 0.88; for $F \geq 3$, 0.90, 0.90 and 0.95; for $F = 4$, 0.95, 0.87 and 0.95. According to these results, they

proposed a clinical management algorithm using the combination of FibroScan and Fibrotest as first-line evaluation of fibrosis. Based on this algorithm, liver biopsy could have been avoided in 140 (77%) of the 183 patients examined for the diagnosis of significant fibrosis. Despite these efforts, available tests even when they are combined distinguish with accuracy only two stages of the fibrosis spectrum: F0/F1 (minimal) and F3/F4 (advanced). Intermediate levels of fibrosis are not reliably detected.

8. The limitations of the assessment of the non-invasive tests

Several authors have suggested that the discrepancies between liver biopsy and non-invasive tests are mainly caused by limitations of liver biopsy and semi-quantitative scoring systems [28, 30]. In the study by Ziol et al. [30], the results showed that the diagnostic performances of elasticity measurements were better in large specimens than in small ones. This suggests that the real diagnostic performance of liver elastography may be underestimated because of the sampling error of biopsy. In the study by Cales et al., the results of the blood tests were also better in patients having biopsy of large hepatic specimens [218].

Moreover, non-invasive tests, which are continuous variables, have been correlated with categorical variables at histology. These categorical variables do not have an arithmetical progression, e.g. in the METAVIR scoring system, the percentage of fibrosis in stage 2 (F2) is neither twice the percentage of stage 1 (F1), nor half the percentage of stage 4 (F4) [5]

Lastly, the scoring systems take into account the degree of distortion of the hepatic architecture as well as the extent of fibrosis, which are not necessarily well correlated.

Patients with cirrhosis may present only thin bundles of fibrotic tissue surrounding the liver nodules. Presumably, liver elasticity is more closely related to the amount of fibrosis than to

the degree of architecture distortion. This point needs to be further evaluated by morphometric studies.

V. MR ELASTOGRAPHY

In collaboration with the Laboratory Ondes et Acoustique (Université Paris Diderot, Paris, France), we have developed and validated a three-dimensional (3D) method of MR elastography of liver. We have assessed the performance of this technique for the detection and staging of liver fibrosis. When compared to transient ultrasound elastography, this new method presents several advantages: the use of compressional waves that permits good penetration throughout the liver, the assessment of the whole three-dimensional displacement vector, and the analysis of a much larger liver volume.

1. Theory

The theory behind the elastography method used has been previously described in detail by Sinkus et al. [197, 198]. Briefly, the propagation of an acoustic wave in a locally homogeneous isotropic viscoelastic medium is described by the following partial differential equation for the total displacement $\vec{u}$:

$$\rho \partial_t^2 \vec{u} = \mu \nabla^2 \vec{u} + (\lambda + \mu) \nabla (\nabla \cdot \vec{u}) + \zeta \partial_t \nabla^2 \vec{u} + (\xi + \zeta) \partial_t \nabla (\nabla \cdot \vec{u}) \quad (1)$$

where ρ is the density of the material, μ the shear modulus, λ the second Lamé coefficient, ζ the shear viscosity accounting for attenuation within the medium and ξ the viscosity of the compressional wave.

To completely remove the contributions from the compressional wave we utilize the curl-operator [25] which leads in steady-state to the following equation of motion:

$$-\rho \omega^2 \vec{q} = (\mu + i\omega\zeta) \nabla^2 \vec{q} \quad , \quad \vec{q} = \nabla \times \vec{u} \quad (2)$$

Since in Eq. (2) all other quantities are measured (or assumed to be known), the method enables estimation of the shear viscoelastic parameters μ and ζ . We, as other authors [219],

used a viscoelastic rather than a linear elastic reconstruction because living tissues do not only have elastic properties but also viscous properties. Ignoring the viscous component easily results in overestimation for the shear modulus because effects originating from viscosity are blamed on the elasticity.

2. Experimental setup

Our setup [220] utilizes the dynamic approach to MR elastography with a continuous mechanical excitation at one frequency leading to a stationary steady-state wave pattern, which appears shortly after the onset of vibration (*Fig. 6*). Low-frequency longitudinal mechanical waves of 65 Hz are thus transmitted into the right liver by a transducer (length: 17.4 cm; width: 12.2 cm; height: 7.3 cm; piston diameter: 6.5 cm) that is placed against the last ribs at the back of the patient in supine position and that contains a coil in which an oscillating current is fed. In the static magnetic field, the Lorentz force induce a torque causing longitudinal oscillations of the piston. The desired shear waves are obtained through mode conversion at interfaces. The technique to excite with longitudinal waves is a way to overcome the strong attenuation of shear waves, because attenuation of compressional waves at low frequencies is negligible. Moreover, it has already been shown that the transcostal approach used in our setup yields better shear waves in the liver than the subcostal approach and that similar results of elasticity are obtained with both approaches [221]. It seems likely that the driver vibrates the entire rib cage and that ribs behave as independent in-phase actuators that create, according to the Huygen principle, a flat wave front in the liver. Images are obtained on a 1.5-T whole-body MR scanner (Gyrosan Intera; Philips Medical Systems, Best, the Netherlands) using a four elements synergy body coil. The MR elastography pulse sequence is a motion-sensitized spin-echo sequence with sinusoidal

displacement encoding gradients, phase-locked to the mechanical excitation. The pulse generator for the transducer is triggered via the standard trigger output channel of the spectrometer. Five slices are acquired with a slice thickness of 4 mm. The field of view is 250 mm and the matrix size 64^2 leading to an isotropic voxel size of $4 \times 4 \times 4 \text{ mm}^3$. The echo time and the repetition time (61/431 ms) are chosen as integer multiples of the basic period of the mechanical wave. Thereby, this sequence measures the total displacement field along a certain direction at one instance of the oscillatory cycle. The number of signals averaged is two and four dynamics are obtained by changing the phase offset between the mechanical excitation and the MR sequence to assess the amplitude and phase of the displacement (after Fourier transformation). The scan is repeated twice to obtain the other two orthogonal components of the 3D displacement vector. The phase maps are transformed into shear elasticity and viscosity maps, according to Eq. (2). Viscoelastic maps corresponding to the three central slices are obtained. The central slice is systematically chosen to obtain the most accurate results. The largest rectangular region of interest that fitted into the liver was then placed on the central slice. The shear elasticity (kPa) and viscosity (Pa.s) of the liver were measured as the mean value within this large region of interest on the elasticity and viscosity maps. The intra-subject heterogeneity of the liver elasticity and viscosity was measured as the standard deviation of the measurements within the large region of interest on the elasticity and viscosity maps for each patient. The inter-subject heterogeneity of liver elasticity and viscosity was calculated as the standard deviation of the mean elasticity and viscosity measurements for each fibrosis stage.

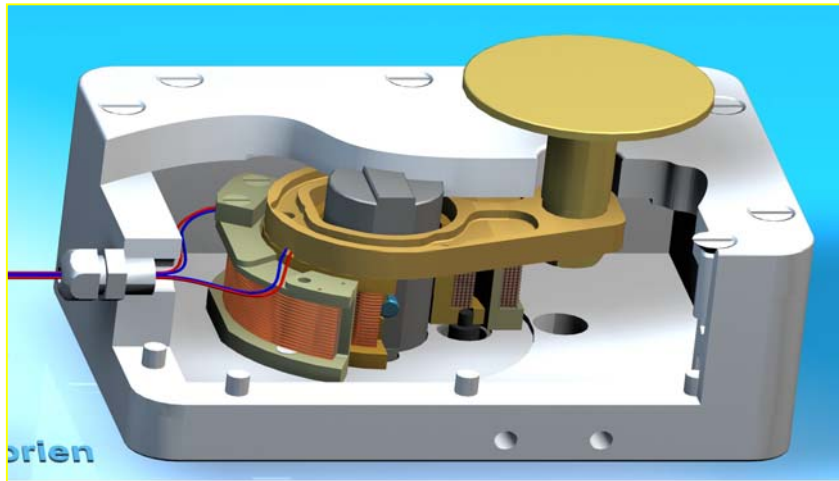


Figure 6. *Low-frequency longitudinal mechanical waves of 65 Hz are thus transmitted into the right liver by a transducer (length: 17.4 cm; width: 12.2 cm; height: 7.3 cm; piston diameter: 6.5 cm) that is placed against the last ribs at the back of the patient in supine position and that contains a coil in which an oscillating current is fed. In the static magnetic field, the Lorentz force induce a torque causing longitudinal oscillations of the piston (From: Philips Medical Systems, Best, The Netherlands).*

3. First study: preliminary study

We validated the feasibility of MR elastography [220]:

- first, by testing it on a phantom,
- second, by determining normal liver elasticity and viscosity values and by evaluating the reproducibility of MR elastography in healthy volunteers,
- third, by assessing MR elastography in patients with liver fibrosis.

3.1. Phantom

MR elastography was tested on a cube-shaped box (15 cm³) filled with agarose gel to show the ability to obtain uniform elasticity and viscosity measurements. Homogeneous elasticity and viscosity maps were observed in the phantom (*Fig. 7*). The mean shear elasticity and its standard deviation within the phantom were 1.50 ± 0.04 kPa. The corresponding figure for the shear viscosity were 0.49 ± 0.08 Pa·s [220].

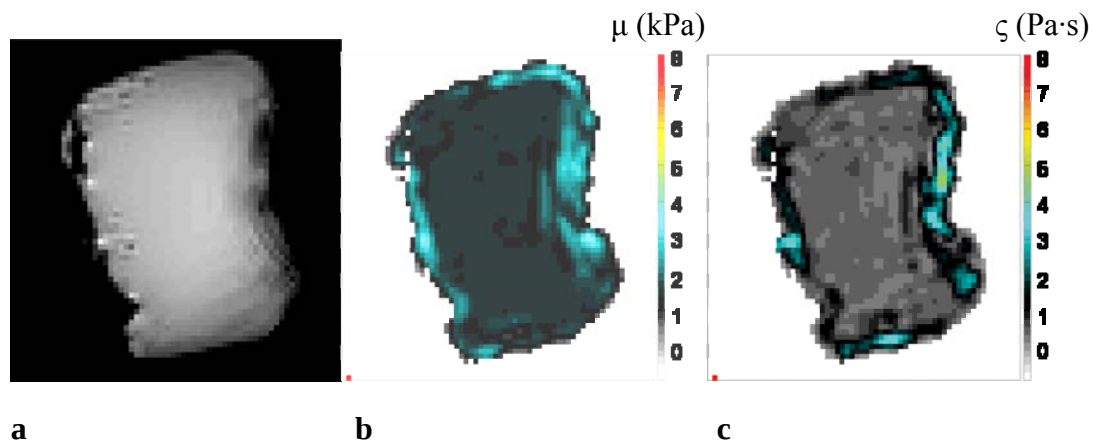


Figure 7. Reconstructed images of the central slice of a phantom at MR elastography. The magnitude image (**a**), and the corresponding elasticity (μ expressed in kPa) (**b**) and viscosity (ζ expressed in Pa·s) maps (**c**) show the homogeneity of the phantom (From: Huwart L, et al. Liver fibrosis: non-invasive assessment with MR elastography. *NMR Biomed* 2006)

3.2. Subjects

The MR elastography examinations could be performed in all the patients and the volunteers, which is explained by the adequate penetration of the longitudinal waves throughout the liver (*Fig. 8*).

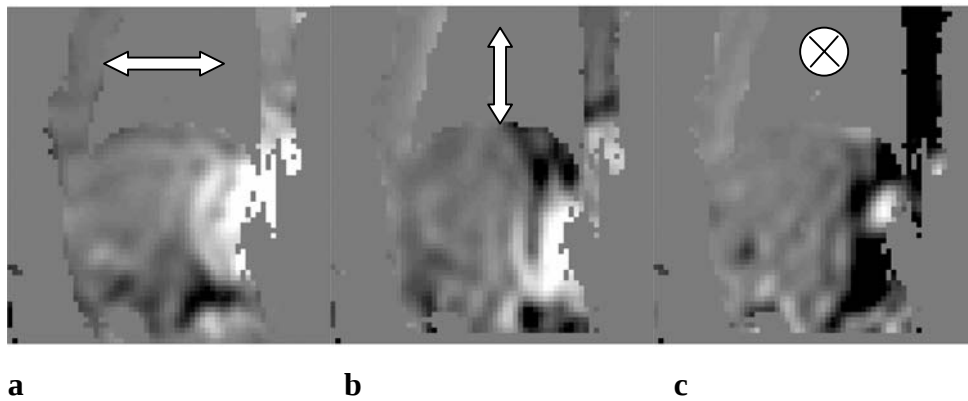


Figure 8. *The reconstructed phase images (one dynamic) for the central slice of a patient with F0 histology show good wave penetration throughout the liver at MR elastography. **a:** x-wave (corresponding to the phase-encoding direction); **b:** y-wave (corresponding to the frequency-encoding direction); **c:** z-wave (corresponding to the slice-selection direction) (From: Huwart L. et al. Liver fibrosis: non-invasive assessment with MR elastography. *NMR Biomed* 2006).*

a. Healthy volunteers

Five healthy adult volunteers were imaged once a day on three different days to assess the reproducibility of the method. The healthy volunteers had a mean shear elasticity of 2.06 ± 0.26 kPa and a mean shear viscosity of 1.72 ± 0.15 Pa·s. The within-subject coefficients of variation of the elasticity and viscosity were respectively 9% and 7%, showing a good reproducibility of the method for both elasticity and viscosity.

b. Patients

The patient group included 25 consecutive adult patients who had liver biopsy for suspicion of chronic liver disease. Two patients had ascites diagnosed by imaging. The cause of chronic liver disease was viral in 17 patients (hepatitis B in 1 patient and hepatitis C in 16 patients), alcoholic in 4, unknown in 3 and autoimmune in 1 patient.

i. Histological analysis

Percutaneous liver biopsy was performed by senior hepatologist using the Menghini technique with a 1.4-mm diameter needle (Hepafix[®]; Braun, Melsungen, Germany). In patients with ascites and/or trouble of blood coagulation, liver biopsy was performed through a transjugular approach using a catheter needle (Cook[®], Bjaeverskov, Denmark). After biopsy, the liver samples were fixed in formalin for 24 hours, paraffin-embedded and stained with hematoxylin-eosin and Masson's trichrome. All biopsy specimens were analysed by a senior hepatopathologist blinded to the results of MR elastography and to the biological

and clinical data. The stage of fibrosis was evaluated semiquantitatively according to the METAVIR scoring system [116, 222]. All liver samples contained at least 10 portal spaces or obvious regenerating nodules. The length of the liver biopsies was 25.8 ± 12.8 mm (mean $\pm$ standard deviation). The METAVIR score was F0 in 9 patients, F1 in 2, F2 in 3, F3 in 1, and F4 in 10.

ii. Measurements with MR elastography

It was observed that the visco-elastic maps of the liver became more heterogeneous with increasing fibrosis. This is in agreement with the known variability in the distribution of fibrosis within the liver [5]. Figures 9 and 10 show the magnitude image, shear elasticity and viscosity maps of a patient with F0 histology and a patient with cirrhosis (F4).

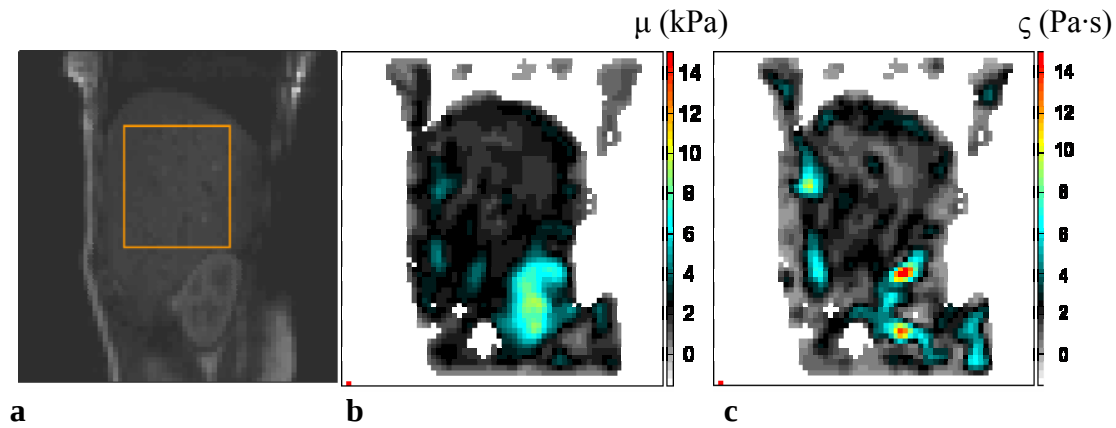


Figure 9. Reconstructed images of the central slice of a patient with F0 histology at MR elastography. The magnitude image (a) shows the region of interest used for the assessment of the mean elasticity and viscosity. The corresponding elasticity (b) and viscosity maps (c) show the homogeneity of liver in a patient with F0 histology (From: Huwart L. et al. Liver fibrosis: non-invasive assessment with MR elastography. *NMR Biomed* 2006).

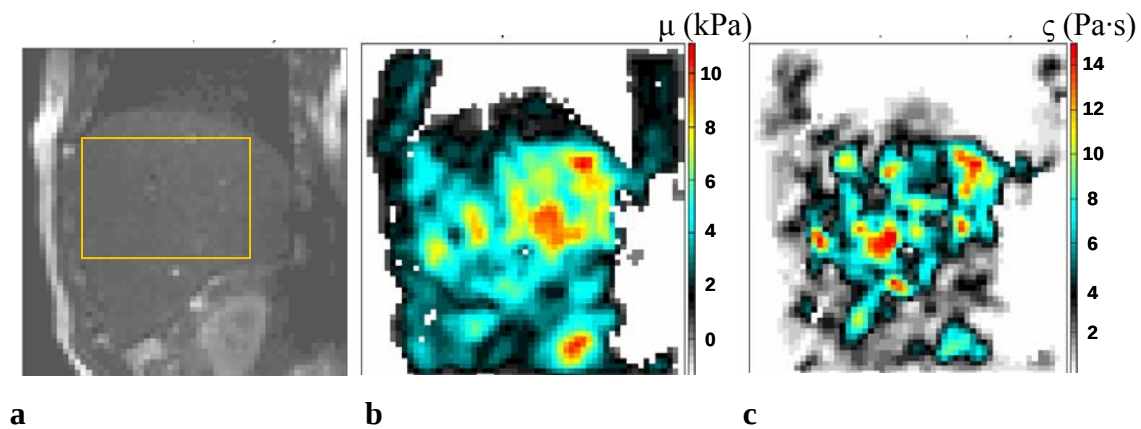


Figure 10. Reconstructed images of the central slice of a patient with cirrhosis at MR elastography. The magnitude image (a) shows the region of interest used for the assessment of the mean elasticity and viscosity. The corresponding elasticity (b) and viscosity maps (c) show the heterogeneity of liver fibrosis (From: Huwart L. et al. Liver fibrosis: non-invasive assessment with MR elastography. *NMR Biomed* 2006).

The shear viscoelastic parameters of the liver increased according to the stage of liver fibrosis. The patients without substantial fibrosis (F0 - F1) had a mean shear elasticity of 2.24 ± 0.23 kPa, the patients with substantial fibrosis (F2 - F3) had a mean elasticity of 2.56 ± 0.24 kPa, and the patients with cirrhosis (F4) had a mean elasticity of 4.68 ± 1.61 kPa. The mean shear viscosity was 2.39 ± 0.86 Pa·s in the patients without substantial fibrosis, 2.27 ± 0.38 Pa·s in the patients with substantial fibrosis, and 5.19 ± 1.85 Pa·s in the patients with cirrhosis. There was a statistically significant difference of elasticity between the patients with F0 - F1 fibrosis versus F2 - F3 ($p = 0.05$), F2 - F3 versus F4 ($p = 0.0162$), and F0 - F1 versus F4 ($p = 0.0001$). There was also a statistically significant difference of viscosity between the patients with F2 - F3 fibrosis versus F4 ($p = 0.0072$), and F0 - F1 versus F4 ($p = 0.0006$). However, no significant difference of viscosity was observed between the patients with F0 - F1 fibrosis versus F2 - F3 ($p = 0.6953$). In contrast to viscosity measurements, the elasticity measurements thus separated the patients with substantial fibrosis ($\geq F2$) from the patients without it.

4. Second study: clinical study

After the validation of the feasibility of MR elastography, we assessed MR elastography for the staging of liver fibrosis in a cohort of patients with suspicion of chronic liver disease, using histology as the reference standard [223].

4.1. Patients

Ninety-six consecutive adult patients who had liver biopsy in the department of gastroenterology of St-Luc University Hospital for suspicion of chronic liver disease were

prospectively included in the study. MR elastography was performed within two days of liver biopsy. After the inclusion, three patients were dropped from the study because of claustrophobia during MR elastography and five patients were excluded because the liver biopsies were unsuitable for fibrosis staging. Thus, the final study group was composed of 88 patients. Thirteen patients had ascites diagnosed by imaging. The cause of chronic liver disease was viral in 69 (chronic hepatitis C in 66 and chronic hepatitis B in 3), alcohol abuse in 10, autoimmune disease in 2 (autoimmune hepatitis in 1 and primary biliary cirrhosis in 1), α_1 -antitrypsin deficiency in 1, and unclassified in 6 patients.

4.2. Histological analysis

The length of the liver biopsies was 34.5 ± 10.5 mm (mean $\pm$ standard deviation). Twenty-two out of 88 patients (25%) had a Metavir score F0, 13 (15%) had F1, 15/88 (17%) F2, 14/88 (16%) F3, and 24/88 (27%) F4.

4.3. Measurements with MR elastography and comparison with APRI

The elasticity, the viscosity, and the intra-subject heterogeneity of elasticity and viscosity increased according to the stage of liver fibrosis. The measurements were correlated to the fibrosis stage: $r = 0.86$, $P < .001$ for elasticity, $r = 0.75$, $P < .001$ for viscosity, $r = 0.70$, $P < .001$ for intra-subject heterogeneity of elasticity, $r = 0.68$, $P < .001$ for intra-subject heterogeneity of viscosity.

For each threshold, the area under the ROC of elasticity was larger than that of the other measurements. It was significantly larger than that of APRI for each threshold ($P = .003$ for $F \geq 1$, $P < .001$ for $F \geq 2$, $P = .003$ for $F \geq 3$, and $P = .004$ for $F = 4$) and than those of intra-

subject heterogeneity of elasticity, viscosity, and intra-subject heterogeneity of viscosity for $F \geq 1$ ($P = .001$, $P = .004$ and $P = .001$ respectively) and for $F \geq 2$ ($P \leq .001$).

The most discriminant cut-off values of elasticity were 2.4 kPa for $F \geq 1$, 2.5 kPa for $F \geq 2$, 3.1 kPa for $F \geq 3$, and 4.3 kPa for $F = 4$. The sensitivities, specificities, positive and negative predictive values obtained with the optimized cut-off values are detailed in Table 2.

The elasticity measurements allowed to clearly separate the intermediate fibrosis stages. This high accuracy is clinically important because:

- According to the American Association for the Study of Liver Diseases, patients with hepatitis C genotype-1 infection should be treated only when substantial fibrosis ($\geq F2$) is observed [12, 224].
- Patients with advanced fibrosis and cirrhosis should be screened for portal hypertension and hepatocellular carcinoma [213, 225, 226].

The viscosity measurements were less accurate than the elasticity measurements to stage liver fibrosis. Even if living tissues have both elastic and viscous properties and a visco-elastic model seems thus to be more appropriate than a single elastic one, the mathematical model used to calculate the viscoelastic parameters should be discussed. We used the so-called Voigt (or Kelvin) model, in which shear elasticity and shear viscosity are considered to be independent. However, a more detailed analysis reveals that these two parameters are related by the so-called Kramers-Kronig relations [227]. Further studies remain to be done to show if an improved model taking these relations into account gives more accurate results of elasticity and viscosity.

It was also observed that the intra-subject heterogeneity of elasticity and viscosity measurements increased with the fibrosis stage. However, the clinical relevance of the intra-subject heterogeneity of elasticity and viscosity appeared to be limited, because the mean elasticity measurements alone had a high accuracy.

Higher correlations were observed between the visco-elastic parameters and the exponential function of the METAVIR score: $r = 0.94$, $P < .001$ for elasticity, $r = 0.85$, $P < .001$ for viscosity, $r = 0.79$, $P < .001$ for intra-subject heterogeneity of elasticity, $r = 0.77$, $P < .001$ for intra-subject heterogeneity of viscosity. The correlation coefficients with the exponential function of the METAVIR score were significantly better than with the non transformed METAVIR score ($P \leq .006$). This observation reflects the exponential accumulation of fibrous tissue within the liver as showed by studies using morphometry [5].

Patients with cirrhosis (F4) were also clearly identified in our study. They had a large range of shear elasticity and viscosity values (4.68 ± 1.61 kPa and 5.19 ± 1.85 Pa·s). The inter-subject heterogeneity of elasticity for cirrhosis (F4) was very large. This may be explained by the variable macroscopic pattern of cirrhosis that may appear as micronodular, macronodular or incomplete septal cirrhosis. Moreover, the inter-subject heterogeneity of elasticity in cirrhosis may be related to the severity of the disease as showed in studies using ultrasound elastography [27, 204, 217].

Table 2. *Most discriminant elasticity cut-off values (kPa) and corresponding sensitivities, specificities, positive and negative predictive values with 95% confidence intervals in parentheses for METAVIR scores $F \geq 1$, $F \geq 2$, $F \geq 3$ and $F = 4$.*

	$F \geq 1$	$F \geq 2$	$F \geq 3$	$F = 4$
Elasticity cut-off (kPa)	2.4	2.5	3.1	4.3
Sensitivity	0.83 (0.72 - 0.91)	0.98 (0.90 - 1.00)	0.95 (0.82 - 0.99)	1.00 (0.86 - 1.00)
Specificity	1.00 (0.85 - 1.00)	1.00 (0.90 - 1.00)	1.00 (0.93 - 1.00)	1.00 (0.94 - 1.00)
Positive predictive value	1.00 (0.94 - 1.00)	1.00 (0.93 - 1.00)	1.00 (0.90 - 1.00)	1.00 (0.86 - 1.00)
Negative predictive value	0.67 (0.48 - 0.92)	0.97 (0.85 - 1.00)	0.96 (0.87 - 1.00)	1.00 (0.94 - 1.00)

5. Third study: comparison with APRI and ultrasound elastography

The results of our previous study showed that MR elastography was an accurate method to stage liver fibrosis. Therefore, we wanted first to further compare this emerging method with other non-invasive tests - APRI and FibroScan -; and second, to assess the reproducibility of MR elastography. Assessment of reproducibility is important to evaluate the potential of MR elastography in the follow-up of patients.

The third study was a single-centre, prospective, blind comparison of MR elastography, ultrasound elastography, and APRI in a consecutive series of patients who had liver biopsy in the department of gastroenterology of St-Luc University Hospital, Université Catholique de Louvain for chronic liver disease. MR elastography, ultrasound elastography and APRI measurements were performed within two days of liver biopsy.

To assess the reproducibility of MR and ultrasound elastography, these examinations were repeated within one month in all patients in whom MR elastography, ultrasound elastography, APRI measurements and analysis of liver biopsy were technically successful and who accepted to have repeated examinations.

5.1. Patients

One hundred and forty-six patients had liver biopsy for chronic liver disease. Five patients refused to participate and 141 patients entered the study. Ultrasound elastography could be performed in $118/141 = 84\%$ of the patients. Ultrasound elastography was unsuccessful in 13 patients with ascites. Ten other failures were caused by obesity. Magnetic resonance elastography could be performed in $133/141 = 94\%$ of the patients ($P = 0.016$ versus ultrasound elastography). The eight failures were caused by claustrophobia in three patients,

obesity in two, and low hepatic signal related to hemochromatosis in three patients.

Measurements of APRI were obtained in $141/141 = 100\%$ of the patients. Liver biopsies were considered suitable for fibrosis staging (i.e. contained at least 10 portal tracts or obvious regenerating nodules) in $127/141 = 90\%$ of the patients.

Further analysis was performed in the 96 patients in whom MR elastography, ultrasound elastography, APRI measurements and histological analysis were successful. There were 45 men and 51 women. The cause of chronic liver disease was chronic viral hepatitis in 65 patients (chronic hepatitis C in 60 and chronic hepatitis B in 5), alcohol abuse in 14, non-alcoholic steatohepatitis in 8, α_1 -antitrypsin deficiency in 1, drug toxicity in 2, and remained unknown in 6 patients.

5.2. Measurements with MR and US elastography

a. MR elastography

Only the shear elasticity (kPa) of the liver was measured as the mean value within the largest rectangular region of interest that fitted into the liver on the elasticity map of the central slice.

b. US elastography

One-dimensional transient ultrasound elastography measurements were performed with a FibroScan (EchoSens, Paris, France). This method measures the velocity of the shear wave, which is directly related to Young's elastic modulus (kPa). It should be noted that, within tissues, the Young's modulus equals three times the shear elasticity modulus measured with the three-dimensional MR elastographic method.

5.3. Histological analysis

The length of the liver biopsies was 30 ± 11 mm (range, 12 – 66 mm) and was ≥ 25 mm in 70/96 (73%) of the patients. The distribution of fibrosis stage at the consensus reading was F0 in 22/96 (23%) of the patients, F1 in 22 (23%), F2 in 19 (20%), F3 in 15 (15%) and F4 in 18 (19%). The two pathologists were initially in agreement for 81 of the 96 liver biopsy specimens analyzed (weighted kappa coefficient, 0.90; 95% confidence interval, 0.84-0.95) with no significant rating bias. Portal inflammation was graded P0 in 17/96 (17%) of the patients, P1 in 43 (45%), P2 in 35 (37%), P3 in 1 (1%) and P4 in 0 (0%). Lobular inflammation was graded L0 in 32/96 (33%) of the patients, L1 in 34 (35%), L2 in 30 (32%), L3 in 0 (0%) and L4 in 0 (0%). Steatosis was graded 0 in 40/96 (42%) of the patients, 1 in 32 (34%), 2 in 18 (19%) and 3 in 6 (5%).

5.4. Influence of inflammatory activity and steatosis

In three-way analysis of variance exploring the possible effect of fibrosis, portal and/or lobular inflammation and steatosis on MR elasticity, fibrosis was the only factor significantly affecting MR elasticity ($P < 0.001$). In addition, no significant interaction was found.

5.5. Reproducibility

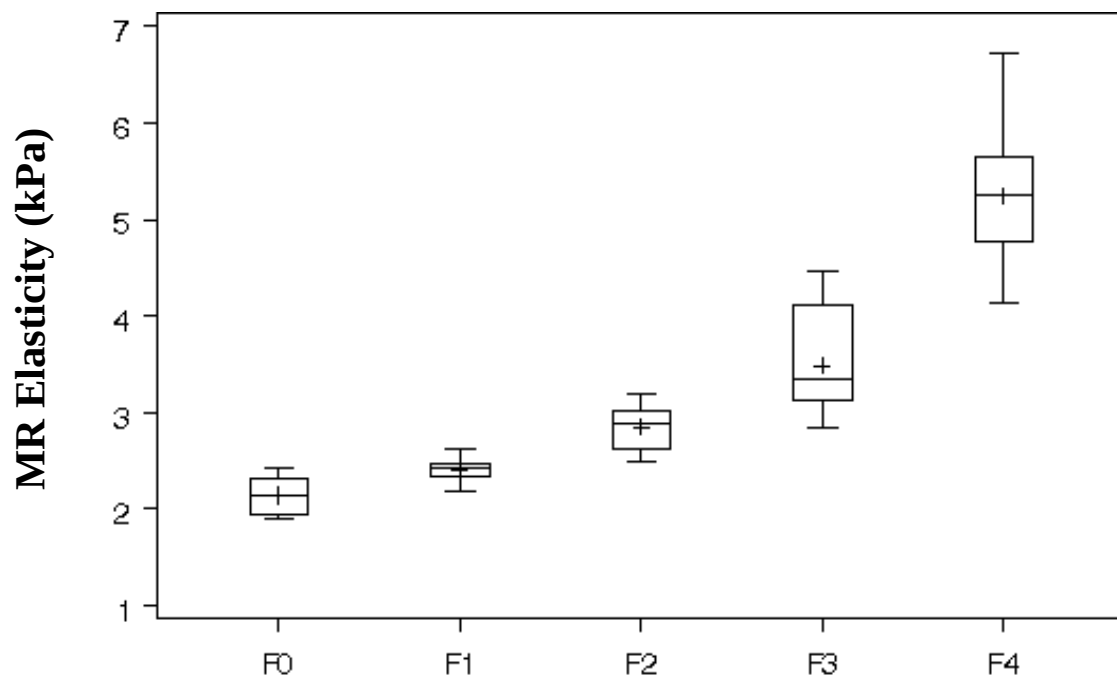
The reproducibility of MR and ultrasound elastography could be assessed in 56 patients. The intraclass correlation coefficients of MR elasticity and ultrasound elasticity were respectively 0.97 (95% confidence interval, 0.92 - 0.99) and 0.94 (95% confidence interval, 0.51 – 0.97).

The 95% confidence intervals of the inter-observer differences of measurements were (- 0.016 - + 0.088) for MR elasticity and (0.028 – 2.192) for ultrasound elasticity. The coefficients of repeatability were 0.385 for MR elastography and 8.149 for ultrasound elastography.

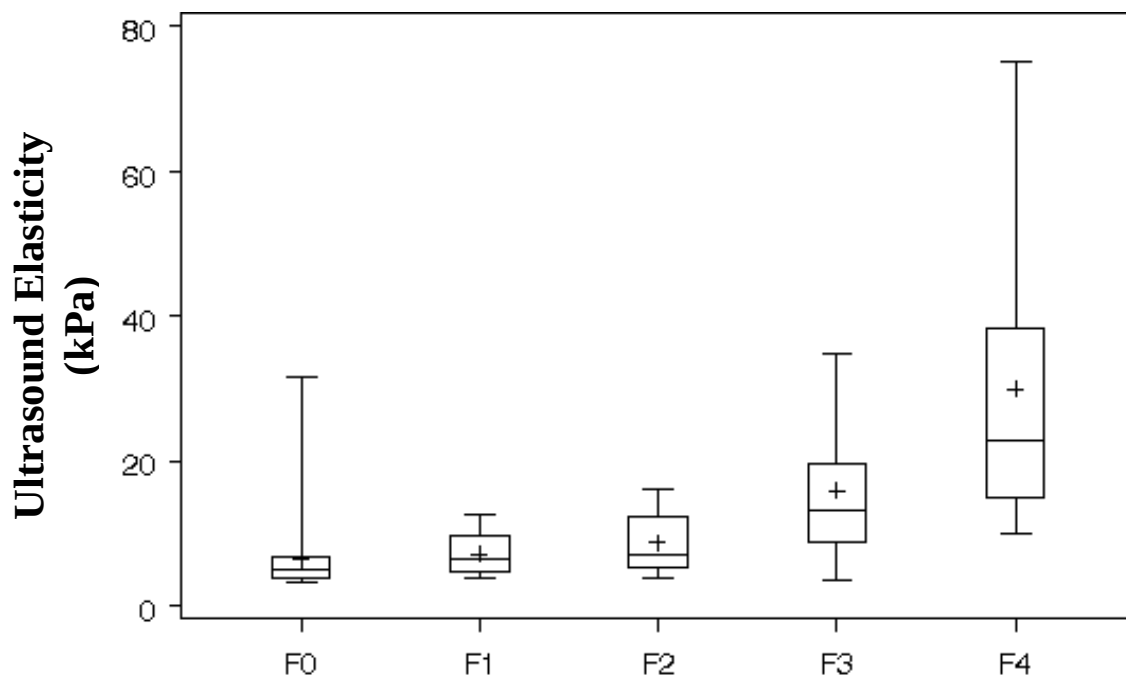
5.6. Comparison with APRI and US elastography

In the 96 patients in whom elastography, APRI measurements and histological analysis were successful, MR elasticity, ultrasound elasticity, and APRI increased according to the stage of liver fibrosis (*Fig. 11*). The measurements were correlated to the fibrosis stage: $r = 0.84$, $P < 0.0001$ for MR elasticity; $r = 0.56$, $p < 0.0001$ for ultrasound elasticity; and $r = 0.36$, $P < 0.0001$ for APRI.

Table 3 shows the areas under the ROC curves of MR elasticity, ultrasound elasticity, and APRI, and the combinations of the non-invasive methods for the different fibrosis thresholds: F0 versus F1 - F4 ($F \geq 1$), F0 - F1 versus F2 - F4 ($F \geq 2$), F0 - F2 versus F3 - F4 ($F \geq 3$), and F0 - F3 versus F4 ($F = 4$). The areas under the ROC curve of MR elastography were significantly larger than those of ultrasound elastography, APRI, and the combination of ultrasound elastography and APRI ($P = 0.003$, < 0.0001 and 0.005 for $F \geq 1$; $P = 0.0001$, < 0.0001 and 0.0002 for $F \geq 2$; $P = 0.01$, 0.0005 and 0.02 for $F \geq 3$; and $P = 0.01$, 0.008 and 0.04 for $F = 4$) (*Fig. 12*). Moreover, the areas under the ROC curves of MR elastography were not significantly different from those of the combinations including MR elastography, i.e. MR elastography and ultrasound elastography, MR elastography and APRI, and MR elastography, ultrasound elastography and APRI ($P > 0.05$).



a. METAVIR Score



b. METAVIR Score

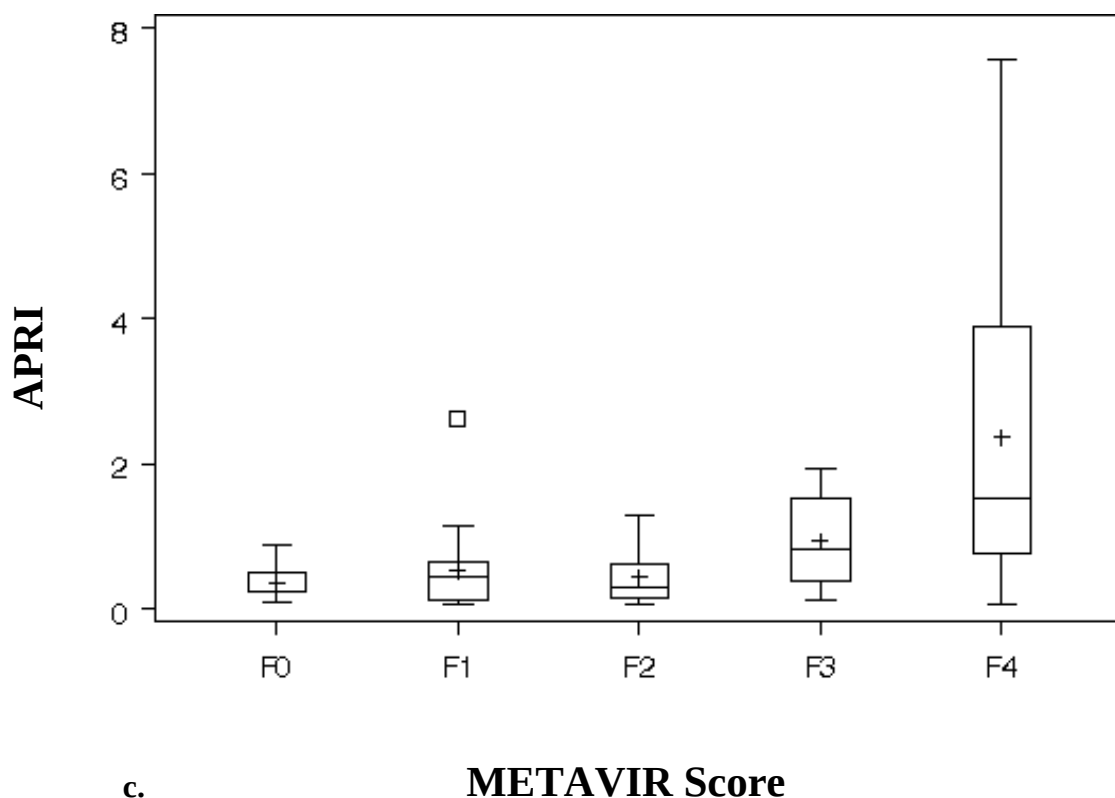


Figure 11. Box plots of MR elasticity (**a**), ultrasound elasticity (**b**), and APRI (**c**) for each METAVIR fibrosis stage. Boundary of boxes closest to zero indicates 25th percentile, line within boxes shows median and boundary of boxes furthest from zero indicates 75th percentile. The crosses within boxes indicate the mean. Error bars indicate the smallest and the largest values that are within 1.5 box-lengths of 25th and 75th percentiles. Outliers are represented as individual points. In (**c**), one outlier has not been represented in the F4 group to maintain the clarity of the graph (From: Huwart L. et al. Magnetic resonance elastography for the non-invasive staging of liver fibrosis. *Submitted to Gastroenterology* 2007).

Table 3. Areas under ROC curves with 95% confidence intervals in parentheses for MR elastography, ultrasound elastography, APRI, and combinations of methods according to METAVIR stages.

	$F \geq 1$	$F \geq 2$	$F \geq 3$	$F = 4$
MR elastography	0.962 (0.929-0.995)	0.994 (0.985-1.0)	0.985 (0.968-1.0)	0.998 (0.993-1.0)
Ultrasound elastography	0.803 (0.701-0.904)	0.837 (0.756-0.918)	0.906 (0.838-0.975)	0.930 (0.877-0.982)
APRI	0.676 (0.565-0.787)	0.709 (0.603-0.814)	0.816 (0.717-0.915)	0.820 (0.688-0.952)
MR elastography and ultrasound elastography	0.961 (0.928-0.995)	0.993 (0.984-1.0)	0.985 (0.966-1.0)	0.997 (0.991-1.0)
MR elastography and APRI	0.959 (0.923-0.995)	0.992 (0.982-1.0)	0.989 (0.972-1.0)	0.998 (0.995-1.0)
Ultrasound elastography and APRI	0.814 (0.714-0.915)	0.849 (0.772-0.926)	0.936 (0.884-0.988)	0.944 (0.891-0.997)
MR elastography, ultrasound elastography and APRI	0.963 (0.930-0.996)	0.992 (0.981-1.0)	0.988 (0.970-1.0)	0.999 (0.995-1.0)

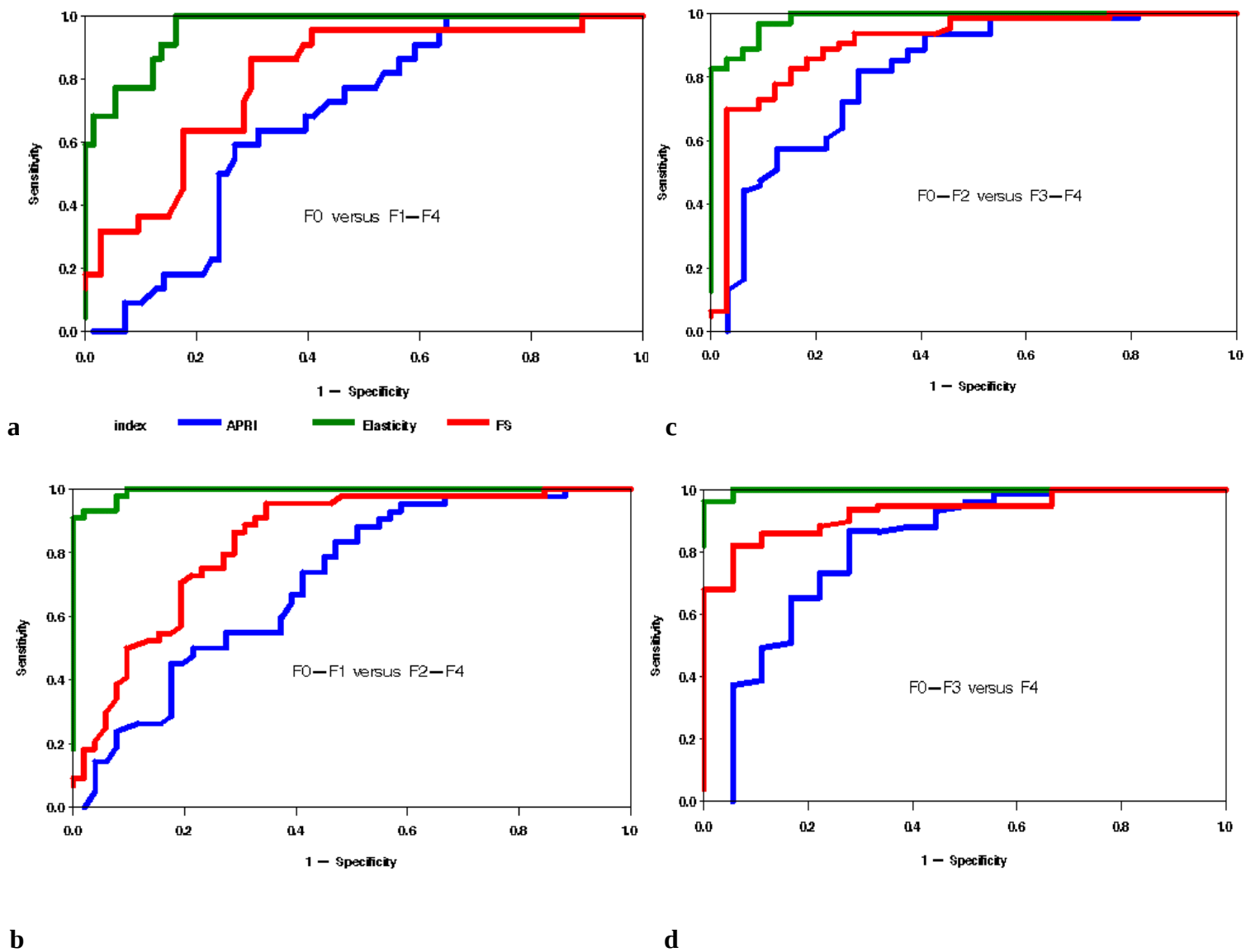


Figure 12. ROC curves for MR elasticity (green), US elasticity (red) and APRI (blue) at METAVIR fibrosis scores thresholds of (a) $F \geq 1$, (b) $F \geq 2$, (c) $F \geq 3$ and (d) $F = 4$ (From: Huwart L. et al. Magnetic resonance elastography for the non-invasive staging of liver fibrosis. *Submitted to Gastroenterology*. 2007).

The APRI index was measured because of its simplicity and its fair effectiveness. However, APRI was less reliable than the elasticity measurements to stage liver fibrosis. These results are in agreement with previous studies showing that the serum scoring systems accurately distinguish only two stages of the fibrosis spectrum, namely minimal and advanced fibrosis. These scoring systems are less effective for differentiating intermediate fibrosis stages.

Compared to ultrasound elastography, The MR elastography method is also superior to ultrasound elastography for staging liver fibrosis. As already stated above, this superiority can be explained by several reasons. First, MR elastography allows the whole three-dimensional displacement vector to be evaluated. This improves the reconstruction of the shear viscoelastic parameters of the liver relative to ultrasound elastography that is a one-dimensional method.

Second, the elasticity of one or several sections of the liver is examined with MR elastography whereas transient ultrasound elastography analyses a hepatic sample approximated by a 40-mm length cylinder [28, 30]. The volume assessed with MR elastography is thus far more representative of the hepatic parenchyma. This is important to avoid the sampling variability caused by the heterogeneity of advanced fibrosis.

Third, liver elasticity can be assessed with MR elastography in patients who are obese or have ascites because the compressional waves have good penetration throughout the liver. The only practical problem for obese patients is that the patients should fit into the magnet bore, which diameter is reduced by the presence of the transducer. In contrast, the penetration of the shear waves used with ultrasound elastography is poor in obese patients [205, 207]. Foucher et al. showed that a body mass index greater than 28 kg/m^2 was a cause of failure of ultrasound elastography [207]. Moreover, as explained by Sandrin et al. [28], elasticity measurements with ultrasound elastography can not be performed in patients with ascites. This may be a limitation to study the severity of cirrhosis or to predict complications of cirrhosis [27].

Moreover, MR elastography can be integrated into a more complete hepatic MR imaging examination including morphological and perfusion imaging for the detection of hepatocellular carcinomas and steatosis, and the assessment of liver function [221]. In contrast, transient ultrasound elastography of the liver is performed with a dedicated machine that permits to obtain elasticity measurements.

MR elastography has some disadvantages. It can not be performed in livers with high iron overload because of signal to noise limitations. The examination time of MR elastography is longer than that of ultrasound elastography. The future replacement of spin-echo acquisition sequences by echo-planar sequences should allow obtaining much faster acquisition times at MR elastography. The use of fast imaging has already been proposed in previous pilot MR elastography studies. Single-shot echo-planar sequences have been used for MR elastography of the brain [228] and fast spoiled gradient-echo and balanced steady-state free precession sequences have been used for MR elastography of the liver [221, 229, 230]. Lastly, the cost of MR elastography is higher than that of ultrasound elastography. However, the accuracy of MR elastography being higher than that of ultrasound elastography, it would be interesting to compare the patient outcome efficacy and the cost-effectiveness of the two methods.

In conclusion, the results of this study showed that MR elastography is superior to ultrasound elastography and APRI measurements for staging liver fibrosis. This suggests that MR elastography should be the preferred non-invasive method for accurate assessment and follow-up of liver fibrosis.

VI. CONCLUSIONS AND PERSPECTIVES

The results of our research show that MR elastography emerges among the non-invasive tests, as an examination with superior accuracy for staging liver fibrosis. This is explained because 3D MR elastography allows for a direct assessment of liver fibrosis, in contrast to indirect blood tests, and permits a more elaborate assessment of the visco-elastic properties of the liver relative to 1D transient ultrasound elastography.

Several additional conditions are needed for the routine clinical use of MR elastography:

- The improvement of the modelling of shear elasticity and viscosity.
- The validation of the results of MR elastography in other centers.
- The integration in a regular hepatic MR protocol: as for diffusion weighted imaging, MR elastography should in the future be nothing else than a push-button and rapid sequence. The replacement of the spin-echo sequence by an echo-planar sequence is a major step in this direction.
- The acceptance by the clinicians: in contrast to what has been reported with 1D transient ultrasound elastography, 3D MR elastography provides standardized cut-off values of elasticity between fibrosis stages, improving the clinical usefulness.

To fulfill these specifications, we will initiate multicentric studies.

Despite the superiority of MR elastography relative to ultrasound elastography, ultrasound elastography remains a first-line examination to screen for liver fibrosis because of its easiness to perform and its low cost. MR elastography should be used as a second line examination when discrepant results are suspected or when MRI of the liver is needed (e.g. for the detection of hepatocellular carcinomas).

Moreover, the detection and staging of liver fibrosis is an evolving topic. The diagnostic methods are continuously improved. For ultrasound elastography, new 2D methods are

created. Using ultrasonic focused beams, it is possible to remotely generate mechanical vibration sources radiating low-frequency, shear waves inside tissues. Relying on this concept, supersonic shear imaging creates such a source and makes it move at a supersonic speed. In analogy with the "sonic boom" created by a supersonic aircraft, the resulting shear waves interfere constructively along a Mach cone, creating two intense plane shear waves. These waves propagate through the medium and are progressively distorted by tissue heterogeneities. An ultrafast scanner is able to both generate this supersonic source and image (5000 frames/s) the propagation of the resulting shear waves. Using inversion algorithms, the shear elasticity of medium can be mapped quantitatively from this propagation movie [231, 232]. Moreover, with this method, ultrasound elastography can be combined with anatomical ultrasound, allowing for the detection of hepatocellular carcinoma and steatosis.

Biochemical tests are also evolving by the use of proteomics and glycomics, which provide a large-scale survey of up to several hundred (glyco)proteins from a single sample [150, 151, 233, 234]. Proteome-based screening has potential not only as an improved test of fibrogenesis but also for new insight into the pathobiology of fibrosis.

Fibrogenesis can be further studied in vivo with molecular imaging. Preliminary studies in animals have shown that proteins and enzymes that are upregulated (such as matrix metalloproteinases or membranous PDGFR beta receptors in activated stellate cells during fibrogenesis) can be targeted with activable fluorescent, isotopic and magnetic probes for optical imaging, SPECT and MRI [235, 236].

This picture of the evolution in the diagnosis of liver fibrosis would be not complete without speaking about the advances in histological examination. Proteomic analysis can be performed on biopsy samples to stage liver fibrosis and detect impairment of key mitochondrial processes including fatty acid oxidation and oxidative phosphorylation, and

response to oxidative stress and reactive oxygen species that occur during advanced fibrosis in hepatitis C [237].

All these progresses will improve the understanding of fibrogenesis and lead to the development of accurate, reproducible, non-invasive tests for detecting and grading liver fibrosis. These tests are urgently needed not only to diagnose liver fibrosis, but also to assess the response to the variety of novel antifibrotic treatments that are developed.

VII. REFERENCES

1. Wong LL, McFall P, Wong LM. The cost of dying of end-stage liver disease. *Arch Intern Med* **1997**;157:1429-1432
2. Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest* **2005**;115:209-218
3. Bravo AA, Sheth SG, Chopra S. Liver biopsy. *N Engl J Med* **2001**;344:495-500
4. Bataller R, North KE, Brenner DA. Genetic polymorphisms and the progression of liver fibrosis: a critical appraisal. *Hepatology* **2003**;37:493-503
5. Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* **2003**;38:1449-1457
6. Colloredo G, Guido M, Sonzogni A, Leandro G. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the smaller the sample, the milder the disease. *J Hepatol* **2003**;39:239-244
7. Maharaj B, Maharaj RJ, Leary WP, et al. Sampling variability and its influence on the diagnostic yield of percutaneous needle biopsy of the liver. *Lancet* **1986**;1:523-525
8. Ratziu V, Charlotte F, Heurtier A, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* **2005**;128:1898-1906
9. Regev A, Berho M, Jeffers LJ, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* **2002**;97:2614-2618
10. Siddique I, El-Naga HA, Madda JP, Memon A, Hasan F. Sampling variability on percutaneous liver biopsy in patients with chronic hepatitis C virus infection. *Scand J Gastroenterol* **2003**;38:427-432
11. Soloway RD, Baggenstoss AH, Schoenfield LJ, Summerskill WH. Observer error and sampling variability tested in evaluation of hepatitis and cirrhosis by liver biopsy. *Am J Dig Dis* **1971**;16:1082-1086

12. Friedman SL. Liver fibrosis -- from bench to bedside. *J Hepatol* **2003**;38 Suppl 1:S38-53
13. Rockey DC. Antifibrotic therapy in chronic liver disease. *Clin Gastroenterol Hepatol* **2005**;3:95-107
14. Rockey DC, Bissell DM. Noninvasive measures of liver fibrosis. *Hepatology* **2006**;43:S113-120
15. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. *N Engl J Med* **2003**;348:800-807
16. Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. *Hepatology* **1999**;29:971-975
17. Dufour JF, DeLellis R, Kaplan MM. Reversibility of hepatic fibrosis in autoimmune hepatitis. *Ann Intern Med* **1997**;127:981-985
18. Marcellin P, Chang TT, Lim SG, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. *N Engl J Med* **2003**;348:808-816
19. Poynard T, McHutchison J, Manns M, et al. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology* **2002**;122:1303-1313
20. Fontana RJ, Lok AS. Noninvasive monitoring of patients with chronic hepatitis C. *Hepatology* **2002**;36:S57-64
21. Forns X, Ampurdanes S, Llovet JM, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* **2002**;36:986-992
22. Guechot J, Laudat A, Loria A, Serfaty L, Poupon R, Giboudeau J. Diagnostic accuracy of hyaluronan and type III procollagen amino-terminal peptide serum assays

- as markers of liver fibrosis in chronic viral hepatitis C evaluated by ROC curve analysis. *Clin Chem* **1996**;42:558-563
23. Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* **2001**;357:1069-1075
 24. Patel K, Lajoie A, Heaton S, et al. Clinical use of hyaluronic acid as a predictor of fibrosis change in hepatitis C. *J Gastroenterol Hepatol* **2003**;18:253-257
 25. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* **2003**;38:518-526
 26. Castera L, Foucher J, Bertet J, Couzigou P, de Ledinghen V. FibroScan and FibroTest to assess liver fibrosis in HCV with normal aminotransferases. *Hepatology* **2006**;43:373-374; author reply 375-376
 27. Foucher J, Chanteloup E, Vergniol J, et al. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. *Gut* **2006**;55:403-408
 28. Sandrin L, Fourquet B, Hasquenoph JM, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* **2003**;29:1705-1713
 29. Yeh WC, Li PC, Jeng YM, et al. Elastic modulus measurements of human liver and correlation with pathology. *Ultrasound Med Biol* **2002**;28:467-474
 30. Ziol M, Handra-Luca A, Kettaneh A, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* **2005**;41:48-54
 31. Kiernan F. *The anatomy and physiology of the liver*: Philos Trans R Soc Lond, **1833**:711-770

32. Rappaport AM. The microcirculatory hepatic unit. *Microvasc Res* **1973**;6:212-228
33. Rappaport AM. The microcirculatory acinar concept of normal and pathological hepatic structure. *Beitr Pathol* **1976**;157:215-243
34. Rappaport AM. Hepatic blood flow: morphologic aspects and physiologic regulation. *Int Rev Physiol* **1980**;21:1-63
35. Rappaport AM, Borowy ZJ, Lougheed WM, Lotto WN. Subdivision of hexagonal liver lobules into a structural and functional unit; role in hepatic physiology and pathology. *Anat Rec* **1954**;119:11-33
36. McCuskey RS. Morphological mechanisms for regulating blood flow through hepatic sinusoids. *Liver* **2000**;20:3-7
37. MacSween R, Desmet VJ, Roskams T, Scothorne RJ. *Developmental anatomy and normal structure*. In: Mac Sween RN, Burt AD, Portmann BC, Ishak KG, Scheuer PG, Anthony PP, eds. *Pathology of the liver*, 4th ed. London, England: Churchill Livingstone, **2002**; 1-66.
38. Arthur MJ. Matrix degradation in liver: a role in injury and repair. *Hepatology* **1997**;26:1069-1071
39. Ashkenas J, Muschler J, Bissell MJ. The extracellular matrix in epithelial biology: shared molecules and common themes in distant phyla. *Dev Biol* **1996**;180:433-444
40. Kim TH, Mars WM, Stolz DB, Petersen BE, Michalopoulos GK. Extracellular matrix remodeling at the early stages of liver regeneration in the rat. *Hepatology* **1997**;26:896-904
41. Hepatic stellate cell nomenclature. *Hepatology* **1996**;23:193
42. Blomhoff R, Wake K. Perisinusoidal stellate cells of the liver: important roles in retinol metabolism and fibrosis. *Faseb J* **1991**;5:271-277

43. Burt AD. C. L. Oakley Lecture (1993). Cellular and molecular aspects of hepatic fibrosis. *J Pathol* **1993**;170:105-114
44. Burt AD. Pathobiology of hepatic stellate cells. *J Gastroenterol* **1999**;34:299-304
45. Hendriks HF, Bosma A, Brouwer A. Fat-storing cells: hyper- and hypovitaminosis A and the relationships with liver fibrosis. *Semin Liver Dis* **1993**;13:72-80
46. Marra F. Hepatic stellate cells and the regulation of liver inflammation. *J Hepatol* **1999**;31:1120-1130
47. Ramadori G. The stellate cell (Ito-cell, fat-storing cell, lipocyte, perisinusoidal cell) of the liver. New insights into pathophysiology of an intriguing cell. *Virchows Arch B Cell Pathol Incl Mol Pathol* **1991**;61:147-158
48. Rockey D. The cellular pathogenesis of portal hypertension: stellate cell contractility, endothelin, and nitric oxide. *Hepatology* **1997**;25:2-5
49. Rockey DC, Chung JJ. Inducible nitric oxide synthase in rat hepatic lipocytes and the effect of nitric oxide on lipocyte contractility. *J Clin Invest* **1995**;95:1199-1206
50. Zhang JX, Pegoli W, Jr., Clemens MG. Endothelin-1 induces direct constriction of hepatic sinusoids. *Am J Physiol* **1994**;266:G624-632
51. Mathew J, Geerts A, Burt AD. Pathobiology of hepatic stellate cells. *Hepatogastroenterology* **1996**;43:72-91
52. Schirmacher P, Geerts A, Pietrangelo A, Dienes HP, Rogler CE. Hepatocyte growth factor/hepatopoietin A is expressed in fat-storing cells from rat liver but not myofibroblast-like cells derived from fat-storing cells. *Hepatology* **1992**;15:5-11
53. Skrtic S, Wallenius V, Ekberg S, Brenzel A, Gressner AM, Jansson JO. Insulin-like growth factors stimulate expression of hepatocyte growth factor but not transforming growth factor beta1 in cultured hepatic stellate cells. *Endocrinology* **1997**;138:4683-4689

54. Wisse E, van't Noordende JM, van der Meulen J, Daems WT. The pit cell: description of a new type of cell occurring in rat liver sinusoids and peripheral blood. *Cell Tissue Res* **1976**;173:423-435
55. Musso O, Rehn M, Saarela J, et al. Collagen XVIII is localized in sinusoids and basement membrane zones and expressed by hepatocytes and activated stellate cells in fibrotic human liver. *Hepatology* **1998**;28:98-107
56. Ekataksin W, Wake K. Liver units in three dimensions: I. Organization of argyrophilic connective tissue skeleton in porcine liver with particular reference to the "compound hepatic lobule". *Am J Anat* **1991**;191:113-153
57. Gebhardt R. Metabolic zonation of the liver: regulation and implications for liver function. *Pharmacol Ther* **1992**;53:275-354
58. Christoffels VM, Sassi H, Ruijter JM, Moorman AF, Grange T, Lamers WH. A mechanistic model for the development and maintenance of portocentral gradients in gene expression in the liver. *Hepatology* **1999**;29:1180-1192
59. Romert P, Quistorff B, Bhenke O. Histological evaluation of the zonation of colloidal gold uptake by the rat liver. *Tissue Cell* **1993**;25:19-32
60. Schiano TD, Kim-Schluger L, Gondolesi G, Miller CM. Adult living donor liver transplantation: the hepatologist's perspective. *Hepatology* **2001**;33:3-9
61. Chen CH, Chen PJ, Chu JS, Yeh KH, Lai MY, Chen DS. Fibrosing cholestatic hepatitis in a hepatitis B surface antigen carrier after renal transplantation. *Gastroenterology* **1994**;107:1514-1518
62. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* **1997**;349:825-832

63. Poynard T, Ratziu V, Charlotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis c. *J Hepatol* **2001**;34:730-739
64. Powell EE, Edwards-Smith CJ, Hay JL, et al. Host genetic factors influence disease progression in chronic hepatitis C. *Hepatology* **2000**;31:828-833
65. Benhamou Y, Di Martino V, Bochet M, et al. Factors affecting liver fibrosis in human immunodeficiency virus-and hepatitis C virus-coinfected patients: impact of protease inhibitor therapy. *Hepatology* **2001**;34:283-287
66. Lagging LM, Westin J, Svensson E, et al. Progression of fibrosis in untreated patients with hepatitis C virus infection. *Liver* **2002**;22:136-144
67. Arthur MJ. Fibrogenesis II. Metalloproteinases and their inhibitors in liver fibrosis. *Am J Physiol Gastrointest Liver Physiol* **2000**;279:G245-249
68. Pinzani M. Liver fibrosis. *Springer Semin Immunopathol* **1999**;21:475-490
69. Gabele E, Brenner DA, Rippe RA. Liver fibrosis: signals leading to the amplification of the fibrogenic hepatic stellate cell. *Front Biosci* **2003**;8:d69-77
70. Milani S, Herbst H, Schuppan D, Kim KY, Riecken EO, Stein H. Procollagen expression by nonparenchymal rat liver cells in experimental biliary fibrosis. *Gastroenterology* **1990**;98:175-184
71. Pinzani M, Gesualdo L, Sabbah GM, Abboud HE. Effects of platelet-derived growth factor and other polypeptide mitogens on DNA synthesis and growth of cultured rat liver fat-storing cells. *J Clin Invest* **1989**;84:1786-1793
72. Gressner AM, Weiskirchen R, Breitkopf K, Dooley S. Roles of TGF-beta in hepatic fibrosis. *Front Biosci* **2002**;7:d793-807

73. Bachem MG, Riess U, Melchior R, Sell KM, Gressner AM. Transforming growth factors (TGF alpha and TGF beta 1) stimulate chondroitin sulfate and hyaluronate synthesis in cultured rat liver fat storing cells. *FEBS Lett* **1989**;257:134-137
74. Olaso E, Friedman SL. Molecular regulation of hepatic fibrogenesis. *J Hepatol* **1998**;29:836-847
75. Cho JJ, Hocher B, Herbst H, et al. An oral endothelin-A receptor antagonist blocks collagen synthesis and deposition in advanced rat liver fibrosis. *Gastroenterology* **2000**;118:1169-1178
76. Racine-Samson L, Rockey DC, Bissell DM. The role of alpha1beta1 integrin in wound contraction. A quantitative analysis of liver myofibroblasts in vivo and in primary culture. *J Biol Chem* **1997**;272:30911-30917
77. Marra F. Leptin and liver fibrosis: a matter of fat. *Gastroenterology* **2002**;122:1529-1532
78. Ikejima K, Takei Y, Honda H, et al. Leptin receptor-mediated signaling regulates hepatic fibrogenesis and remodeling of extracellular matrix in the rat. *Gastroenterology* **2002**;122:1399-1410
79. Kamada Y, Tamura S, Kiso S, et al. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. *Gastroenterology* **2003**;125:1796-1807
80. Ortiz V, Berenguer M, Rayon JM, Carrasco D, Berenguer J. Contribution of obesity to hepatitis C-related fibrosis progression. *Am J Gastroenterol* **2002**;97:2408-2414
81. Crawford J. *Liver cirrhosis*. In: Mac Sween RN, Burt AD, Portmann BC, Ishak KG, Scheuer PG, Anthony PP, eds. *Pathology of the liver*, 4th ed. London, England: Churchill Livingstone, **2002**; 575-620.

82. Murata K, Ochiai Y, Akashio K. Polydispersity of acidic glycosaminoglycan components in human liver and the changes at different stages in liver cirrhosis. *Gastroenterology* **1985**;89:1248-1257
83. Rappaport AM, MacPhee PJ, Fisher MM, Phillips MJ. The scarring of the liver acini (Cirrhosis). Tridimensional and microcirculatory considerations. *Virchows Arch A Pathol Anat Histopathol* **1983**;402:107-137
84. Schaffner F, Poper H. Capillarization of hepatic sinusoids in man. *Gastroenterology* **1963**;44:239-242
85. Picchiotti R, Mingazzini PL, Scucchi L, et al. Correlations between sinusoidal pressure and liver morphology in cirrhosis. *J Hepatol* **1994**;20:364-369
86. Syrota A, Vinot JM, Paraf A, Roucayrol JC. Scintillation splenoportography: hemodynamic and morphological study of the portal circulation. *Gastroenterology* **1976**;71:652-659
87. Wanless IR, Wong F, Blendis LM, Greig P, Heathcote EJ, Levy G. Hepatic and portal vein thrombosis in cirrhosis: possible role in development of parenchymal extinction and portal hypertension. *Hepatology* **1995**;21:1238-1247
88. Gerber MA, Thung SN. Liver stem cells and development. *Lab Invest* **1993**;68:253-254
89. Grisham JW. Migration of hepatocytes along hepatic plates and stem cell-fed hepatocyte lineages. *Am J Pathol* **1994**;144:849-854
90. Sell S. Heterogeneity and plasticity of hepatocyte lineage cells. *Hepatology* **2001**;33:738-750
91. Anthony PP, Ishak KG, Nayak NC, Poulsen HE, Scheuer PJ, Sobin LH. The morphology of cirrhosis: definition, nomenclature, and classification. *Bull World Health Organ* **1977**;55:521-540

92. Fauerholdt L, Schlichting P, Christensen E, Poulsen H, Tygstrup N, Juhl E.
Conversion of micronodular cirrhosis into macronodular cirrhosis. *Hepatology*
1983;3:928-931
93. Hammel P, Couvelard A, O'Toole D, et al. Regression of liver fibrosis after biliary
drainage in patients with chronic pancreatitis and stenosis of the common bile duct. *N*
Engl J Med **2001**;344:418-423
94. Kweon YO, Goodman ZD, Dienstag JL, et al. Decreasing fibrogenesis: an
immunohistochemical study of paired liver biopsies following lamivudine therapy for
chronic hepatitis B. *J Hepatol* **2001**;35:749-755
95. Dixon JB, Bhathal PS, Hughes NR, O'Brien PE. Nonalcoholic fatty liver disease:
Improvement in liver histological analysis with weight loss. *Hepatology*
2004;39:1647-1654
96. Pares A, Caballeria J, Bruguera M, Torres M, Rodes J. Histological course of
alcoholic hepatitis. Influence of abstinence, sex and extent of hepatic damage. *J*
Hepatol **1986**;2:33-42
97. Perez-Tamayo R. Cirrhosis of the liver: a reversible disease? *Pathol Annu* **1979**;14 Pt
2:183-213
98. Arthur MJ. Reversibility of liver fibrosis and cirrhosis following treatment for
hepatitis C. *Gastroenterology* **2002**;122:1525-1528
99. Desmet VJ, Roskams T. Reversal of cirrhosis: evidence-based medicine?
Gastroenterology **2003**;125:629-630; author reply 630-631
100. Desmet VJ. Comments on cirrhosis reversal. *Dig Liver Dis* **2005**;37:909-916
101. Desmet VJ, Roskams T. Cirrhosis reversal: a duel between dogma and myth. *J*
Hepatol **2004**;40:860-867

102. Issa R, Zhou X, Constandinou CM, et al. Spontaneous recovery from micronodular cirrhosis: evidence for incomplete resolution associated with matrix cross-linking. *Gastroenterology* **2004**;126:1795-1808
103. Hirose A, Ono M, Saibara T, et al. Angiotensin II type 1 receptor blocker inhibits fibrosis in rat nonalcoholic steatohepatitis. *Hepatology* **2007**;45:1375-1381
104. Jonsson JR, Clouston AD, Ando Y, et al. Angiotensin-converting enzyme inhibition attenuates the progression of rat hepatic fibrosis. *Gastroenterology* **2001**;121:148-155
105. Gonzalo T, Beljaars L, van de Bovenkamp M, et al. Local inhibition of liver fibrosis by specific delivery of a platelet-derived growth factor kinase inhibitor to hepatic stellate cells. *J Pharmacol Exp Ther* **2007**;321:856-865
106. Bataller R, Brenner DA. Hepatic stellate cells as a target for the treatment of liver fibrosis. *Semin Liver Dis* **2001**;21:437-451
107. Brunt EM. Grading and staging the histopathological lesions of chronic hepatitis: the Knodell histology activity index and beyond. *Hepatology* **2000**;31:241-246
108. Knodell RG, Ishak KG, Black WC, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* **1981**;1:431-435
109. Ishak KG. Chronic hepatitis: morphology and nomenclature. *Mod Pathol* **1994**;7:690-713
110. Ludwig J. The nomenclature of chronic active hepatitis: an obituary. *Gastroenterology* **1993**;105:274-278
111. Scheuer PJ. Changing views on chronic hepatitis. *Histopathology* **1986**;10:1-4
112. Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. *J Hepatol* **1991**;13:372-374

113. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* **1994**;19:1513-1520
114. Batts KP, Ludwig J. Chronic hepatitis. An update on terminology and reporting. *Am J Surg Pathol* **1995**;19:1409-1417
115. Hunt N, Fleming K. Reproducibility of liver biopsy grading and staging. *Liver* **1999**;19:169-170
116. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group. *Hepatology* **1994**;20:15-20
117. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* **1995**;22:696-699
118. Ghany MG, Kleiner DE, Alter H, et al. Progression of fibrosis in chronic hepatitis C. *Gastroenterology* **2003**;124:97-104
119. Kyrlagkitis I, Portmann B, Smith H, O'Grady J, Cramp ME. Liver histology and progression of fibrosis in individuals with chronic hepatitis C and persistently normal ALT. *Am J Gastroenterol* **2003**;98:1588-1593
120. Wright M, Goldin R, Fabre A, et al. Measurement and determinants of the natural history of liver fibrosis in hepatitis C virus infection: a cross sectional and longitudinal study. *Gut* **2003**;52:574-579
121. Zarski JP, Mc Hutchison J, Bronowicki JP, et al. Rate of natural disease progression in patients with chronic hepatitis C. *J Hepatol* **2003**;38:307-314
122. Poynard T, Mathurin P, Lai CL, et al. A comparison of fibrosis progression in chronic liver diseases. *J Hepatol* **2003**;38:257-265

123. Goodman ZD, Becker RL, Jr., Pockros PJ, Afdhal NH. Progression of fibrosis in advanced chronic hepatitis C: evaluation by morphometric image analysis. *Hepatology* **2007**;45:886-894
124. O'Brien MJ, Keating NM, Elderiny S, et al. An assessment of digital image analysis to measure fibrosis in liver biopsy specimens of patients with chronic hepatitis C. *Am J Clin Pathol* **2000**;114:712-718
125. Caballero T, Perez-Milena A, Masseroli M, et al. Liver fibrosis assessment with semiquantitative indexes and image analysis quantification in sustained-responder and non-responder interferon-treated patients with chronic hepatitis C. *J Hepatol* **2001**;34:740-747
126. Duchatelle V, Marcellin P, Giostra E, et al. Changes in liver fibrosis at the end of alpha interferon therapy and 6 to 18 months later in patients with chronic hepatitis C: quantitative assessment by a morphometric method. *J Hepatol* **1998**;29:20-28
127. Kage M, Shimamatu K, Nakashima E, Kojiro M, Inoue O, Yano M. Long-term evolution of fibrosis from chronic hepatitis to cirrhosis in patients with hepatitis C: morphometric analysis of repeated biopsies. *Hepatology* **1997**;25:1028-1031
128. Manabe N, Chevallier M, Chossegros P, et al. Interferon-alpha 2b therapy reduces liver fibrosis in chronic non-A, non-B hepatitis: a quantitative histological evaluation. *Hepatology* **1993**;18:1344-1349
129. Sakaida I, Nagatomi A, Hironaka K, Uchida K, Okita K. Quantitative analysis of liver fibrosis and stellate cell changes in patients with chronic hepatitis C after interferon therapy. *Am J Gastroenterol* **1999**;94:489-496
130. Wright M, Thursz M, Pullen R, Thomas H, Goldin R. Quantitative versus morphological assessment of liver fibrosis: semi-quantitative scores are more robust than digital image fibrosis area estimation. *Liver Int* **2003**;23:28-34

131. Cadranel JF, Rufat P, Degos F. Practices of liver biopsy in France: results of a prospective nationwide survey. For the Group of Epidemiology of the French Association for the Study of the Liver (AFEF). *Hepatology* **2000**;32:477-481
132. Benhamou Y, Bochet M, Di Martino V, et al. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfecting patients. The Multivirc Group. *Hepatology* **1999**;30:1054-1058
133. Marcellin P, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. *Hepatology* **2002**;36:S47-56
134. Abdi W, Millan JC, Mezey E. Sampling variability on percutaneous liver biopsy. *Arch Intern Med* **1979**;139:667-669
135. Gronbaek K, Christensen PB, Hamilton-Dutoit S, et al. Interobserver variation in interpretation of serial liver biopsies from patients with chronic hepatitis C. *J Viral Hepat* **2002**;9:443-449
136. Rousselet MC, Michalak S, Dupre F, et al. Sources of variability in histological scoring of chronic viral hepatitis. *Hepatology* **2005**;41:257-264
137. Petz D, Klauck S, Rohl FW, Malfertheiner P, Roessner A, Rocken C. Feasibility of histological grading and staging of chronic viral hepatitis using specimens obtained by thin-needle biopsy. *Virchows Arch* **2003**;442:238-244
138. Westin J, Lagging LM, Wejstal R, Norkrans G, Dhillon AP. Interobserver study of liver histopathology using the Ishak score in patients with chronic hepatitis C virus infection. *Liver* **1999**;19:183-187
139. Goldin RD, Goldin JG, Burt AD, et al. Intra-observer and inter-observer variation in the histopathological assessment of chronic viral hepatitis. *J Hepatol* **1996**;25:649-654
140. Pinzani M. Non-invasive evaluation of hepatic fibrosis: don't count your chickens before they're hatched. *Gut* **2006**;55:310-312

141. Burroughs AK, Cholongitas E. Non-invasive tests for liver fibrosis: encouraging or discouraging results? *J Hepatol* **2007**;46:751-755
142. Koda M, Matunaga Y, Kawakami M, Kishimoto Y, Suou T, Murawaki Y. FibroIndex, a practical index for predicting significant fibrosis in patients with chronic hepatitis C. *Hepatology* **2007**;45:297-306
143. Naveau S, Poynard T, Benattar C, Bedossa P, Chaput JC. Alpha-2-macroglobulin and hepatic fibrosis. Diagnostic interest. *Dig Dis Sci* **1994**;39:2426-2432
144. Adams LA, Bulsara M, Rossi E, et al. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. *Clin Chem* **2005**;51:1867-1873
145. Rossi E, Adams L, Prins A, et al. Validation of the FibroTest biochemical markers score in assessing liver fibrosis in hepatitis C patients. *Clin Chem* **2003**;49:450-454
146. Poynard T, Imbert-Bismut F, Munteanu M, et al. Overview of the diagnostic value of biochemical markers of liver fibrosis (FibroTest, HCV FibroSure) and necrosis (ActiTest) in patients with chronic hepatitis C. *Comp Hepatol* **2004**;3:8
147. Lackner C, Struber G, Liegl B, et al. Comparison and validation of simple noninvasive tests for prediction of fibrosis in chronic hepatitis C. *Hepatology* **2005**;41:1376-1382
148. Sebastiani G, Alberti A. Non invasive fibrosis biomarkers reduce but not substitute the need for liver biopsy. *World J Gastroenterol* **2006**;12:3682-3694
149. Rosenberg WM, Voelker M, Thiel R, et al. Serum markers detect the presence of liver fibrosis: a cohort study. *Gastroenterology* **2004**;127:1704-1713
150. Callewaert N, Van Vlierberghe H, Van Hecke A, Laroy W, Delanghe J, Contreras R. Noninvasive diagnosis of liver cirrhosis using DNA sequencer-based total serum protein glycomics. *Nat Med* **2004**;10:429-434

151. Poon TC, Hui AY, Chan HL, et al. Prediction of liver fibrosis and cirrhosis in chronic hepatitis B infection by serum proteomic fingerprinting: a pilot study. *Clin Chem* **2005**;51:328-335
152. Staudt LM. Molecular diagnosis of the hematologic cancers. *N Engl J Med* **2003**;348:1777-1785
153. Morra R, Munteanu M, Bedossa P, et al. Diagnostic value of serum protein profiling by SELDI-TOF ProteinChip compared with a biochemical marker, FibroTest, for the diagnosis of advanced fibrosis in patients with chronic hepatitis C. *Aliment Pharmacol Ther* **2007**;26:847-858
154. Aube C, Winkfield B, Oberti F, et al. New Doppler ultrasound signs improve the non-invasive diagnosis of cirrhosis or severe liver fibrosis. *Eur J Gastroenterol Hepatol* **2004**;16:743-751
155. Aube C, Oberti F, Koralı N, et al. Ultrasonographic diagnosis of hepatic fibrosis or cirrhosis. *J Hepatol* **1999**;30:472-478
156. Dodd GD, 3rd, Baron RL, Oliver JH, 3rd, Federle MP. Spectrum of imaging findings of the liver in end-stage cirrhosis: part I, gross morphology and diffuse abnormalities. *AJR Am J Roentgenol* **1999**;173:1031-1036
157. Harbin WP, Robert NJ, Ferrucci JT, Jr. Diagnosis of cirrhosis based on regional changes in hepatic morphology: a radiological and pathological analysis. *Radiology* **1980**;135:273-283
158. Lafortune M, Matricardi L, Denys A, Favret M, Dery R, Pomier-Layrargues G. Segment 4 (the quadrate lobe): a barometer of cirrhotic liver disease at US. *Radiology* **1998**;206:157-160
159. Brown JJ, Naylor MJ, Yagan N. Imaging of hepatic cirrhosis. *Radiology* **1997**;202:1-16

160. Van Beers B, Trigaux JP, Grandin C, et al. Regenerative nodules in hepatic cirrhosis. Value of gradient-echo MR imaging with a surface coil. *Acta Radiol* **1992**;33:245-250
161. Elizondo G, Weissleder R, Stark DD, et al. Hepatic cirrhosis and hepatitis: MR imaging enhanced with superparamagnetic iron oxide. *Radiology* **1990**;174:797-801
162. Lucidarme O, Baleston F, Cadi M, et al. Non-invasive detection of liver fibrosis: Is superparamagnetic iron oxide particle-enhanced MR imaging a contributive technique? *Eur Radiol* **2003**;13:467-474
163. Aguirre DA, Behling CA, Alpert E, Hassanein TI, Sirlin CB. Liver fibrosis: noninvasive diagnosis with double contrast material-enhanced MR imaging. *Radiology* **2006**;239:425-437
164. Annet L, Materne R, Danse E, Jamart J, Horsmans Y, Van Beers BE. Hepatic flow parameters measured with MR imaging and Doppler US: correlations with degree of cirrhosis and portal hypertension. *Radiology* **2003**;229:409-414
165. Materne R, Van Beers BE, Smith AM, et al. Non-invasive quantification of liver perfusion with dynamic computed tomography and a dual-input one-compartmental model. *Clin Sci (Lond)* **2000**;99:517-525
166. Huwart L, Michoux N, Van Beers BE. [Magnetic resonance imaging of angiogenesis in tumors]. *J Radiol* **2007**;88:331-338
167. Gowland P, Mansfield P, Bullock P, Stehling M, Worthington B, Firth J. Dynamic studies of gadolinium uptake in brain tumors using inversion-recovery echo-planar imaging. *Magn Reson Med* **1992**;26:241-258
168. Lim AK, Patel N, Eckersley RJ, et al. Can Doppler sonography grade the severity of hepatitis C-related liver disease? *AJR Am J Roentgenol* **2005**;184:1848-1853

169. Van Beers BE, Materne R, Annet L, et al. Capillarization of the sinusoids in liver fibrosis: noninvasive assessment with contrast-enhanced MRI in the rabbit. *Magn Reson Med* **2003**;49:692-699
170. Taouli B, Vilgrain V, Dumont E, Daire JL, Fan B, Menu Y. Evaluation of liver diffusion isotropy and characterization of focal hepatic lesions with two single-shot echo-planar MR imaging sequences: prospective study in 66 patients. *Radiology* **2003**;226:71-78
171. Ichikawa T, Haradome H, Hachiya J, Nitatori T, Araki T. Diffusion-weighted MR imaging with a single-shot echoplanar sequence: detection and characterization of focal hepatic lesions. *AJR Am J Roentgenol* **1998**;170:397-402
172. Koinuma M, Ohashi I, Hanafusa K, Shibuya H. Apparent diffusion coefficient measurements with diffusion-weighted magnetic resonance imaging for evaluation of hepatic fibrosis. *J Magn Reson Imaging* **2005**;22:80-85
173. Boulanger Y, Amara M, Lepanto L, et al. Diffusion-weighted MR imaging of the liver of hepatitis C patients. *NMR Biomed* **2003**;16:132-136
174. Kim T, Murakami T, Takahashi S, Hori M, Tsuda K, Nakamura H. Diffusion-weighted single-shot echoplanar MR imaging for liver disease. *AJR Am J Roentgenol* **1999**;173:393-398
175. Yamada I, Aung W, Himeno Y, Nakagawa T, Shibuya H. Diffusion coefficients in abdominal organs and hepatic lesions: evaluation with intravoxel incoherent motion echo-planar MR imaging. *Radiology* **1999**;210:617-623
176. Lewin M, Poujol-Robert A, Boelle PY, et al. Diffusion-weighted magnetic resonance imaging for the assessment of fibrosis in chronic hepatitis C. *Hepatology* **2007**

177. Taouli B, Martin AJ, Qayyum A, et al. Parallel imaging and diffusion tensor imaging for diffusion-weighted MRI of the liver: preliminary experience in healthy volunteers. *AJR Am J Roentgenol* **2004**;183:677-680
178. Annet L, Peeters F, Abarca-Quinones J, Leclercq I, Moulin P, Van Beers BE. Assessment of diffusion-weighted MR imaging in liver fibrosis. *J Magn Reson Imaging* **2007**;25:122-128
179. Cho SG, Kim MY, Kim HJ, et al. Chronic hepatitis: in vivo proton MR spectroscopic evaluation of the liver and correlation with histopathologic findings. *Radiology* **2001**;221:740-746
180. Angus PW, Dixon RM, Rajagopalan B, et al. A study of patients with alcoholic liver disease by ³¹P nuclear magnetic resonance spectroscopy. *Clin Sci (Lond)* **1990**;78:33-38
181. Meyerhoff DJ, Boska MD, Thomas AM, Weiner MW. Alcoholic liver disease: quantitative image-guided P-31 MR spectroscopy. *Radiology* **1989**;173:393-400
182. Oberhaensli R, Rajagopalan B, Galloway GJ, Taylor DJ, Radda GK. Study of human liver disease with P-31 magnetic resonance spectroscopy. *Gut* **1990**;31:463-467
183. Cox IJ, Menon DK, Sargentoni J, et al. Phosphorus-31 magnetic resonance spectroscopy of the human liver using chemical shift imaging techniques. *J Hepatol* **1992**;14:265-275
184. Jalan R, Sargentoni J, Coutts GA, et al. Hepatic phosphorus-31 magnetic resonance spectroscopy in primary biliary cirrhosis and its relation to prognostic models. *Gut* **1996**;39:141-146
185. Menon DK, Sargentoni J, Taylor-Robinson SD, et al. Effect of functional grade and etiology on in vivo hepatic phosphorus-31 magnetic resonance spectroscopy in cirrhosis: biochemical basis of spectral appearances. *Hepatology* **1995**;21:417-427

186. Meyerhoff DJ, Karczmar GS, Weiner MW. Abnormalities of the liver evaluated by 31P MRS. *Invest Radiol* **1989**;24:980-984
187. Munakata T, Griffiths RD, Martin PA, Jenkins SA, Shields R, Edwards RH. An in vivo 31P MRS study of patients with liver cirrhosis: progress towards a non-invasive assessment of disease severity. *NMR Biomed* **1993**;6:168-172
188. Rajanayagam V, Lee RR, Ackerman Z, Bradley WG, Ross BD. Quantitative P-31 MR spectroscopy of the liver in alcoholic cirrhosis. *J Magn Reson Imaging* **1992**;2:183-190
189. Taylor-Robinson SD, Sargentoni J, Bell JD, et al. In vivo and in vitro hepatic 31P magnetic resonance spectroscopy and electron microscopy of the cirrhotic liver. *Liver* **1997**;17:198-209
190. Taylor-Robinson SD, Sargentoni J, Bell JD, et al. In vivo and in vitro hepatic phosphorus-31 magnetic resonance spectroscopy and electron microscopy in chronic ductopenic rejection of human liver allografts. *Gut* **1998**;42:735-743
191. van Wassenraer-van Hall HN, van der Grond J, van Hattum J, Kooijman C, Hoogenraad TU, Mali WP. 31P magnetic resonance spectroscopy of the liver: correlation with standardized serum, clinical, and histological changes in diffuse liver disease. *Hepatology* **1995**;21:443-449
192. Murphy EJ, Rajagopalan B, Brindle KM, Radda GK. Phospholipid bilayer contribution to 31P NMR spectra in vivo. *Magn Reson Med* **1989**;12:282-289
193. Ruiz-Cabello J, Cohen JS. Phospholipid metabolites as indicators of cancer cell function. *NMR Biomed* **1992**;5:226-233
194. Lim AK, Patel N, Hamilton G, Hajnal JV, Goldin RD, Taylor-Robinson SD. The relationship of in vivo 31P MR spectroscopy to histology in chronic hepatitis C. *Hepatology* **2003**;37:788-794

195. Noren B, Dahlqvist O, Lundberg P, et al. Separation of advanced from mild fibrosis in diffuse liver disease using (31)P magnetic resonance spectroscopy. *Eur J Radiol* **2007**
196. Friedrich-Rust M, Ong MF, Herrmann E, et al. Real-time elastography for noninvasive assessment of liver fibrosis in chronic viral hepatitis. *AJR Am J Roentgenol* **2007**;188:758-764
197. Sinkus R, Tanter M, Catheline S, et al. Imaging anisotropic and viscous properties of breast tissue by magnetic resonance-elastography. *Magn Reson Med* **2005**;53:372-387
198. Sinkus R, Tanter M, Xydeas T, Catheline S, Bercoff J, Fink M. Viscoelastic shear properties of in vivo breast lesions measured by MR elastography. *Magn Reson Imaging* **2005**;23:159-165
199. Royer D, Dieulesaint E. *Elastic waves in solids*. New York: Springer Verlag, **2000**
200. Castera L, Vergniol J, Foucher J, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* **2005**;128:343-350
201. Coco B, Oliveri F, Maina AM, et al. Transient elastography: a new surrogate marker of liver fibrosis influenced by major changes of transaminases. *J Viral Hepat* **2007**;14:360-369
202. Corpechot C, El Naggar A, Poujol-Robert A, et al. Assessment of biliary fibrosis by transient elastography in patients with PBC and PSC. *Hepatology* **2006**;43:1118-1124
203. Fraquelli M, Rigamonti C, Casazza G, et al. Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. *Gut* **2007**
204. Ganne-Carrie N, Ziol M, de Ledinghen V, et al. Accuracy of liver stiffness measurement for the diagnosis of cirrhosis in patients with chronic liver diseases. *Hepatology* **2006**;44:1511-1517

205. Kettaneh A, Marcellin P, Douvin C, et al. Features associated with success rate and performance of fibroscan measurements for the diagnosis of cirrhosis in HCV patients: A prospective study of 935 patients. *J Hepatol* **2007**;46:628-634
206. Gomez-Dominguez E, Mendoza J, Rubio S, Moreno-Monteagudo JA, Garcia-Buey L, Moreno-Otero R. Transient elastography: a valid alternative to biopsy in patients with chronic liver disease. *Aliment Pharmacol Ther* **2006**;24:513-518
207. Foucher J, Castera L, Bernard PH, et al. Prevalence and factors associated with failure of liver stiffness measurement using FibroScan in a prospective study of 2114 examinations. *Eur J Gastroenterol Hepatol* **2006**;18:411-412
208. Prediction of the first variceal hemorrhage in patients with cirrhosis of the liver and esophageal varices. A prospective multicenter study. The North Italian Endoscopic Club for the Study and Treatment of Esophageal Varices. *N Engl J Med* **1988**;319:983-989
209. D'Amico G, Pagliaro L, Bosch J. Pharmacological treatment of portal hypertension: an evidence-based approach. *Semin Liver Dis* **1999**;19:475-505
210. Spiegel BM, Targownik L, Dulai GS, Karsan HA, Gralnek IM. Endoscopic screening for esophageal varices in cirrhosis: Is it ever cost effective? *Hepatology* **2003**;37:366-377
211. Cales P, Desmorat H, Vinel JP, et al. Incidence of large oesophageal varices in patients with cirrhosis: application to prophylaxis of first bleeding. *Gut* **1990**;31:1298-1302
212. Chalasani N, Imperiale TF, Ismail A, et al. Predictors of large esophageal varices in patients with cirrhosis. *Am J Gastroenterol* **1999**;94:3285-3291

213. de Franchis R. Evolving consensus in portal hypertension. Report of the Baveno IV consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* **2005**;43:167-176
214. Giannini E, Botta F, Borro P, et al. Platelet count/spleen diameter ratio: proposal and validation of a non-invasive parameter to predict the presence of oesophageal varices in patients with liver cirrhosis. *Gut* **2003**;52:1200-1205
215. Pilette C, Oberti F, Aube C, et al. Non-invasive diagnosis of esophageal varices in chronic liver diseases. *J Hepatol* **1999**;31:867-873
216. Thomopoulos KC, Labropoulou-Karatza C, Mimidis KP, Katsakoulis EC, Ionomou G, Nikolopoulou VN. Non-invasive predictors of the presence of large oesophageal varices in patients with cirrhosis. *Dig Liver Dis* **2003**;35:473-478
217. Kazemi F, Kettaneh A, N'Kontchou G, et al. Liver stiffness measurement selects patients with cirrhosis at risk of bearing large oesophageal varices. *J Hepatol* **2006**;45:230-235
218. Cales P, Oberti F, Michalak S, et al. A novel panel of blood markers to assess the degree of liver fibrosis. *Hepatology* **2005**;42:1373-1381
219. Paulsen KD, V.H., E.E.W.; Doyley, M.M.; Weaver, J.B. Magnetic Resonance Elastography in the breast: initial reconstructions of damping coefficient. In: *IEEE International Symposium on Biomedical Imaging*. Washington, **2002**:157- 160
220. Huwart L, Peeters F, Sinkus R, et al. Liver fibrosis: non-invasive assessment with MR elastography. *NMR Biomed* **2006**;19:173-179
221. Rouviere O, Yin M, Dresner MA, et al. MR elastography of the liver: preliminary results. *Radiology* **2006**;240:440-448
222. Bedossa P. An algorithm for the grading of activity in chronic hepatitis C. *Hepatology* **1996**;24:289-293

223. Huwart L, Sempoux C, Salameh N, et al. Liver Fibrosis: Noninvasive Assessment with MR Elastography versus Aspartate Aminotransferase to-Platelet Ratio Index. *Radiology* **2007**;245:458-466
224. Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* **2004**;39:1147-1171
225. Bruix J, Sherman M. Management of hepatocellular carcinoma. *Hepatology* **2005**;42:1208-1236
226. Jalan R, Hayes PC. UK guidelines on the management of variceal haemorrhage in cirrhotic patients. British Society of Gastroenterology. *Gut* **2000**;46 Suppl 3-4:III1-III15
227. Salameh N, Sinkus R, Peeters F, Annet L, ter Beek LC, Van Beers BE. Assessment of liver fibrosis by MR elastography - Voigt model and beyond (Kramers-Kronig). In: Proceedings of the 22nd Annual Meeting of ESMRMB. *ESMRMB*: Basle, **2005**;67
228. Hamhaber U, Sack I, Papazoglou S, Rump J, Klatt D, Braun J. Three-dimensional analysis of shear wave propagation observed by in vivo magnetic resonance elastography of the brain. *Acta Biomater* **2007**;3:127-137
229. Klatt D, Asbach P, Rump J, et al. In vivo determination of hepatic stiffness using steady-state free precession magnetic resonance elastography. *Invest Radiol* **2006**;41:841-848
230. Rump J, Klatt D, Braun J, Warmuth C, Sack I. Fractional encoding of harmonic motions in MR elastography. *Magn Reson Med* **2007**;57:388-395
231. Sandrin L, Catheline S, Tanter M, Hennequin X, Fink M. Time-resolved pulsed elastography with ultrafast ultrasonic imaging. *Ultrason Imaging* **1999**;21:259-272
232. Sandrin L, Tanter M, Catheline S, Fink M. Shear modulus imaging with 2-D transient elastography. *IEEE Trans Ultrason Ferroelectr Freq Control* **2002**;49:426-435

- 233. Paradis V. Glycomics: a new taste of cirrhosis marker. *J Hepatol* **2005**;43:913-914
- 234. Paradis V, Degos F, Dargere D, et al. Identification of a new marker of hepatocellular carcinoma by serum protein profiling of patients with chronic liver diseases. *Hepatology* **2005**;41:40-47
- 235. Michalet X, Pinaud FF, Bentolila LA, et al. Quantum dots for live cells, in vivo imaging, and diagnostics. *Science* **2005**;307:538-544
- 236. Ntziachristos V, Ripoll J, Wang LV, Weissleder R. Looking and listening to light: the evolution of whole-body photonic imaging. *Nat Biotechnol* **2005**;23:313-320
- 237. Diamond DL, Jacobs JM, Paeper B, et al. Proteomic profiling of human liver biopsies: Hepatitis C virus-induced fibrosis and mitochondrial dysfunction. *Hepatology* **2007**

VIII. MANUSCRIPTS RELATED TO THIS THESIS

Original articles

Huwart L, Peeters F, Sinkus R, Annet L, Salameh N, ter Beek LC, Horsmans Y, Van Beers B
Liver fibrosis: non-invasive assessment with MR elastography.
NMR Biomed. 2006 Apr;19(2):173-9 (*Impact factor* = 3,626)

Huwart L, Sempoux S, Salameh N, Jamart J, Annet L, Sinkus R, Peeters F, ter Beek LC,
Horsmans Y, Van Beers BE
Comparison of MR elastography and aspartate aminotransferase to platelets ratio index for the
non-invasive assessment of liver fibrosis
Radiology. 2007 Nov;245(2):458-466 (*Impact factor* = 5,251)

Huwart L, Sempoux C, Vicaut E, Salameh N, Annet L, Danse E, Peeters F, ter Beek LC,
Rahier J, Sinkus R, Horsmans Y, Van Beers BE
Magnetic resonance elastography for the non-invasive staging of liver fibrosis
Submitted to Gastroenterology

Reviews

Huwart L, Michoux N, Van Beers B
Imagerie par résonance magnétique de l'angiogenèse tumorale.
J Radiol 2007;88:331-8

Huwart L, Salameh N, Van Beers BE
L'élastographie du foie, c'est quoi?
Feuillets de Radiologie 2006;46(3):211-215

