REVIEW

Liver stiffness: a novel parameter for the diagnosis of liver disease

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Abstract: The noninvasive quantitation of liver stiffness (LS) by ultrasound based transient elastography using FibroScan® has revolutionized the diagnosis of liver diseases, namely liver cirrhosis. Alternative techniques such as acoustic radiation impulse frequency imaging or magnetic resonance elastography are currently under investigation. LS is an excellent surrogate marker of advanced fibrosis (F3) and cirrhosis (F4) outscoring all previous noninvasive approaches to detect cirrhosis. LS values below 6 kPa are considered as normal and exclude ongoing liver disease. LS of 8 and 12.5 kPa represent generally accepted cut-off values for F3 and F4 fibrosis. LS highly correlates with portal pressure, and esophageal varices are likely at values >20 kPa. Many other factors may also increase LS such as hepatic infiltration with tumor cells, mast cells (mastocytosis), inflammatory cells (all forms of hepatitis) or amyloidosis. In addition, LS is directly correlated with the venous pressure (eg, during liver congestion) and is increased during mechanic cholestasis. Thus, LS should always be interpreted in the context of clinical, imaging and laboratory findings. Finally, LS has helped to better understand the molecular mechanisms underlying liver fibrosis. The novel pressure-stiffness-fibrosis sequence hypothesis is introduced.

Keywords: liver stiffness, fibrosis, liver disease, transient elastography

Introduction

All chronic liver diseases whether of toxic, genetic, autoimmune or infectious origin undergo typical histological changes that ultimately lead to fibrosis/cirrhosis, and the excess deposition of matrix. Liver cirrhosis can rapidly decompensate and has a high mortality rate. Patients with cirrhosis suffer from a decreasing hepatic capacity to metabolize and synthesize proteins, peptides and hormones. In addition, progression of fibrosis and regenerating nodules cause an increased vascular portocaval resistance with portal hypertension and an increased hepatic venous pressure gradient (HVPG) of >10 mm Hg. Portal hypertension finally leads to ascites, and vascular collaterals will develop such as esophageal varices. Most patients suffering from cirrhosis eventually die from complications such as spontaneous bacterial peritonitis, variceal bleeding, liver failure or hepatocellular carcinoma (HCC). Especially compensated liver cirrhosis without clinical signs such as spider nevi, encephalopathy, icterus, or ascites is difficult to diagnose. Such patients typically do not show specific symptoms. This is also one important reason why no valid and reliable prevalence data are available for cirrhosis for many countries, although cirrhosis is a major mortality cause in developed countries at the age of 40–60 years.

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Many techniques have been explored in the last decades to allow an early and reliable diagnosis of cirrhosis (see Figure 1). These include both invasive but also noninvasive approaches. Liver biopsy is still considered the gold standard for assessing hepatic cirrhosis. However, it is an invasive procedure, with rare but potentially life-threatening complications.[1] In addition, the accuracy of liver biopsy in assessing fibrosis is limited owing to sampling error (reaching up to 30%) and interobserver variability.[2–6] Other invasive procedures such as laparoscopy and endoscopy are not very sensitive. Likewise, conventional imaging techniques such as ultrasound, magnetic resonance imaging (MRI) and computer tomography (CT) are noninvasive but absolute signs of cirrhosis such as collaterals or nodular aspect of the liver surface are required, rendering these methods rather insensitive. Many efforts have been invested to identify serum markers that allow the diagnosis of cirrhosis from a simple blood test.[7] Unfortunately, although markers such as serum collagen or hyaluron reflect profibrogenic activity, they do not correlate with the absolute amount of matrix deposited in the liver.

Liver cirrhosis per se causes a typical induration of the liver that is sometimes clearly palpable. In fact, palpation of the liver has been used by physicians for centuries as the only valid bedside test to diagnose cirrhosis. Thus, it has been a question of time to develop sophisticated physical methods to truly quantify liver stiffness (LS). The first such approach has been successfully introduced by Sandrin and coworkers in 2003.[8] Meanwhile, many studies on chronic liver diseases have proven that measurement of LS is a rapid and excellent screening test for liver cirrhosis. Alternative approaches are currently explored either based on competing ultrasound or MRI methods, and the future will show which technique will prevail in which clinical setting.

On the other hand, LS has been introduced to the field of hepatology as a novel objective physical parameter that can be followed up as compared to, for example, temperature. Like body temperature, we have learnt in a rather short time that LS is not only determined by the degree of fibrosis but also other clinical settings such as inflammation, cholestasis and liver congestions. This review, therefore, is designed to briefly update the reader on the present knowledge of LS. After an overview about technical aspects and alternative methods, basic conditions are discussed that influence LS. Algorithms are presented on how to use LS values in clinical practices, to consider pitfalls. In addition, the novel pressure–stiffness–fibrosis sequence hypothesis is introduced and briefly discussed that could stimulate the intensive search to identify the molecular mechanisms underlying liver fibrosis. Finally, open LS-related questions are defined that should be addressed by future clinical and basic research studies.

Pathophysiology of liver stiffness
Liver stiffness – definition

Going through the theory of elasticity is far beyond the scope of this review. However some basic notions are useful to
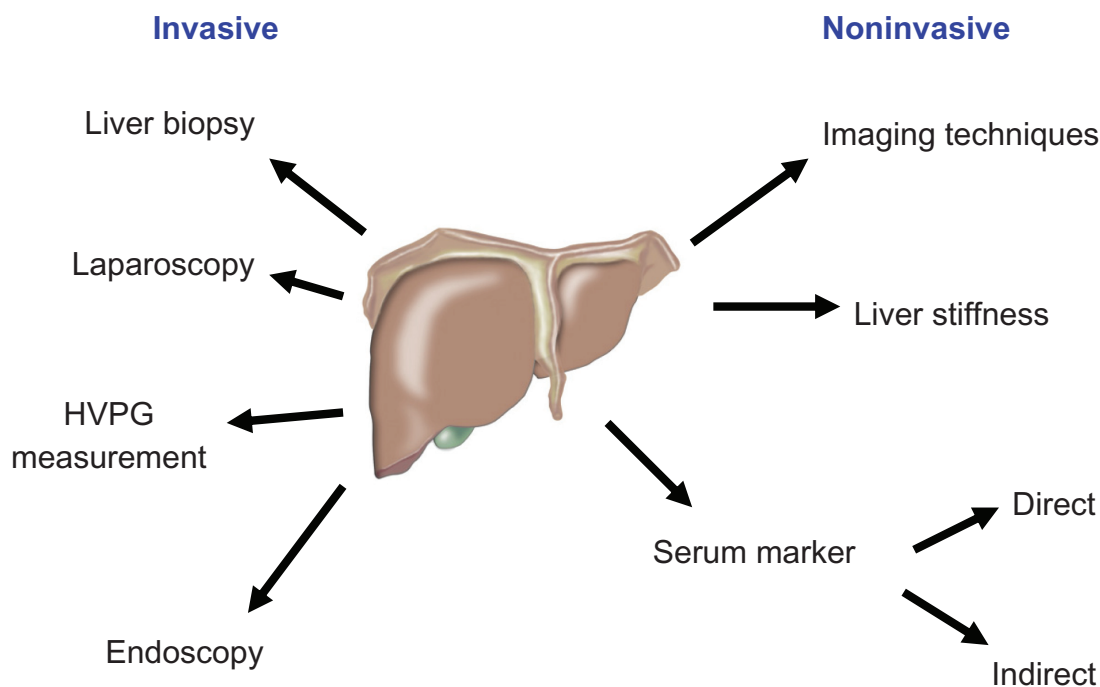

Figure 1 Invasive and noninvasive methods to determine liver fibrosis hepatic venous pressure gradient.

better understand what stiffness means. From a physical and mechanical point of view, stiffness can be defined as the modulus of elasticity or Young's modulus (E). Hooke's law of elasticity is an approximation that states that the extension of a material is directly proportional to the applied stress, $\sigma = E\varepsilon$, where σ is the stress applied to the material, and ε is the strain induced in the material. Stiffness (E) is expressed in kilopascals (kPa) and represents the resistance of material to deformation. While stiff materials, such as concrete, exhibit low strain even at high stress, soft materials such as biological soft tissues exhibit large strain even at low stress.

LS, like any other soft tissue stiffness, depends on many factors. The first and main factor is the extracellular matrix of the organ. The extracellular matrix is a deformable structure that transfers the external forces through the liver. It can be compared to the foundation of a building. A second factor is the constraints that are applied on the organ. The more pressure that is applied to the liver at its boundaries, the stiffer it gets. A third factor is the internal pressure inside the organ – if blood, or another liquid is coming in and out, then stiffness will depend on the resistance that the organ applies to the flow. A fourth and important factor is the viscous effects which influence the time constant over which stiffness is tested. This effect is linked to frequency, ie, stiffness depends on frequency. While liver is soft at very low frequency (on the order of several hertz) which corresponds to manual palpation time-constant, it tends to be much harder at high frequencies (over several tens of kilohertz).

Measurement of liver stiffness using transient elastography (FibroScan®)

The FibroScan® (FS) (Echosens, Paris, France) device is the first elastography technique developed to quantitatively and noninvasively assess soft biological tissue stiffness *in vivo*. Liver was a natural first organ to study due to its size and rather homogenous texture.[8] In principle, shear waves are generated through the liver and LS is deduced from their velocity. FS uses the technique called transient elastography (TE) or vibration-controlled transient elastography (VCTE™). It is based on the controlled generation of a transient shear wave using a servo-controlled vibration of known frequency and amplitude. LS is computed from the velocity of these mechanical waves using the following equation:

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

where E is the Young's modulus or stiffness, ρ is the density, and V_s the shear velocity. The shear velocity measured by VCTE™ is a group velocity around 50 Hz. Minimum and maximum stiffness values that can be measured by FS are 1.5 kPa and 75.0 kPa respectively.

Technically, FS consists in a dedicated acquisition platform that includes a single channel ultrasound analog front end to emit and receive ultrasound signals, and a servo-controlled vibrator for the shear wave generation. The probe itself contains a sophisticated vibrator on the axis of which a single element ultrasound transducer is mounted. As shown in Figure 2, the vibration consists of a sinusoid period with a center frequency of 50 Hz. Its amplitude depends on the probe model: 2 mm peak-to-peak (PP) with the standard probe (model M), 1 mm PP with the pediatric probe (model S), and 3 mm PP with the obese patients dedicated probe (model XL). The shear wave propagation is monitored using ultrafast ultrasound acquisitions.

In the standard examination procedures, LS measurements using FS are performed on the right lobe of the liver in intercostal position (see Figure 3). This prevents direct
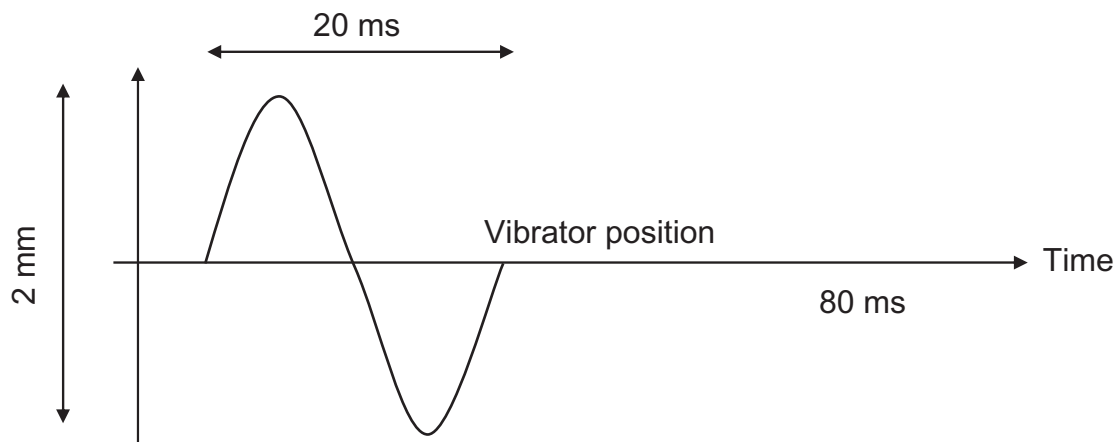
Figure 2 FibroScan® vibration consists of a period with a center frequency of 50 Hz. The standard M probe has a 2 mm peak-to-peak amplitude.
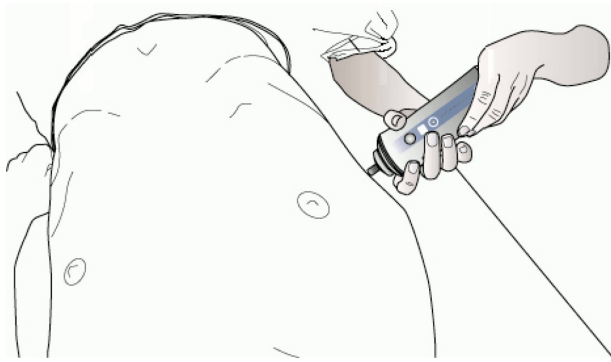
Figure 3 Liver stiffness measurements are performed on the right lobe of the liver in intercostal position using FibroScan®.

compression of the liver that would eventually affect LS values. The patient is lying on his back with the right arm behind the head in order to enlarge intercostals space as much as possible. The operator uses ultrasound M-mode and A-mode images (Figures 4A and B) to locate the liver, and triggers the measurement by pushing on the probe button. The shear wave can be observed on the elastogram image (Figure 4C) which represents the strains induced in the liver as a function of time and depth. It is computed from ultrasound data acquired at a very high frame rate during the shear wave propagation which lasts 80 ms.

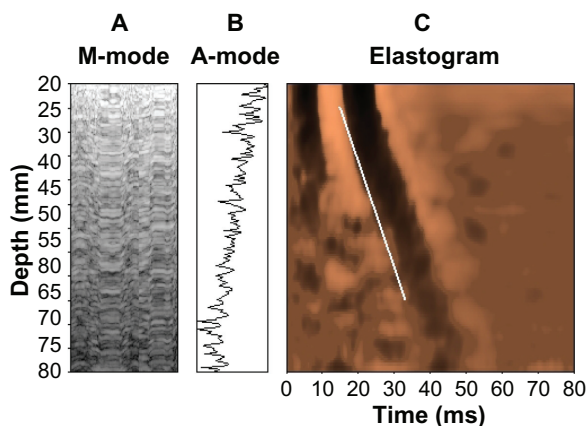## Measurement of liver stiffness using other elastographic techniques and normal stiffness values

Although FS has been the first noninvasive elastographic techniques in practical use to assess LS, other competing-technical approaches have been developed. They are under current cross-validation and it is still too preliminary for a final statement (see Table 1). Magnetic resonance elastography

Figure 4 FibroScan® operator uses **A**) A-mode and **B**) M-mode images to locate the liver. The shear wave velocity is deduced from the **C**) elastogram which represents the strains induced in the liver by the shear wave propagation as a function of time and depth.

(MRE) was introduced in 1995 by Muthupillai[9] and is now commercially available as MR-Touch (General Electric). Rouviere et al[10] measured liver shear stiffness in healthy volunteers and in patients with liver fibrosis. The shear stiffness μ can be deduced from Young's modulus E (as measured by FS) using the simple relationship: $\mu = E/3$. Klatt et al measured the shear elastic modulus in 12 healthy volunteers and two patients.[11] Results obtained on volunteers are close to 6 kPa when converted in Young's modulus. MRE looks very promising. It seems to have a smaller standard deviation and, naturally, offers the combination of magnetic resonance imaging and elastography in one setting for different organs. However, it is an expensive, time consuming, certainly not a bedside procedure, and cannot be used in the setting of metal implants. FS has been directly cross-validated with MRE using artificial phantoms with an excellent correlation of $r = 0.96$.[12,13] A linear correlation between LS and fibrosis stage has been observed in animal fibrosis models using MRE.[14]

In addition to FS, various ultrasound-scanner-compatible elastography procedures are currently being evaluated. FS should not be mismatched with conventional static elastography that is now integrated in many ultrasound devices. The first system based on static elastography was the real time elastography (HI-RTE). It allows a visualization of relative stiffness within a B-ultrasound image using a red and blue color map. However, HI-RTE does not allow the quantitative measurement of stiffness values and, hence, pilot studies did not show a satisfying correlation with fibrosis score as compared to FS.[15]

More recently, several techniques[16–18] based on radiation force[19] have been proposed for LS measurement. These techniques use high intensity ultrasound beams to induce displacements inside the liver remotely. Acoustic Radiation Force Impulse (ARFI) with Virtual Touch™ tissue quantification has been introduced by Siemens (German). First ARFI-based results have been presented at international meetings in cross-validation with FS. Reasonable areas under receiver operating characteristic curves (AUROCS) for F3-4 fibrosis >0.86 have been presented on various diseases with excellent interobserver variability of 0.98[20,21] and a good correlation with FS of $r = 0.65$.[22] In contrast to FS, ascites does not impose a limitation to ARFI. However, up to now, FS seems to outscore the identification of F2-4 fibrosis stages with regards to diagnostic accuracy.[21]

Since the physiological determinants of LS are not completely understood and the detection methods vary considerably, it is still a debate how to define normal LS values. In a recent study, we could demonstrate that simple

Table 1 Comparison of various techniques to assess liver stiffness

	Method	Product name	Vibration mode/source	Frequency	Advantages	Limitations
Static elastography	Quasi-static compression	eg, by Hitachi	None	Not applicable	Widely available in ultrasound scanners	Qualitative only
Magnetic resonance elastography	Shear wave	Optima MR450 w 1.5 T	Continuous mechanical actuator	50–60 Hz	2D/3D stiffness mapping, frequency controlled vibration, other organs	Expensive, metal implants (pace makers, bone implants)
Acoustic radiation force impulse	Shear wave	Acuson S2000	Transient radiation force		Ascites, other organs	Accuracy, limited clinical data
Vibration-controlled transient elastography	Shear wave	FibroScan®	Transient mechanical actuator	50 Hz	Largely validated, frequency controlled vibration	Sensitive to body habitus (obesity, ascites, bowel interpolate)

breath maneuvers such as valsalva, or position changes such as laying to standing position, can dramatically either permanently or temporarily increase LS up to the upper detection limit of 75 kPa.[23] This study could also demonstrate that a horizontal position with normal breathing yields the lowest and most reproducible LS values. According to our experience, LS of <6 kPa can be considered as normal.[23] Confirmation has come from a large screening study obtained on 1067 blood donors median with a medium LS of 4.4 kPa (95th centile 6.7).[24] Tables 1 and 2 give an overview of recently reported stiffness values for liver and other organs obtained with different techniques for normal and pathological conditions.

Liver stiffness assessment by FibroScan® – practical experience

The major success of FS in measuring LS can be mainly explained by its true bedside test character that can be performed within 5–10 minutes. After a rapid training, FS provides a reasonable performance for the diagnosis of cirrhosis that is not influenced substantially by any other feature.[25] FS has an excellent interobserver rate especially without elevated transaminases[26] and a fast learning curve.[27] In addition, no significant difference in LS values have been found whether they were obtained from the fifth, sixth and seventh intercostal space.[28] Thus, in general, FS measurements can be routinely performed in more than 95% of patients. Major limitations are severe obesity and ascites that directly weaken the ultrasound signal.[29] In some collectives such as patients with decompensated cardiac insufficiency, the successful performance of FS can drop down to ca. 50%.[30] However, with the development of the novel XL probe, these obstacles could be drastically overcome. In our own preliminary experience, we found that the XL probe could measure LS in 70% of patients where the normal M probe were not applicable. Moreover, the XL probe could not only be successfully applied to severely obese patients but also to patients with ascites and lean patients with an ultrasound-diffracting subcutaneous fat tissue. It will be interesting to learn in the future why some nonobese people are critical to be measured by FS.

Potential artificial results obtained by FibroScan®

Shear wave propagation in soft biological tissues might be very complex. Thus, LS is calculated as the median of 5 to 10 valid measurements. Outliers are removed and the interquartile range is provided as a mean to check for the quality of the measure. Furthermore, FS implements special algorithms to automatically reject incorrect measurements which are ranked invalid and are thus not included in stiffness calculation. However some cautions must be taken, especially with probe perpendicularity and rib cage intercostal spaces.

Table 2 Stiffness and shear velocity of liver and other organs by various methodological approaches

	Liver	Pancreas	Spleen	Kidney	Ref.
MRE*	~2.2 kPa (60 Hz)	~2.0 kPa (60 Hz)		~7.3 kPa (90 Hz)	107
ARFI	1.16–1.59 m/s	1.4 m/s	2.44 m/s	2.24 m/s	108,46,20,109
VCTE/FS	4–6 kPa (50 Hz)				23,24

Note: *Young's modulus E (as measured by VCTE/FS) is three times higher than the MRE-measured shear stiffness μ according to the following equation: μ = E/3.
Abbreviations: MRE, magnetic resonance elastography; ARFI, acoustic radiation force impulse; VCTE, vibration-controlled transient elastography™; FS, FibroScan®.

First, it is important that the probe is placed perpendicular to the skin surface when measuring LS to prevent overestimation which could happen if the shear wave propagation is misaligned with the ultrasound beam. Second, the probe model should be adapted to the patient morphology so that the ribs do not contribute to the shear wave generation. This would affect the measurement quality by inducing secondary shear waves. Although diffraction effects by ribs are rare, they may lead to confusion and misinterpretation.

Interestingly, shear waves do not propagate through liquids because they are elastic waves and only pressure waves can propagate through liquids such as those which are used by ARFI. For this reason patients with ascites may not be measurable with FS as far as no physical contact exists between the liver and the intercostals wall.

(Patho)physiology of liver stiffness
Liver stiffness as surrogate marker of fibrosis stage

LS has mainly been studied in patients with viral hepatitis B and C (HBV and HCV),[8,25,31–37] and to a lesser extent in alcoholic liver disease (ALD)[38–40] and primary biliary cirrhosis (PBC)/primary sclerosing cholangitis (PSC).[41–43] In contrast, only random and preliminary reports exist on autoimmune hepatitis[44,45] and nonalcoholic liver disease (NALD).[46,47] Table 3 shows the performance of LS to assess fibrosis stages F3 and F4 for various diseases (selected studies). Table 4 compares normal and fibrotic stiffness values obtained by different methods. The major experience of these studies can be summarized as follows:

a. LS correlates well with fibrosis stage typically with an $r > 0.7$ and $P < 0.005$.

b. Advanced fibrosis stage F3 and cirrhosis (F4) are identified via LS with high accuracy (AUROC > 0.9).

This is mainly due to the so called bridging fibrosis (the continuous formation of collagen septa between liver lobuli) that are characteristic for these fibrosis stages. In contrast, fibrosis stages F1 and F2 only mildly increase LS. Therefore, these fibrosis stages are not well discriminated via the measurement of LS.

c. Cut-off values have been defined that allow the diagnosis of advanced fibrosis (F3/F4). Despite some variability, cut-off values of 8.0 and 12.5 kPa are widely accepted to identify patients with F3 and F4 fibrosis, respectively (Figure 5).

It has also become rapidly clear that cut-off values differ between various chronic liver diseases, being tentatively higher in disease with pronounced inflammation or cholestasis such as ALD, PSC or PBC. This is one reason to ask for studies with well defined and homogenous patient populations. Potential causes for varying cut-off values will be discussed below.

Fibrosis assessment by liver stiffness and comparison with other noninvasive fibrosis markers/techniques
Imaging techniques

Since abdominal ultrasound is routinely and rapidly performed in liver patients, a few studies have naturally asked the questions whether LS provides additional information with regard to fibrosis. In comparison to FS, ultrasound is a subjective examination that largely depends upon the experience of the examiner. It is not always clear that only a few ultrasound signs such as nodular aspects of the liver surface, or vascular collaterals are so called sure ultrasound signs of liver cirrhosis (but not splenomegaly or ascites). In an actual larger study on 320 patients with various liver disease, the diagnostic accuracy of LS was significantly superior to ultrasound.[48] In our own experience, FS recognized generally more than twice

Table 3 Liver stiffness and fibrosis stages in various liver diseases

Disease	N	Fibrosis-LS correlation	AUROC F3	AUROC F4	Cut-off F3	Cut-off F4	Ref.
HCV	193		0.9	0.95	9.5	12.5	32
HCV	935		0.89	0.91			25
HCV/HIV	72	0.48; $P < 0.0001$	0.91	0.97		11.9	37
HBV	202	0.65; $P < 0.001$	0.93	0.93		11.0	110
ALD	103	0.72, $P < 0.014$	0.9	0.92	11	19.5	38
ALD	45			0.97		25.8	39
ALD	101	0.72; $P < 0.001$	0.91	0.92	8	11.5	40
NAFLD	246		0.92	0.95	7.9		47
PBC/PSC	101	0.84, $P < 0.0001$	0.95	0.96	9.8	17.3	42
PBC	80			0.96			43

Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; HBV, hepatitis B virus; ALD, alcoholic liver disease; NAFLD, nonalcoholic fatty liver disease; LS, liver stiffness; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; AUROC, areas under receiver operating characteristic curves.

Table 4 Comparison of liver stiffness obtained by various techniques for normal and cirrhotic livers

	Normal	Fibrosis (F3)	Cirrhosis (F4)	Ref.
MRE*	2 kPa (90 Hz)		5 kPa (90 Hz)	10
ARFI	1.5 m/s	1.8 m/s	>1.95 m/s 2.1–2.3 m/s	111 20,109
VCTE/FS	2–6 kPa (50 Hz)	>8 kPa (50 Hz)	>12.5 kPa (50 Hz)	see above23

Note: *Young's modulus E (as measured by FibroScan/VCTE/FS) is three times higher than the MRE-measured shear stiffness μ according to the following equation: $\mu = E/3$.
Abbreviations: MRE, magnetic resonance elastography; ARFI, acoustic radiation force impulse; VCTE, vibration-controlled transient elastography™; FS, FibroScan®.

of patients with F3/4 fibrosis compared to ultrasound.[49] This means in numbers, that more than 20 patients with F3/4 fibrosis were not recognized by routine ultrasound, while FS identified almost all 45 patients. It should be pointed out that these are results for a typical clinical routine ultrasound performed within 15–20 min; the accuracy of ultrasound can certainly be increased by a more meticulous and time consuming procedure. However, the time-intensive ultrasound is still subjective and can typically not be performed during the daily practice in most regular hospitals and outpatient departments. Therefore, as a rule of thumb, the rapid 5–10 min FS recognizes ca. twice as many patients with advanced fibrosis as compared to the routine ultrasound.

Serum markers

Although serum markers that are used within scores such as the Fibrotest, APRI score, etc, are widely explored and have been also cross-validated with FS,[22,35,50–54] the authors, up to now, do not generally recommend their use and FS seems to outscore all of these tests. However, we admit, as will be discussed below, that a combination and a refined algorithm using elastography, serum markers, and imaging techniques may optimize a cost-efficient screening for liver fibrosis in certain settings or spare patients from invasive histology.[55] The major problem is that serum markers reflect the profibrogenic or profibrolytic activity, but do not yield any information about the net deposition of matrix in the liver which are not necessarily correlated to each other.
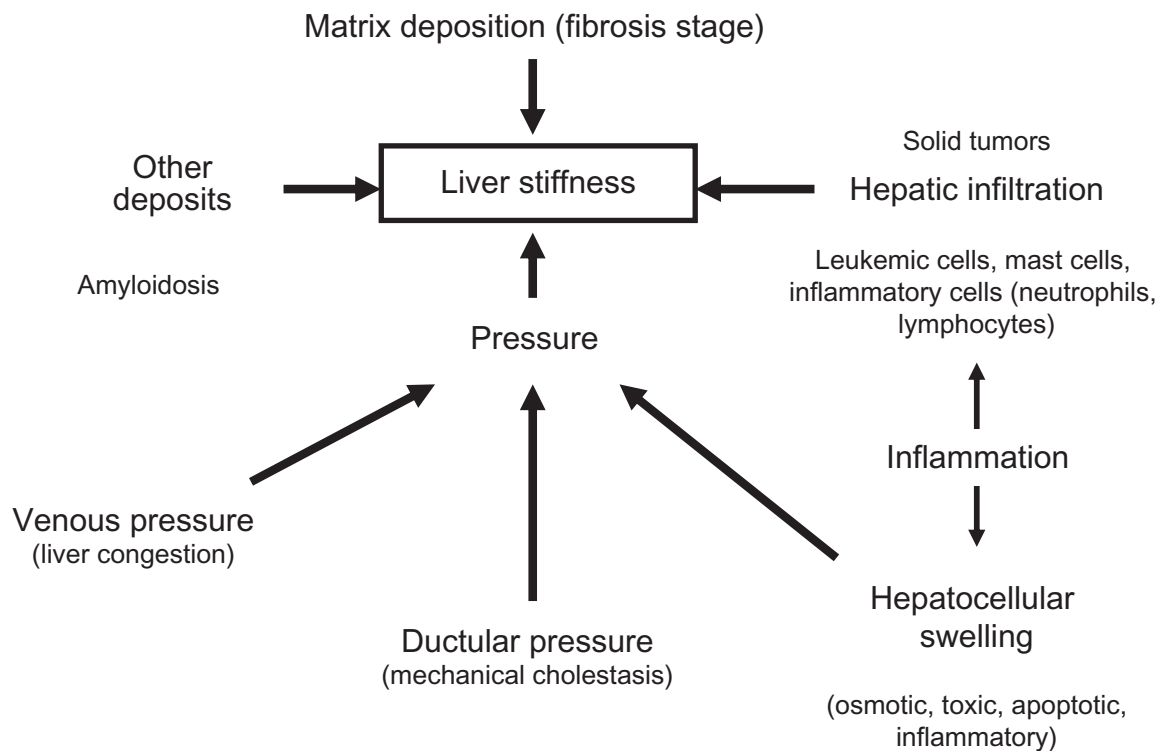
Other factors that increase liver stiffness

It has been rapidly learnt that LS is also increased by other confounding factors such as hepatitis, mechanic cholestasis, liver congestion, cellular infiltrations, and deposition of amyloid irrespective of fibrosis stage (see Figures 5 and 6). These important interferences will now be discussed in more detail. It should be mentioned that steatosis does not increase LS[40,56] although it is often regarded as an essential initial state in chronic liver disease. Rather, steatosis may slightly decrease LS.

Inflammation (hepatitis)

LS can be dramatically increased during laboratory signs of hepatitis[50,57,58] independent of the degree of fibrosis. These conditions may increase LS to a degree that would otherwise suggest advanced liver cirrhosis (ie, stiffness values of 12.5 kPa and above). In our recent studies on patients with
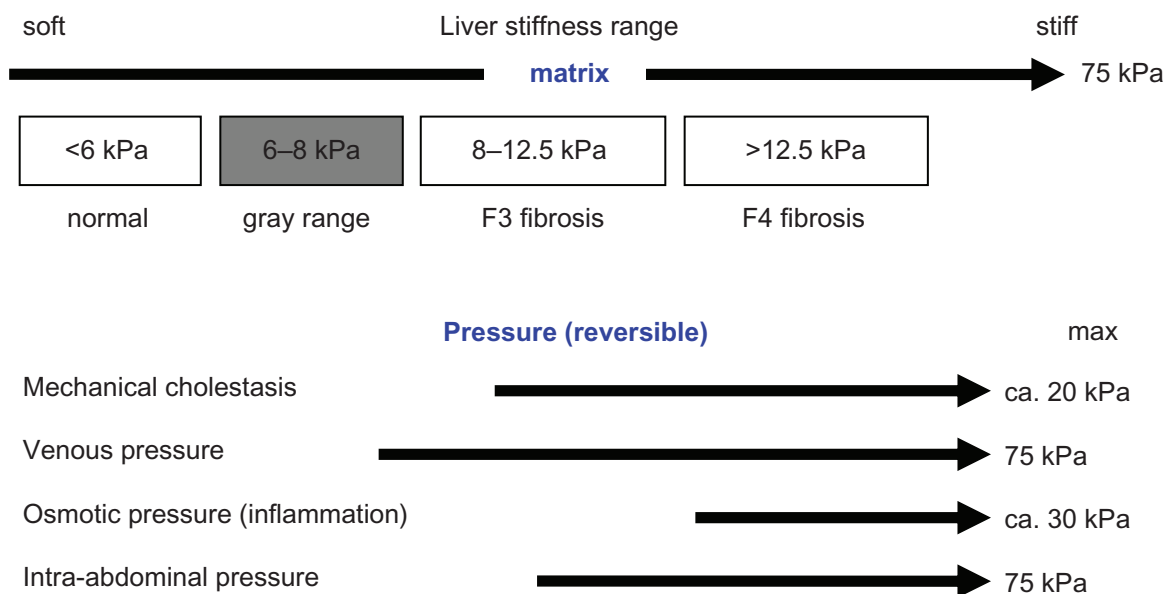
Figure 5 Liver stiffness range caused by matrix deposition (fibrosis) and pressure changes (osmotic, hydrostatic, intra-abdominal).

Matrix deposition (fibrosis stage)

Liver stiffness

Other deposits

Amyloidosis

Solid tumors

Hepatic infiltration

Leukemic cells, mast cells, inflammatory cells (neutrophils, lymphocytes)

Pressure

Venous pressure
(liver congestion)

Ductular pressure
(mechanical cholestasis)

Inflammation

Hepatocellular swelling

(osmotic, toxic, apoptotic, inflammatory)

Figure 6 Not only matrix but also pressure-associated conditions influence liver stiffness.

ALD undergoing alcohol detoxification, LS was initially increased up to 50 kPa but could decrease within 1 week by 30 kPa.[40] In HCV patients with biochemical remission (either spontaneous or after antiviral therapy), LS was lower than in patients with identical fibrosis stage, but elevated alanine transaminase (ALT). The LS dynamic profiles paralleled those of ALT, increasing 1.3- to 3-fold during ALT flares in patients with hepatitis exacerbations.[50] In patients with HBV infection, fibrosis assessment was unreliable if serum transaminases were higher than twice of normal values.[59] In our experience, ongoing biochemical activity of liver disease in form of increased transaminases leads to an overestimation of fibrosis stage, since hepatitis per se increases LS, irrespective of fibrosis. What are the underlying factors leading to increased LS in these patients? In our sequential FS study on 50 patients with ALD undergoing alcohol detoxification we could show the following phenomena:[40]

a. All transaminase levels decreased during alcohol detoxification, and almost all LS values decreased during the observation interval.

b. The higher the decrease in transaminases was, the higher was the decrease of LS.

c. Excluding patients with significant ongoing biochemical activity of hepatitis from fibrosis assessment by FS significantly improved AUROC for F3/4 fibrosis.

d. Additional histological information on inflammation did not further improve the diagnostic accuracy.

This study thus shows that, at least in patients with ALD, serum transaminases truly reflect the degree of hepatitis and that the inflammation is a critical factor determining LS. In our patient population, the decrease of aspartate aminotransferase (AST) correlated better with the decrease of LS as compared to ALT. It is interesting to learn that in HCV infected patients similar observations have been made. Here, AST was found to be the unique variable significantly related ($P = 0.046$) with discordance between biopsy and LS.[60] Subanalysis of histological scores with LS values was also very revealing. Here, necrosis, hepatocyte swelling and the degree of inflammation correlated with LS but not steatosis. This has been partly confirmed in a recent study on patients with nonalcoholic fatty liver disease (NAFLD).[47] We conclude from our study that patients with an AST > 100 U/L lead to an overestimation of fibrosis stage. These patients should be first detoxified from alcohol and LS should be obtained after normalization. A refined algorithm will be discussed below.

Cholestasis

In a recent study on 15 patients with mechanic cholestasis due to tumor obstruction (pancreas carcinoma, Klatskin

tumor, liver metastases, and gastrointestinal stromal tumor [GIST]) or choledocholithiasis, we could demonstrate that mechanical cholestasis per se can drastically and reversibly increase LS.[61] LS correlated significantly with a decrease in bilirubin, but not with gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (AP), AST, or ALT. We further confirmed the direct relation between LS and choletasis in bile duct ligation experiments on landrace pigs. The bile duct ligation over 120 min led to a significant swelling of the liver and a tightly palpable gall bladder. LS values doubled during bile duct ligation and reached values suggesting F3 fibrosis. After removal of the bile duct ligation and a recovery period of 30 min, LS values returned to almost normal values around 6.1 kPa. The reasons underlying the high stiffness in cholestasis are unknown but could be related to tissue swelling, edema and increased intracellular pressure due to impaired bile flow. In addition, cholestasis might be a general phenomenon leading to increased LS in various chronic liver diseases as intrahepatic cholestasis has been shown to correlate strongly with LS in patients with acute hepatitis[58] but also ALD.[40]

Liver congestion and venous pressure

Random observation had suggested earlier that FS is unreliable in patients with liver congestion, for example due to cardiac insufficiency. We could recently demonstrate that the central venous pressure directly controls LS in a reversible manner.[30] Over a wide range, LS is a linear function of intravenous pressure reaching the upper detection limit of 75 kPa at an intravenous pressure of 36 cm water column. We eventually showed in 10 patients with decompensated congestive heart failure that LS is dramatically elevated under such pathological conditions and rapidly decreases during clinical recompensation due to diuretic therapy. Since fibrosis state cannot change within such a short period of time, these findings further underline the direct dependence of LS on venous pressure. The majority of patients with decompensated cardiac failure had initial LS far above the cut-off value of 12.5 kPa which is generally accepted for the diagnosis of F4 fibrosis, reaching up to 51.3 kPa. Although LS decreased in all patients during therapy with diuretics it only fell below 12.5 kPa in two of them while seven remained in the range of F4 fibrosis. Older age as a reason for increased LS can be excluded as a recent study by Sirli and colleagues showed.[62] Thus, increased LS could be due to the onset of cardiac fibrosis in these cases, and fibrosis assessment by FS will be especially challenging in patients with cardiac insufficiency since both fibrosis and venous pressure increase LS. It also remains questionable in this context whether recently reported increased LS in patients with failing Fontan circulation was indeed due to cardiac liver fibrosis,[63] or just elevated central venous pressure since no sequential LS measurements were performed. On a special note, LS may become a useful noninvasive tool for screening cardiac patients and identifying those that are at risk of cardiac cirrhosis since increased venous pressure (but not abnormal liver function tests) has been recognized as major risk factor of cardiac fibrosis.[64]

Liver infiltration, deposits, rare diseases

It is a daily experience of surgeons that hepatic tumor infiltration increases LS. Therefore, focal or nodular masses within the liver should be excluded by ultrasound prior to FS. However, since not all hepatic masses can be detected by ultrasound, one should be aware of such potential misinterpretations of LS measurements. A typical finding during LS measurements in, for example, a metastatic liver, are extremely variable stiffness values that clearly depend on position changing of the probe.[61]

However, also rare and less visible infiltration with mast cells can also lead to dramatically increased LS.[23] We recently reported on a patient with systemic mastocytosis showing an LS of 75 kPa (upper detection limit). The patient had otherwise suspicious signs of liver cirrhosis (splenomegaly, ascites, varices). However, liver synthesis was normal and the differential blood count showed an increased number of mast cells. Diagnosis was ultimately confirmed by liver biopsy. An important noncancerous differential diagnosis of increased LS is amyloidosis. Increased LS due to amyloid deposits has been demonstrated in animal models (submitted by Sandrin L, et al) and humans with amyloidosis A.[65, 66] Interestingly, all these clinical entities showed pronounced hepatomegaly.

Liver stiffness and clinical end points

The ultimate goal of novel medical techniques should be to improve diagnosis or therapy of human disease. Therefore, with regard to LS, we would like to see whether it improves the early recognition of cirrhosis-related complications such as portal hypertension, esophageal varices, primary liver cancer or the response to therapies.

Liver stiffness and portal hypertension

Since fibrosis increases the hepatic vascular resistance and ultimately leads to portal hypertension (see Figure 7), it
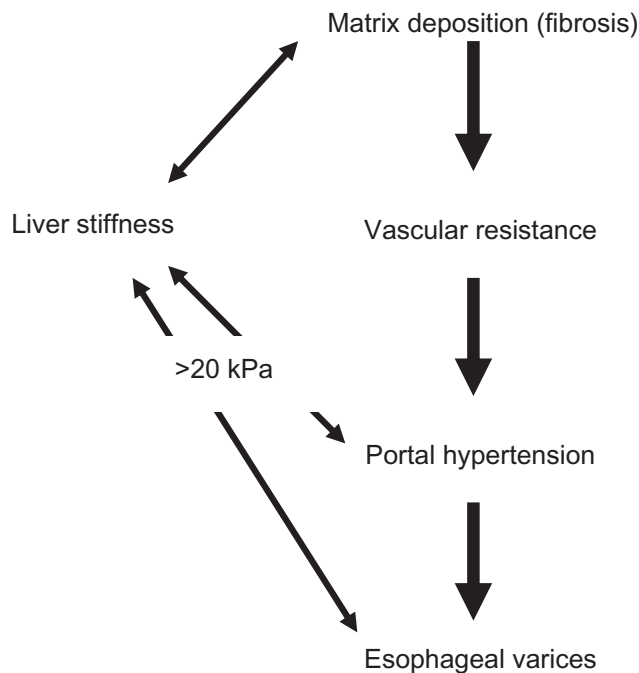
Figure 7 Relation of liver stiffness with clinical fibrosis-related entities such as fibrosis stage, portal hypertension and esophageal bleeding.

was just a matter of time to test whether LS could be used as a diagnostic test for portal hypertensions. Meanwhile, several studies have compared LS directly against invasive hepatic venous pressure gradient (HVPG) or the presence of esophageal varices in adults (0.84–0.86)[54,67–73] and children.[70] As shown in Table 5, there is an excellent direct correlation between LS and HVPG (0.84–0.86)[67–69] with an AUROC for detection of significant HVPG (>6–12 mm Hg) of 0.92–0.99.[67–69] A cut-off value of ca. 20 kPa (13.6–34.9 kPa) predicted significant HVPG.[67–69] Interestingly, lower values were found for HCV (ca. 20 kPa) as compared to ALD (34 kPa). More interestingly, LS correlated with the degree of esophageal varices (r = 0.6, $P < 0.0001$)[71] and the AUROC for the prediction of significant varices was 0.71–0.95 with a comparable cut-off of ca. 20 kPa (see Table 6).[54,69–73]

Figure 8 and Table 7 explain the more complex relation of liver and spleen stiffness with regard to the location of a potential thrombosis in the porto-caval system. This might explain why additional assessment of spleen stiffness could be better to predict portal hypertension and varices.[74] In addition, cirrhosis develops in post or sinusoidal thrombosis,[75,76] but not in presinosoidal idiopathic portal hypertension (IPH).[77] Hence, no increased LS can be detected in patients with IPH and this explains why in some patients a normal LS does not exclude portal hypertension and the presence of varices. Indeed, a recent report documented five patients presented with variceal bleeding,

two with splenomegaly, and one with ascites. All had large esophageal varices. Median HVPG was 8 mm Hg (range 3.5–14.5), clearly underestimating the true portal pressure due to the presinusoidal component of portal hypertension. Median LS was 8.9 kPa (range 6.8–14.9) and was unreliable in predicting the presence of fibrosis or of esophageal varices.[77]

Liver stiffness and disease follow up
Follow up studies in viral hepatitis C patients

Meanwhile, several longitudinal studies have been reported on LS during HCV treatment. Vergniol et al studied 416 patients, of whom 112 started treatment after enrolment. In multivariate analysis, treatment was the only factor independently associated with a fall in LS.[78] Ogawa et al prospectively studied 145 Japanese patients with chronic HCV infection at baseline, at the end of treatment, and at 48 and 96 weeks after the end of treatment. LS significantly decreased in the groups with sustained virological response and biochemical response but not in the nonresponders.[79] Andersen et al prospectively studied 114 Japanese patients with chronic HCV median follow up 47–48 months. In this study, LS was significantly lower for patients with sustained viral response (SVR). The differences were more pronounced in the F2-F4 fibrosis group.[80]

Liver stiffness and alcoholic liver disease follow up

We recently performed a sequential FS study in patients with ALD undergoing alcohol detoxicification[40] to test if inflammation also interferes with LS assessment in ALD, and to provide a clinical algorithm for reliable fibrosis assessment in ALD by FS. We first performed sequential LS analysis before and after normalization of serum transaminases in a learning cohort of 50 patients with ALD admitted for alcohol detoxification. LS decreased in almost all patients within a mean observation interval of 5.3 d. Six patients (12%) would have been misdiagnosed with F3 and F4 fibrosis but LS decreased below critical cut-off values of 8 and 12.5 kPa after normalization of transaminases. Of the serum transaminases, the decrease in LS correlated best with the decrease in glutamic oxaloacetic transaminase (GOT). No significant changes in LS were observed below GOT levels of 100 U/L. After establishing the association between LS and GOT levels, we applied the rule of GOT < 100 U/L for reliable LS assessment in a second validation cohort of 101 patients with histologically confirmed ALD. By excluding those patients with GOT > 100 U/L at the time

Table 5 Liver stiffness and hepatic venous pressure gradient

Patients	N	HVPG vs LS correlation	HVPG (mm Hg)	AUROC for significant portal hypertension	Cut-off for significant portal hypertension	Ref.
HCV	150	0.858; $P < 0.001$		0.945	21 kPa	[112]
HCV, ALD	92	0.76			20.5 kPa (HCV) 34.9 kPa (ALD)	[68]
Liver transplant patients	124	0.84; $P < 0.001$	>6	0.93		[67]
HCV	61	0.81, $P < 0.0001$	>10	0.99	13.6 kPa	[69]
			>12	0.92	17.6 kPa	

Abbreviations: HCV, hepatitis C virus; ALD, alcoholic liver disease; HVPG, hepatic venous pressure gradient; LS, liver stiffness; AUROC, areas under receiver operating characteristic curves.

of LS assessment from this cohort, the AUROC for cirrhosis detection by FS improved from 0.921 to 0.945 while specificity increased from 80 to 90% at a sensitivity of 96%. A similar AUROC could be obtained for lower F3 fibrosis stage if LS measurements were restricted to patients with GOT < 50 U/L. Histological grading of inflammation did not further improve the diagnostic accuracy of LS. In conclusion, coexisting steatohepatitis markedly increases LS in patients with ALD, independent of fibrosis stage. Postponing cirrhosis assessment by FS during alcohol withdrawal until GOT decreases to <100 U/mL significantly improves the diagnostic accuracy.

Liver stiffness and hepatocellular carcinoma

Some studies have tested whether LS allows the prediction of HCC risk since cirrhosis is an independent risk factor of HCC. Foucher et al reported a cut off values for the presence of HCC of 53.7.[81] Several studies have now looked in more detail into the relation of HCC and LS.[54,72,73,82–84] As can be seen from Table 8, an LS of >20 kPa drastically increases the risk for HCC. Not by coincidence, this cut-off value is almost identical with the cut-of value for esophageal varices and significant portal hypertension.

Liver stiffness and surgery
Liver stiffness and liver transplant

Risk stratification of patients on the liver transplant waiting list is still an unresolved challenge, but the limited organ supply asks for more quantitative risk assessment strategies. LS could be a supplemental quantitative method since it recognizes pathological states of the liver that could all worsen the outcome such as fibrosis, inflammation, venous pressure, cholestasis, or portal hypertension. In a post-transplant study on patients infected with HCV, median LS at months 6, 9, and 12 were significantly higher in rapid fibrosers as compared to slow fibrosers. The slope of LS progression in rapid fibrosers was significantly greater than in slow fibrosers, suggesting two different speeds of liver fibrosis progression.[85] Multivariate analysis identified donor age, bilirubin level, and LS as independent predictors of fibrosis progression and portal hypertension in the estimation group.[85] Another study suggested that TE is a reliable tool to assess liver fibrosis in patients with recurrent HCV after living donor liver transplantation.[86]

Table 6 Liver stiffness and prediction of esophageal varices

Patients	n	Cut-off for varices	AUROC	Sensitivity	Specificity	PPV/ NPV	Ref.
HCV	65	17.6 kPa	0.76	0.9			[69]
Children with biliary atresia	49	9.7 kPa		0.97	0.8		[70]
Cirrhosis	165	19.5 kPa	0.83	0.84		47/93	[71]
HBV LSM-spleen diameter to platelet ratio score (LSPS)	90		0.95	0.947			[72]
HCV		21.5 kPa		0.76	0.78		[54]
HIV/HCV coinfected patients with liver cirrhosis	102	21 kPa	0.71			100	[73]

Abbreviations: HCV, hepatitis C virus; HBV, hepatitis B virus; HIV, human immunodeficiency virus; ALD, alcoholic liver disease; NAFLD, nonalcoholic fatty liver disease; LS, liver stiffness; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; AUROC, areas under receiver operating characteristic curves; PPV, positive predictive value; NPV, negative predictive value.
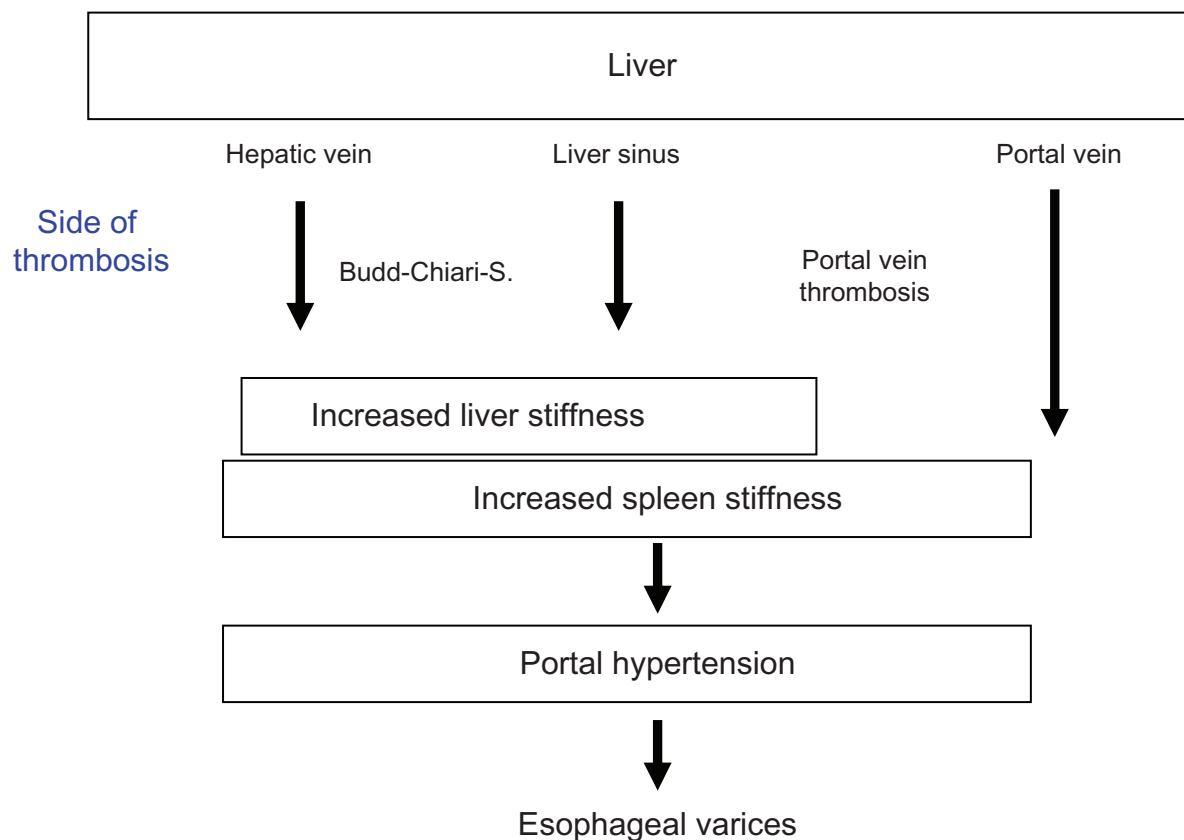
Figure 8 Liver stiffness is increased in post-sinusoidal thrombosis (eg, Budd-Chiari-Syndrome) but not in pre-sinusoidal thrombosis (eg, portal vein thrombosis). Additional measurement of spleen stiffness closes the diagnostic gap.

Liver stiffness and hepatectomy

Tactile stiffness sensors have been evaluated in the pre-FS era with success in patients with partial hepatectomy to predict the sufficient remain liver mass.[87–89] It remains open whether FS will add to the evaluation of critical liver mass especially in fibrotic patients prior to partial hepatectomy.

Present algorithm to diagnose liver disease via liver stiffness

Various algorithms have been presented mainly for viral hepatitis to use LS in combination with blood tests to improve the noninvasive diagnosis of liver fibrosis or to spare at least some patients from the invasive liver biopsy.[55,90] Given the many interfering factors that modulate LS, however, we are somewhat skeptical about using such approaches. Such statistical approaches aim to automate a complex diagnostic decision procedure. At the end, a unique patient requires an individual differential diagnosis, and a careful balance of the various risks has to be kept. Just to mention one example, many patients with viral hepatitis do have additional liver diseases such as alcoholic liver disease or suboptimal dietary condition. These are all factors that can dramatically worsen the outcome of chronic hepatitis in a synergistic manner.[91] With this regard, at least to us, it is more useful to view LS

Table 7 Liver stiffness and portal hypertension by pre-and postsinusoidal thrombosis

Thrombosis	Disease	Fibrosis	Portal hypertension	Liver stiffness
Presinusoidal	Portal vein thrombosis	no	yes	normal
	Idiophatic portal hypertension	no	yes	normal, slightly elevated
Sinusoidal thrombosis	hepatic veno-occlusive disease (sinusoidal obstruction syndrome)	yes	yes	elevated
Postsinusoidal thrombosis	Budd-chiari syndrome	yes	yes	elevated

Table 8 Liver stiffness and risk of hepatocellular carcinoma

Patients	N	Liver stiffness	HCC likelihood	Ref.
HCV	262	<10 kPa 10.1 to 15 kPa 15.1 to 25 kPa >25 kPa	0.22 0.73 1.3 5.0 (stratum-specific likelihood ratios)	82
HCV, prosepctive study	984	10.1–15 kPa 15.1–20 kPa 20.1–25 kPa > 25 kPa	16.7 20.9 25.6 45.5 (hazard ratio, as compared to LSM ≤ 10 kPa)	83
HCV, ALD	265		Patients with HCC had higher LS than patients without HCC; 35.3 vs 19.0 kPa	84
HBV LSM-spleen diameter to platelet ratio score (LSPS)	90		0.95	72
HCV		21.5 kPa		54
HIV/HCV-coinfected patients with liver cirrhosis	102	21 kPa	0.71	73

Abbreviations: HCV, hepatitis C virus; ALD, alcoholic liver disease; HBV, hepatitis B virus; HIV, human immunodeficiency virus; LS, liver stiffness; HCC, hepatocellular carcinoma.

as a novel physical parameter such as, for example, body temperature – which can be objectively measured and should then be interpreted in the full clinical context. We propose this more open and critical procedure since misinterpretations or biases can rapidly harm the patient and delay other important diagnostic or therapeutic measures.

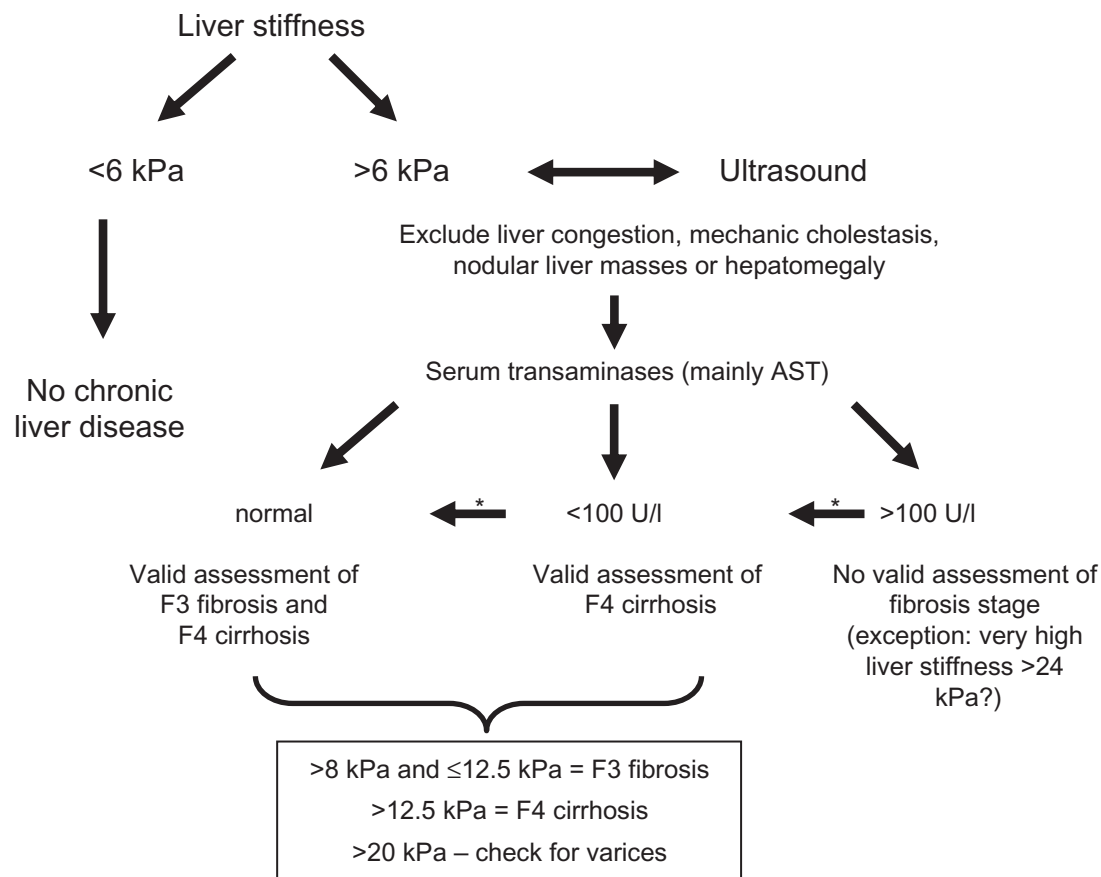A general actual scheme for the interpretation of LS is shown in Figure 9.

Although, the definition of normal stiffness values are still under discussion and need to be defined for various populations with regard to age, gender, or other factors, recent populations of healthy blood donors or the influence of position changes and breath maneuvers suggest an LS < 6 kPa as normal.[23,24] Moreover, at least in our experience, an LS < 6 kPa seems to exclude any manifest liver disease since all potential confounding factors such as inflammation, cholestasis or congestion increase LS. LS measurements are therefore an ideal screening tool to exclude any severe ongoing liver disease. Of course, one should be aware that other pathological conditions such as fatty liver or even terminal liver failure do not increase LS further, or may even decrease LS, but these conditions are easily discernible within the clinical context. If LS is higher than 6 kPa an ultrasound is required to exclude mechanic cholestasis,[61] liver congestion[30] or nodular masses.

Typically, we obtain the ultrasound before stiffness measurements since other valuable information such as splenomegaly, ascites or signs of liver disease can be detected. In addition, the location of an optimal stiffness measurement

is identified. Thus, it becomes rapidly clear that a valid interpretation of LS is only possible in association with a qualified abdominal ultrasound.

If ultrasound does not reveal any of the stiffness-modulating factors above, serum transaminases should be obtained. If the serum transaminases are normal, LS can be directly used to quantitate the degree of fibrosis. If the serum transaminases, mainly AST, are below 100 U/L, the diagnosis of F4 fibrosis is highly accurate while F3 fibrosis should be viewed with caution. At AST levels higher than 100 U/L, an accurate determination of fibrosis stage is not possible. It should be mentioned that these transaminase cut-off values have been obtained for patients with ALD[40] and future studies are required to determine the conditions for other liver diseases.

The context-related interpretation of LS is more difficult in the case of several stiffness-related factors such as inflammation/fibrosis or liver congestion/cardiac cirrhosis. However, under certain conditions, a decision is still possible. For instance, in the case of ALD, the diagnosis of F4 cirrhosis can be made at LS > 24 kPa despite ongoing severe alcoholic steatohepatitis.[40] Such upper cut-off values need to be confirmed and defined for all other liver diseases in larger populations (see Figure 9). In addition, if possible, therapeutic interventions may help to more accurately differentiate fibrosis stage from other LS-increasing confounding factors. Thus, if liver congestion in a patient with congestive heart failure can be clearly cured by therapy with diuretics

Liver stiffness

<6 kPa   >6 kPa   ⟷   Ultrasound

Exclude liver congestion, mechanic cholestasis,
nodular liver masses or hepatomegaly

Serum transaminases (mainly AST)

No chronic
liver disease

normal   ←*   <100 U/l   ←*   >100 U/l

Valid assessment of
F3 fibrosis and
F4 cirrhosis

Valid assessment of
F4 cirrhosis

No valid assessment of
fibrosis stage
(exception: very high
liver stiffness >24
kPa?)

>8 kPa and ≤12.5 kPa = F3 fibrosis
>12.5 kPa = F4 cirrhosis
>20 kPa – check for varices

Figure 9 Estimated increase of liver stiffness by various clinical conditions irrespective of fibrosis.
**Note:** *alcohol withdrawal.

(as confirmed by ultrasound and blood tests), an increased but stable LS could directly be used to quantitate fibrosis stage. Under certain circumstances it is possible to estimate the contribution of venous pressure, mechanic cholestasis and inflammation (hepatitis). Figure 10 gives typical empirical values for stiffness changes as obtained from previous reports.[30,40,61] Thus, during mechanic cholestasis by gallstones, an increase of bilirubin by 1 mg/dl will cause a medium increase in LS by ca. 1 kPa.

Liver stiffness as molecular mechanism of liver fibrosis

The molecular mechanisms of liver fibrosis are poorly understood despite extensive research activities over many decades.[92–94] Consequently, no targeted treatment options exist to directly prevent progression of matrix deposition. It is intriguing that all chronic liver diseases eventually lead to liver cirrhosis and the sequence of steatosis, steatohepatitis and fibrosis/cirrhosis is generally accepted as causative. However, it is not known which of the intermediated steps are just bystanders or obligatory. In fact, most, if not all, liver diseases show various forms of inflammation and

steatosis. It is also notable that in most scenarios, eg, ALD or HCV, only a minority of patients (ca. 15%) progress to cirrhosis.[91] This generates some optimism that there are genetic or environmental causes that determine fibrosis progression and that fibrosis progression is not an essential and constitutional process. This optimism is further nourished by the established knowledge that early causative treatment of liver diseases not only stops fibrosis progression but can even introduce the complete reversal of fibrosis. Unfortunately, the conditions that define the "points of no return" are not known.

LS and its direct relation to pressure[30,61] may serve as an eye-opener for mechanical stretch as a longtime neglected potential stimulus of matrix deposition. It is indeed fascinating to see that all possible conditions of liver cirrhosis increase LS, and that these conditions are not always related to inflammation (which is typically regarded as a common road to liver fibrosis of all liver diseases). Thus, mechanical stop of bile flow or hepatic vein blood flow dramatically increase LS, and both conditions are known to cause cirrhosis. Both conditions increase hydrostatic pressure in distinct compartments and ultimately lead to specific cirrhosis patterns (cardiac cirrhosis,
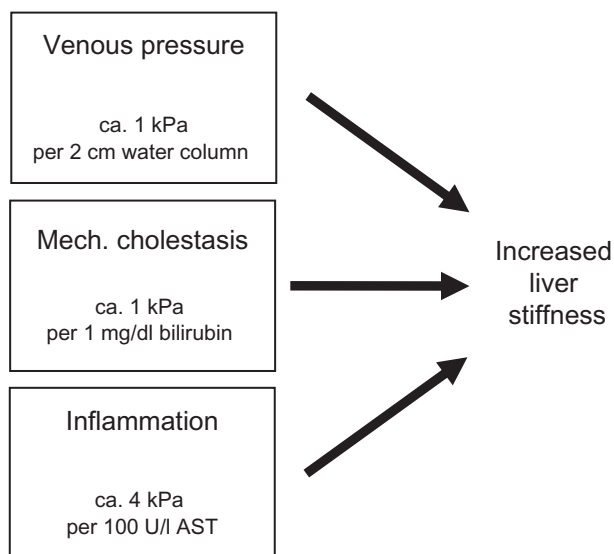
Figure 10 Present diagnostic algorithm of liver stiffness. For details see text.
*Arrows indicate cured hepatitis eg, detoxification from alcohol or cure from hepatitis C virus.

biliary fibrosis). Although both conditions may also lead to remarkable signs of inflammation or hepatocellular necrosis, they are typically not as pronounced as compared to inflammatory liver diseases such as ALD or viral hepatitis. On the other hand, it has become clear that inflammatory conditions increase LS irrespective of fibrosis.[57,58] This is not a surprise since "tumor" (swelling) has been known since the ancient times as a classical sign of inflammation besides "calor" (heat), *functio laesa* and "rubor" (reddening). It is, however, undisputable that inflammation-caused tissue swelling regardless of its multifactorial cause, is also caused by pressure that is more related to osmotic pressure. Thus, in fact, all conditions that ultimately lead to cirrhosis cause increased LS, and this increased LS is initially related to increased pressure of various origins and in various compartments. It is very obvious that matrix and connective tissue are in balance with various kinds of pressures.

These observations and thoughts yield to the following new paradigm that we would like to call pressure-stiffness-fibrosis sequence hypothesis (see Figure 11): during chronic liver diseases, the accumulation of interstitial liquid and inflammatory infiltrate yield to an increase of local stress and stretch of blood vessels or bile ducts. Therefore, increased mechanical stretch would stimulate the production of collagen (fibrotic tissue) which would result in a permanent stiffness increase as if the liver was adapting its structure to mechanical conditions. Interestingly, increased LS values related to fibrotic tissue could be a long-term consequence of a short-term stiffness increase due to the inflammatory episode related

to the chronic liver diseases. Portal hypertension would then be the consequence of increased vascular resistance either caused by inflammation or matrix-related increase of stiffness. Indeed, an increased rate of esophageal variceal bleeding is observed in patients with ALD in the phase of fulminant alcoholic steatohepatitis and in the absence of end stage cirrhosis, and these patients are known to reach high but reversible LS values.

Some very recent molecular findings may support the pressure-matrix-stiffness sequence hypothesis. Thus, mechanical stretch induces transforming growth factor (TGF)-β synthesis in hepatic stellate cells, which is known to be highly expressed under profibrogenic conditions.[95] From animal experiments it was recently concluded that increases in LS precede fibrosis and potentially myofibroblast activation.[96] Thus, matrix stiffness could be a major denominator of the equilibrium of matrix-bound growth factors.[97,98] These findings point to a regulatory interlink between physical forces of gravity, hemodynamic stress, and movement in tissue development that are still a poorly understood area of research.[99] Intercellular mechanical coupling of stress fibres via adherens junctions, intracellular calcium oscillations, and mechanosensitive ion channels have been discussed to control cell-dense tissue by coordinating the activity of myofibroblasts.[100] The pressure-matrix-stiffness hypothesis would also encourage a more in-depth look into the regulation of cell volume[101,102] and aquaporin regulation.[103] In addition, also a relation to vasoactive hormones such as natriuretic peptides seems to be attractive which are increased in all patients with edematous disorders which lead to an increase in atrial tension or central blood volume, such as renal failure or liver cirrhosis with ascites.[104] Indeed, continuous intravenous infusion of atrial natriuretic peptide prevented liver fibrosis in rat.[105]

Liver stiffness and future perspectives

The noninvasive ability to measure LS has opened a new realm for both the diagnosis but also the molecular understanding of liver fibrosis. We will observe a rapid technical improvement of ultrasound and MRI-based elastography techniques. In addition, stiffness measurements of other organs such as spleen, pancreas or kidney will be possible. Hopefully, miniaturization will open stiffness measurements via endoscopic procedures. Modified technologies such as FS will be able to quantitate the degree for liver steatosis. Thus, a novel physical parameter has been developed to quantify hepatic steatosis. This VCTE-based ultrasonic attenuation is called 'CAP', for 'controlled attenuation parameter' and demonstrates good
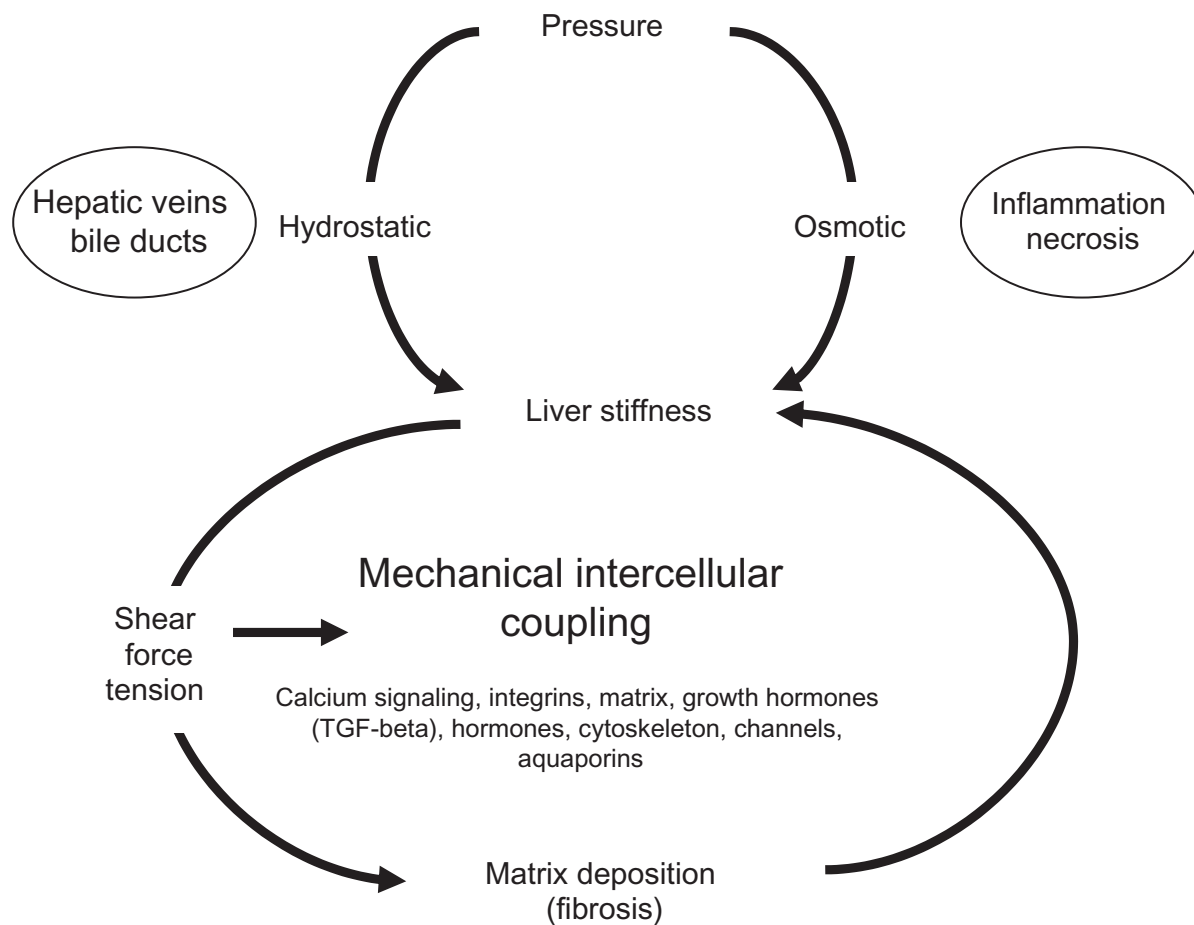
Figure 11 Pressure-stiffness-matrix sequence hypothesis. Either hydrostatic (venous or bile) or osmotic (eg, inflammation) pressure increases liver stiffness which, in turn, initiates increased matrix deposition via mechanical intercellular signaling. Matrix deposition finally leads to an irreversible increase of liver stiffness that is independent of pressure. These events may ultimately enter a vicious cycle causing end-stage liver disease. For more details see text.

performance for diagnosis of fatty infiltration in more than 10% of hepatocytes.[106] With regards to LS, upcoming studies have to clarify the following open questions:

- Can we identify a direct quantitative relation between type and histological localization of hepatitis, serum transaminases and LS?
- What is the diagnostic value of LS in more complex clinical settings, eg, a patient with combined alcoholic liver fibrosis, steatohepatitis, and cardiomyopathy?
- Could LS be part of prognostic scores for patients on the liver transplantation waiting list?
- What other factors or rare diseases increase LS?
- Could we use LS as a novel parameter to measure venous pressure in the context of intensive care settings or cardiology?
- How valuable is LS in the neonatal screening for inborn liver diseases?
- What are the gender and age specific normal stiffness values?

- What are the population-wide prevalence rates of inceased LS and fibrosis?

The area of LS will booster many basic research activities, and novel miniaturized equipment is urgently required that will allow LS measurements on small animals such as mice. These are some of the questions that need to be addressed in the future:

- What are the genetic and molecular determinants of LS?
- What are the kinetics of LS in various fibrosis models?
- What are the kinetics of stiffness resolution in these models and is there a point of no return?
- Is there a critical cut-off value for stiffness that causes fibrosis?
- What is the role of vasoactive hormones, mechanosensing channels, and water channels such as aquaporins on LS and fibrosis?
- Are there pharmacological or other therapeutic approaches to modulate LS and treat liver fibrosis?

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Disclosures

SM reports no conflict of interest. LS developed VCTE (Fibroscan) and is currently Director of Research and Development at Echosens.

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