

# Diagnostic et évaluation de la gravité des maladies chroniques du foie : impact de l'elastographie par ondes de cisaillement " supersonic shear imaging "

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# THÈSE PRÉSENTÉE POUR OBTENIR LE GRADE DE

# DOCTEUR DE

# L'UNIVERSITÉ DE BORDEAUX

ÉCOLE DOCTORALE SCIENCES DE LA VIE ET DE LA SANTE

SPÉCIALITÉ BIOIMAGERIE

Par Christophe CASSINOTTO

# DIAGNOSTIC ET EVALUATION DE LA GRAVITE DES MALADIES CHRONIQUES DU FOIE

IMPACT DE L'ELASTOGRAPHIE PAR ONDES DE CISAILLEMENT « SUPERSONIC SHEAR IMAGING »

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Soutenue le 23 Novembre 2016

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#### **ABBREVIATIONS**

ARFI : Acoustic Radiation Force Impulse APRI : Aspartate Aminotransferase to Platelet Ratio Index cACLD : compensated advanced chronic liver disease kPa : kiloPascal LSM : Liver Stiffness Median NAFLD : Non-Alcoholic Fatty Liver Disease PBH : Ponction-Biopsie Hépatique IQR : InterQuartile Range SSI : Supersonic Shear Imaging SWE : Shear Wave Elastography

#### **INTRODUCTION**

Les maladies chroniques du foie constituent un problème majeur de santé publique, étant responsables d'une morbi-mortalité spécifique touchant les pays du monde entier. Leur prise en charge ainsi que leur pronostic sont largement basés sur la présence et le développement d'une fibrose hépatique, qui peut survenir lors de toute hépatopathie chronique, qu'elle soit d'origine virale, métabolique, alcoolique, autoimmune ou héréditaire. L'aggravation progressive de la fibrose hépatique va aboutir chez un certain nombre de patients au développement d'une cirrhose et de ses complications potentielles comme la décompensation hépatique, l'ascite, les hémorragies digestives sur rupture de varices oesophagiennes, l'encéphalopathie et le carcinome hépato-cellulaire (1-4). L'évaluation précise du degré de fibrose hépatique apporte au clinicien une estimation du pronostic dès la prise en charge initiale de ces patients, mais permet également la réalisation d'une surveillance évolutive, et intervient dans la décision et le choix d'un traitement (5,6). La ponction-biopsie hépatique (PBH) est toujours considérée comme la référence pour l'évaluation et la quantification de la fibrose hépatique. Cependant, cette méthode diagnostique est invasive, souvent douloureuse, et peut s'accompagner de complications rares mais sévères (7). Son efficacité diagnostique est également limitée par une variabilité liée à la représentativité des échantillons, ainsi que par des discordances inter et intraobservateurs (8).

L'évaluation non-invasive de la fibrose hépatique, apparue au début du XXIème siècle a permis de révolutionner la prise en charge des malades atteints d'une maladie chronique du foie. Deux approches distinctes ont été développées ces dernières années :

- l'une biologique consistant au dosage de marqueurs sanguins de fibrose hépatique. Ainsi, le Fibrotest (Biopredictive, Paris, France ; Fibrosure-Labcorp, Burlington, VT), l'APRI (aspartate aminotransferase to platelet ratio index), ou le FIB-4 score ont montré une utilité certaine dans le diagnostic de fibrose ou de cirrhose (9-14).
- Une approche « physique », qui consiste à réaliser une mesure de l'élasticité hépatique. L'évaluation de la vitesse de propagation d'une onde dite de cisaillement permet d'approcher certaines propriétés physiques intrinsèques du parenchyme hépatique comme l'élasticité. Plus le parenchyme est fibreux donc « dur », plus l'onde de cisaillement se propage rapidement.

Le premier outil développé suivant cette approche physique est le Fibroscan® (Echosens, Paris, France) dont la première étude fut publiée en 2002. De nombreuses

études ont depuis confirmé son efficacité et sa reproductibilité dans le diagnostic de fibrose sévère et de cirrhose chez les patients atteints d'hépatopathie chronique (15-17). Au-delà de l'impact diagnostique, son intérêt est également pronostique. Chez les patients cirrhotiques, il existe une relation entre le score du Fibroscan® et la survenue des complications de la cirrhose (18). Une récente étude a aussi montré que l'élasticité hépatique mesurée par Fibroscan® était pronostique de la survie à 5 ans des patients atteints d'hépatite virale C (19).

Le Fibroscan® a cependant de nombreuses limites. Si ses performances sont bonnes pour le diagnostic de fibrose sévère ou de cirrhose, elles restent perfectibles pour le diagnostic de fibrose significative. De plus, des échecs de mesure ou des résultats non interprétables surviennent respectivement dans 5 et 15% des cas, avec comme principal facteur limitant l'obésité (20). En effet, l'accumulation de tissu adipeux sous-cutané atténue la transmission de l'onde de cisaillement et des faisceaux ultrasonores qui vont mesurer sa propagation. Compte tenu que la prévalence de l'obésité et de ses manifestations hépatiques associées augmente de manière alarmante dans les pays occidentaux, cette limite est un frein potentiel à l'utilisation de cette technique en pratique clinique. Pour pallier à cette insuffisance, une nouvelle sonde de Fibroscan®, la sonde « XL », dédiée aux patients en surcharge pondérale, a récemment été développée (21). Son évaluation est en cours dans plusieurs études cliniques mais déjà ses résultats préliminaires sont prometteurs. Enfin, Le Fibroscan® n'est pas couplé à un appareillage ultrasonographique, et est donc limité à la mesure de l'élasticité hépatique, sans analyse morphologique hépatique réalisable.

Depuis 2009, une nouvelle méthode diagnostique est apparue dans le domaine de l'élastographie hépatique. L'ARFI (Acoustic Radiation Force Impulse) est basé sur des principes physiques proches de ceux du Fibroscan®, mais présente toutefois quelques différences fondamentales. Contrairement au Fibroscan®, le système d'élastométrie impulsionnelle est directement incorporé sur un appareil d'échographie standard (Acuson S2000 ; Siemens Medical Solutions, Mountain view, Calif), et l'onde de cisaillement est localisée, émise à partir d'une région fixée par l'opérateur. Ainsi, une évaluation de l'élasticité hépatique reste réalisable chez des patients en forte surcharge pondérale ou lorsque s'interpose un épanchement ascitique. Plusieurs études préliminaires ont confirmé l'intérêt diagnostique de cette nouvelle méthode, qui semble, comme le Fibroscan®, présenter une performance diagnostique satisfaisante surtout pour le diagnostic de fibrose sévère ou de cirrhose (22-24).

Une société française (Supersonic Imagine, Aix-en-Provence, France) a très récemment conçu et développé une nouvelle évolution technologique dans le domaine

de l'élastographie hépatique. La méthode Supersonic Shear Imaging (SSI), autrement appelée Shear Wave Elastography, est également basée sur le principe de mesure de la vitesse de propagation d'une onde de cisaillement dans les tissus mous. Comme l'ARFI et contrairement au Fibroscan, cette méthode est incorporée sur un appareil échographique, et ne nécessite pas de vibrateur externe pour produire l'onde de cisaillement. Contrairement à l'ARFI et au Fibroscan où l'évaluation de la propagation d'une seule onde de cisaillement est réalisée à chaque mesure, la méthode SSI permet l'évaluation synchrone de la propagation de plusieurs fronts d'ondes de cisaillement et donc la réalisation d'une multitude de mesures de vitesses de cisaillement émises sur une large gamme de fréquence (25, 26). Cette imagerie quantitative va permettre d'approcher l'élasticité d'un tissu sur une surface par la réalisation d'une cartographie couleur. En plaçant une région d'intérêt au centre de cette surface, la valeur calculée correspondra à la moyenne de nombreuses valeurs au sein de l'aire analysée. Même si cette nouvelle technique paraît très prometteuse, très peu d'études ont pour l'instant évalué son efficacité dans le domaine des maladies chroniques du foie. L'élastographie hépatique étant un domaine en constante évolution, il est indispensable d'étudier en profondeur chaque nouvelle technologie mise à disposition sur le marché afin d'en connaître les avantages et inconvénients par rapport aux techniques existantes, et donc de préciser leur application dans la pratique clinique et l'évaluation non-invasive de la fibrose hépatique.

Ce document présente 3 études dont le but est d'analyser les performances diagnostiques de l'élastographie « Supersonic Shear Imaging » ainsi que sa place dans l'évaluation non-invasive des maladies chroniques du foie :

1. **Dans une première étude**, nous avons prospectivement analysé et comparé les performances diagnostiques de l'élastographie SSI par rapport au FibroScan et l'ARFI pour le staging de la fibrose hépatique sur une série de 349 patients avec une maladie chronique du foie diagnostiquée et gradée par PBH.

2. **Dans une seconde étude**, nous avons prospectivement étudié l'impact diagnostique de l'élastographie SSI sur le foie et la rate chez une population de 401 patients avec cirrhose pour l'évaluation de la gravité de la maladie cirrhotique.

3. **Dans une troisième étude**, nous avons réalisé une étude prospective bicentrique (Angers et Bordeaux) analysant la performance diagnostique de l'élastographie SSI, du FibroScan et de l'ARFI dans l'évaluation non-invasive de la fibrose hépatique sur une série de 291 patients avec stéatopathie non-alcoolique.

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## Elastography: French innovations in the spotlight

#### Cassinotto C.

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In fifteen years elastography has undoubtedly got a mature technique status! But, who would have anticipated in the early 2000s that elastography would have become in a couple of years this routine diagnostic tool responsible for major changes in the management of so many conditions?

The story started in 1999 when researchers from the "Laboratoire Ondes et Acoustique" (LOA) of Paris VII-Diderot University proposed to quantify the stiffness of biological tissues using measurement of displacements induced by the propagation of pulsed shear waves [1]. Some of them designed and marketed the FibroScan® (Echosens, Paris, France) that was the first machine dedicated to the liver parenchyma stiffness measurement, because elastography was originally specifically designed for the liver. This innovation was followed by an impressive success as well as a uniform acknowledgment of the added medical value of this new technology. Later, in 2007, the French «Haute Autorité de Santé» approved the use in liver stiffness measurements for the staging of fibrosis in hepatitis C patients. This use was further approved by the European Association for the Study of the Liver (EASL) in 2011 [2]. Another key date was 2011 when a group of hepatologists in Bordeaux finally demonstrated that liver stiffness measurement was an independent prognostic factor of 5-years survival in patients with initial diagnosis of C viral hepatitis [3]. Since that time, the clinical impact of liver stiffness measurement has been validated worldwide [4].

Since then, elastography has become a rapidly developing field with frequent technological innovations. In this regard, each manufacturer or vendor offers an elastography program. But once again, the "LOA" of Paris VII-Diderot University played a pivotal role in this frantic technological race, with the development of Supersonic Shear Imaging (SSI), also named 2D-shear wave elastography (2D-SWE). SSI elastography available on Aixplorer<sup>®</sup> US machines (Supersonic Imagine, Aix-en-

Provence, France) is also based on the measurement of the velocity of a local shear wave through soft tissues, but herein the radiation force induced by an ultrasonic beam is combined with an ultrafast imaging sequence capable of catching in real time the propagation of the resulting shear waves. By generating a real-time color mapping of the elasticity encoded pixel by pixel in an image superimposed on the standard B-mode, SSI allows a quantitative imaging of the tissue elasticity.

Although the SSI technique is quite recent, first studies demonstrated the potential clinical relevance and the accuracy of this new tool especially in the field of liver fibrosis or breast lesions [5]. In this issue of Diagnostic & Interventional Imaging, two well-designed studies confirmed the great role that SSI can play in the area of diagnostic ultrasonography. First, a nicely performed study by Guibal et al. demonstrated the high degrees of diagnostic accuracy of SSI for the staging of liver fibrosis [6]. Second, a well-designed study by Dorado-Cortez et al. investigated the reliability of SSI in the field of muscle elastography [7]. Both studies nicely participate to the mounting evidence that SSI is an accurate and reliable elastography technique.

However, there is still a long way until elastography reaches its zenith. First, performing reliable stiffness measurements is not always easy and requires some experience, therefore raising the issue of the training of operators as well as the identification and validation of reliability criteria for each different elastography techniques. Second, each elastography technique has its own stiffness values, ranges and cut-offs. Regarding the liver, we noted in our experience and works that by chance, SSI values and cut-off values were quite close to those obtained with the FibroScan<sup>®</sup>, which is convenient for its wide dissemination among hepatologists already familiar with the range of FibroScan® values for many years. However, the multiple available elastography techniques may conceptually mirror the Tower of Babel [8]. Of note, should a patient have follow-up evaluation with different techniques, it will become difficult or even impossible to obtain a reliable assessment of possible changes. Ideally, all manufacturers and elastography researchers should work together to find how a harmonization of values should be possible between the different techniques, and if not possible, to find at least the way of converting or calibrating values between these different techniques. This issue of interplatform reproducibility has been addressed using magnetic resonance imaging [9] but not with ultrasound. Such a study would serve as a template for ultrasound [9].

Finally, the widespread implementation of elastography modules on most ultrasound machines had the advantage to expand the application scope of elastography to others organs than liver, such as breast, thyroid, kidney, spleen, muscles, or even vessel parietal wall. As evidences are not as mature in these organs as they are for the liver, many advances and progress are expected for these other applications in the coming years. As it has been done for the liver, we will also need to increase our understanding of technical and patient factors that might affect elastography performances, regardless the type of organ or disease studied. Nevertheless, the implementation of elastography on almost all ultrasound machines allowed the radiological community to become the cornerstone regarding the development of elastography, from routine clinical application to future research trends. We have to prove now that we are capable to deal with and overcome all the challenges that elastography will have to face in the near future.

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# PRE-REQUIS : ELASTOGRAPHIE POUR LE DIAGNOSTIC NON-INVASIF DE LA FIBROSE HEPATIQUE

Au début du 21<sup>ème</sup> siècle sont apparus les scores non-invasifs sanguins de fibrose hépatique ainsi que la mesure de la dureté du foie par FibroScan® qui ont permis de révolutionner la prise en charge des malades atteints d'hépatopathie chronique. En quelques années, ces méthodes non-invasives de diagnostic de la fibrose hépatique ont connu un tel essor qu'en 2007, la Haute Autorité de Santé a validé leur intérêt clinique en recommandant soit la PBH, soit le FibroTest, soit le FibroScan® dans la prise en charge initiale d'une hépatite virale C sans comorbidité (1). Nous présentons ci-dessous les bases physiques des principales méthodes actuellement disponibles ainsi que les études scientifiques qui ont permis leur validation.

## Elastographie : bases physiques

L'élastographie est une technique qui permet d'approcher les propriétés mécaniques des tissus. Les différents concepts d'élastographie reposent sur la même base : **estimer la dureté d'un tissu via l'évaluation quantitative du module de Young :** 

Soit par l'étude de la déformation du tissu soumis à une contrainte (Fig. 1)



Figure 1 : schéma descriptif du concept physique du module de Young

Cette approche peut être rapprochée de la mesure physique quantitative du geste qualitatif de palpation du médecin.

Soit par la mesure de propagation d'ondes de cisaillement sensibles à la dureté du tissu :

# E (module de Young) = $3.0.v^2$

Où  $\varrho$  est la masse volumique (constante  $\approx 1000 \text{ kg/m}^2$ ) et v la vitesse de l'onde en m/s.

Plus la vitesse v est élevée, plus le milieu est dur.

Ainsi, en fonction de la méthode utilisée pour évaluer le module de Young, et du régime appliqué pour évaluer la déformation du tissu ou la propagation de l'onde, plusieurs techniques d'élastographie ont été développés (Fig. 2) :



Figure 2 : organigramme résumant les différentes techniques d'élastographie disponibles en 2013

Dans ce document, nous nous sommes concentrés sur les techniques basées sur un régime transitoire, qui sont les principales utilisées en pratique clinique courante, et celles dont nous disposons actuellement au CHU de Bordeaux.

L'élasticité hépatique est donc le reflet direct de la dureté du foie et non de la fibrose hépatique. En effet, si dans les maladies chroniques du foie, la fibrose est le principal facteur responsable d'une augmentation de la dureté du parenchyme hépatique, il n'est pas le seul, et bien d'autres facteurs confondants ont été décrits (Fig. 3) :



Figure 3 : facteurs intervenant dans la valeur d'élasticité hépatique

## La mesure de l'élasticité hépatique par FibroScan

## • Technique

Le FibroScan (EchoSens, Paris, France), ou élastométrie impulsionnelle, est la méthode de référence dans l'évaluation non-invasive de la fibrose hépatique, car la première mise au point, et donc la plus étudiée. Le FibroScan est composé d'un vibreur monté sur un transducteur ultrasonore, le tout constituant la sonde de FibroScan (Figures 4-6). Après pression sur un bouton, le vibreur placé en région intercostale droite transmet une impulsion à la surface de la peau, ce qui va engendrer une onde mécanique dont la vitesse de propagation sera mesurée par ultrasons sur une longueur de 4 cm de long et 1 cm de diamètre. L'estimation de l'élasticité du tissu à partir de la mesure de la vitesse de l'onde est exprimée en kPa (kilopascal). Plus le foie est dur, plus l'onde mécanique induite par le FibroScan se déplace rapidement, et plus les valeurs en kPa sont élevées.



Figure 4 : schéma représentant la sonde de FibroScan et l'impulsion mécanique en regard du lobe droit hépatique



Figure 5 : sonde de FibroScan



Figure 6 : FibroScan : vue d'ensemble de l'appareillage

# • Applications cliniques

Le FibroScan a été à l'origine du formidable essor de l'élastographie hépatique dans les années 2000. Il a jeté les bases de l'intérêt clinique de l'élastographie hépatique dans des domaines aussi variés que :

- ✓ Le dépistage (2)
- ✓ Le diagnostic de la fibrose (3,1)
- ✓ Le suivi des patients traités (4)
- ✓ L'évaluation de la sévérité de la cirrhose (5)
- ✓ Le prognostic des patients (6-8)

Les valeurs obtenues sont comprises entre 2 et 75 kPa. Le résultat final est la médiane de 10 mesures. L'appareil affiche aussi l'interquartile range (IQR), et le taux de réussite (nombre de mesures obtenues par rapport au nombre de tirs effectués). La durée totale de l'examen n'excède pas 5 minutes. Il a été démontré qu'une mesure pourra être considérée comme fiable ou valide essentiellement en fonction du ratio IQR/LSM (liver stiffness median) (Tableau 1) (9).

		Elasticité médiane (kPa)					
		< 7,1	≥ 12,5				
IQR/LSM	≤ 0,10	Excellent					
	0,10 - 0,30	Moyen					
	> 0,30	Mauvais					

Tableau 1 : classification décrivant la fiabilité de la mesure d'élasticité hépatique en fonction de la valeur d'élasticité et du ratio IQR/LSM. Source : Boursier et al. Hepatology 2013 (9)

La valeur moyenne d'élasticité hépatique chez les sujets « normaux » c'est-à-dire dépourvus d'hépatopathie chronique, est de  $5,5 \pm 1,6$  kPa (10). Les valeurs d'élasticité hépatique sont plus importantes chez les hommes que chez les femmes, chez les sujets obèses et chez les sujets avec syndrome métabolique.

C'est certainement pour le diagnostic de la cirrhose que le FibroScan a le plus grand intérêt, car sa performance est excellente, quelle que soit l'étiologie de la maladie hépatique. C'est dans les hépatites virales, et notamment l'hépatite C que la performance du FibroScan® a été la plus étudiée, mais il existe maintenant nombre d'études scientifiques qui valident l'utilisation du FibroScan dans la plupart des étiologies de maladies chroniques du foie, dont la NAFLD (11,12).

Cependant, dans certains cas, aucune valeur n'est obtenue malgré 10 tirs (4 à 10% des cas selon les études). Les facteurs associés à un échec de mesure sont l'ascite, l'index de masse corporelle ainsi que l'épaisseur de la paroi thoracique et le tour de taille (13). Devant ces limites, les concepteurs du FibroScan ont développé vers 2008 une sonde de FibroScan dite « XL », adaptée aux patients en surcharge pondérale.

## • La sonde XL de FibroScan

La nouvelle sonde de FibroScan, appelée sonde XL, a été conçue spécialement pour les malades en surcharge pondérale avec une fréquence d'ultrasons plus basse, un transducteur ultrasonore plus sensible, une mesure de l'élasticité hépatique plus profonde et une amplitude de vibration plus importante (Tableau 2) (10).

Characteristics	M probe	XL probe
US central frequency	3.5 MHz	2.5 MHz
Tip diameter	9 mm	12 mm
Measurement depths (below skin surface)	2.5 – 6.5 cm	3.5 – 7.5 cm
Vibration amplitude (peak to peak)	2 mm	3 mm

Tableau 2 : caractéristiques respectives des sondes M et XL de FibroScan

En quelques années, la littérature est déjà abondante sur la sonde XL, montrant son intérêt dans le diagnostic de la fibrose hépatique quelle que soit son étiologie. Il a notamment été montré que les performances diagnostiques des deux sondes semblent identiques mais avec des valeurs seuils pour l'interprétation des résultats plus basses avec la sonde XL qu'avec la sonde M (14). La sonde XL permet le diagnostic de la fibrose hépatique avec la même performance que la sonde M mais avec beaucoup moins d'échecs de mesure (2% d'échecs avec la sonde XL versus 10% avec la sonde M). Et chez les patients avec BMI > 25 kg/m2, la mesure avec la sonde XL est possible dans 75% des cas d'échec de mesure de la sonde M (15,16).

# La mesure de l'élasticité hépatique par Acoustic Radiation Force Impulse (ARFI)

#### • Technique

La technologie ARFI (Acoustic Radiation Force Impulse) consiste en un système impulsionnelle d'élastométrie incorporée sur un appareil échographique conventionnel (Acuson S2000) développé par Siemens (Siemens Medical Solutions, Mountain View, Calif). L'avantage d'un tel système est d'utiliser l'imagerie échographique temps réel pour cibler une région d'intérêt où l'opérateur souhaite réaliser la mesure d'élasticité. Dans la zone ainsi sélectionnée, la sonde d'échographie émet une onde (ou pulse) ultrasonore localisée, de haute intensité et de courte durée, suivie d'une série d'autres ondes diagnostiques qui vont permettre d'analyser la propagation de l'onde initiale au sein du tissu excité (Fig. 7, 8). La séquence ARFI est constituée d'impulsions de référence, suivie d'une impulsion d'excitation qui génère l'onde de cisaillement, et enfin d'impulsions de suivi qui enregistrent la réponse à la déformation du tissu induite par l'onde de cisaillement. La vitesse de l'onde de cisaillement est recueillie latéralement de part et d'autre d'une fenêtre centrale de 5 mm de profondeur sur 4 mm de largeur, positionnée au sein d'une région d'intérêt de 1cm sur 6mm (17). Les résultats sont exprimés en mètres par seconde. Contrairement au FibroScan, l'onde de cisaillement est transmise au tissu à distance de la sonde émettrice, les mesures d'élasticité pouvant donc être réalisées en présence d'ascite, ou d'une épaisseur pariétale importante.



Figure 7 : schéma représentant la séquence d'acquisition d'une mesure d'élasticité en mode ARFI



Figure 8 : exemple de mesure d'élasticité hépatique en mode ARFI réalisée sur échographe Acuson S2000

Pour éviter la compression tissulaire liée au positionnement de la sonde, les mesures s'effectuent par voie inter-costale droite au niveau du parenchyme hépatique droit, chez un patient en décubitus dorsal, a jeun, lors d'apnées coutes ou avec une respiration lente. La région d'intérêt est positionnée à distance de la zone souscapsulaire et des grosses structures vasculaires, en choisissant une fenêtre échographique où au moins 6 cm d'épaisseur de parenchyme hépatique sont visualisables. Comme pour le FibroScan, il est recommandé de réaliser plusieurs échantillonnages afin d'obtenir 10 mesures valides. La médiane de ces 10 mesures est la valeur d'élasticité finale retenue (Fig. 9). Les valeurs sont comprises entre 0,5 et 4,4 m/sec.

Mesures d	e la vitess	e de cisaillement
	Site 1	
	Vc (m/s)	Prof. (cm)
	1,02	3,9 🔛
	0,98	3,9 🔛
	0,94	3,9 🔛
	0,96	3,9 🔛
	0,89	3,9 🔛
	1,00	3,9 🔛
	0,92	3,9 🔛
	1,32	3,9 🔛
	0,99	3,9 🔛
	1,00	3,9 🔛
	0,97	3,9 🔛
	0,95	3,9 🔛
	1,01	3,9 🔛
Médian	0,98	
Moy	1,00	
Ect typ	0,10	
EIQ	0,05	

Figure 9 : rendu de résultats en mode ARFI avec l'échographe Acuson S2000

## • Applications cliniques

La technologie ARFI a commencée à être appliquée à l'évaluation non-invasive de la fibrose hépatique à partir de l'année 2009. Plusieurs études puis méta-analyses ont rapidement confirmé que les performances de l'ARFI étaient satisfaisantes pour le diagnostic de fibrose sévère et de cirrhose (Tableau 3).

Auteurs	Auteurs Design Patients Pathologies	Patients	Pathologies	AUROC			Cut-offs		
			≥F2	≥F3	F4	≥F2	≥F3	F4	
Nierhoff 2013 (18)	Méta-analyse	3951	Multi-étiologie	0.84	0.89	0.91	1.35	1.61	1.87
Friedrich-Rust 2012 (19)	Méta-analyse	518	Multi-étiologie	0.87	0.91	0.93	1.34	1.55	1.80
Sporea 2012 (20)	Multicentrique rétrospectif	914	VHC	0.79	0.83	0.84	1.33	1.43	1.55

Tableau 3 : performances diagnostiques de l'ARFI pour grader la fibrose hépatique

Comparativement au FibroScan, il a été montré que l'ARFI a des performances comparables pour le diagnostic de fibrose significative et de cirrhose (Tableau 4).

Auteurs	eurs Patients Pathologies	ARFI			FibroScan			
			≥F2	≥F3	F4	≥F2	≥F3	F4
Lupsor 2009 (21)	112	VHC	0.85	0.87	0.91	0.94	0.93	0.95
Sporea 2012 (22)	223	Multi-étiologie	0.89		0.95	0.95		0.99
Rizzo 2011 (23)	139	VHC	0.86	0.94	0.89	0.78	0.83	0.80
Cassinotto 2013 (24)	321	Multi-étiologie	0.81	0.85	0.88	0.88	0.89	0.91
Bota 2013 Méta- analyse (25)	1163	Multi-étiologie	0.85		0.93	0.87		0.93

Tableau 4 : comparaison des performances diagnostiques de l'ARFI et du FibroScan. En jaune foncé lorsque la supériorité est significative, jaune clair les résultats d'une méta-analyse réalisée en 2013 et ne retrouvant pas de différence significative entre les 2 techniques. Si la technologie ARFI a montré son intérêt potentiel dans le diagnostic et la stadification de la fibrose hépatique, d'autres études restent nécessaires pour affiner les valeurs seuils diagnostiques des différents degrés de fibrose, potentiellement variables selon l'étiologie de l'hépatopathie chronique, rechercher les critères de qualité d'une mesure fiable et les facteurs associés à des mesures d'élasticité non fiables. De plus, il faudra probablement se positionner pour homogénéiser et résoudre le problème de l'unité de mesure de l'élasticité hépatique. En effet, l'Acuson S2000 de Siemens exprime les résultats d'ARFI en mètre par seconde (m/sec), ce qui est assez perturbant pour des cliniciens maintenant habitués dans leur pratique quotidienne à utiliser le kiloPascal pour exprimer la dureté du foie. Enfin, comme le Fibroscan, l'ARFI est une technique d'évaluation unidimensionnelle. Une seule mesure est réalisée à chaque échantillonnage, évaluant la dureté d'une zone de parenchyme de petite taille, prédéfinie et non modifiable.

Une dernière limite de l'ARFI que nous avions décrite en 2013 est la baisse de ses performances diagnostiques chez les patients en forte surcharge pondérale (Fig. 10) (24). En effet, les échecs de mesure avec l'ARFI sont rares, mais en contrepartie, la fiabilité de ces mesures semble décroître avec la qualité de la fenêtre acoustique qui est intimement liée à l'épaisseur de paroi que les ondes ont à traverser et donc à l'indice de masse corporelle du patient. Cette notion renforce la nécessité de mettre au point des critères de qualité des mesures d'élastographie réalisées avec l'ARFI.



Figure 10 : Evolution des performances diagnostiques de l'ARFI en fonction de la limite supérieure d'indice de masse corporelle de la population étudiée. BMI= body mass index ; AUROC= Area under the receiver operating curve.

## La mesure de l'élasticité hépatique par Supersonic Shear Imaging (SSI)

## • Technique

L'élastographie SSI, aussi connue sous le nom de Shear Wave Elastography (SWE) et maintenant appelée 2D-SWE, a été mise au point par le laboratoire Ondes et Acoustique de Paris-VII, qui est l'unité de recherche également à l'origine du FibroScan. Comme avec l'ARFI, la technologie SSI est implémentée sur un échographe standard, l'Aixplorer (Supersonic Imagine, Aix-en-Provence, France), et réalisable avec une sonde d'échographie convexe standard. La technique est, comme avec l'ARFI, basée sur la mesure de la vitesse de propagation d'ondes de cisaillement générées par ultrasons. A la différence de l'ARFI, les forces radiaires générées par ultrasons ne sont pas appliquées à un point donné mais sont focalisées à des profondeurs croissantes générant un front d'onde de cisaillement qui se propage dans la zone explorée. La même sonde émettrice réceptionne à très haute fréquence les données de vitesse de propagation de cette multitude d'ondes de cisaillement. Le résultat est rendu sous forme d'une cartographie temps réel de l'élasticité pixel par pixel codée en couleur dans une image superposée au mode B standard (Fig. 11) (26-28).



Figure 11 : représentation d'une mesure d'élasticité hépatique en mode SSI avec l'échographe Aixplorer de Supersonic Imagine

Cette technologie innovante a été mise au point grâce au développement de l'imagerie ultra-rapide (UltraFast<sup>TM</sup> Imaging) qui est basée sur 3 principes :

- une onde plane
- une très grande fréquence de répétition de l'onde plane (jusqu'à 20000 Hz)
- et une très grande vitesse de traitement des données de réception (plusieurs GBytes/s)

En échographie conventionnelle, le temps d'acquisition est dépendant de la profondeur de l'écho le plus distant et du nombre de lignes composant l'image. Pour remplir 128 lignes, un faisceau est « focusé » 128 fois pour remplir la totalité de l'image. En imagerie ultra-rapide, une seule onde plane est émise pour la totalité de l'image, le temps d'acquisition ne dépendant plus alors que de la profondeur de l'écho le plus profond (Fig. 12).



Figure 12 : représentation schématique de la technique d'émission de l'onde plane avec la technologie UltraFast Imaging (Source : d'après J. Bercoff et C. Casalegno, Supersonic Imagine)

La création de l'onde de cisaillement est basée sur une technologie ARFI, avec une force radiaire émise à un point donnée en « focusant » un faisceau ultrasonore, et entraînant la propagation latérale d'une onde de cisaillement (Fig. 13).



Figure 13 : émission du faisceau radiaire ultrasonore et propagation de l'onde de cisaillement basée sur des principes d'ARFI (Source : d'après J. Bercoff et C. Casalegno, Supersonic Imagine)

Avec le SSI, les forces radiaires à l'origine des ondes de cisaillement seront successivement appliquées à 4 profondeurs croissantes sur la même ligne, et ce à une vitesse plus rapide que la vitesse de l'onde de cisaillement elle-même (Fig. 14).



Figure 14 : génération d'un front d'onde de cisaillement en mode SSI (Source : Mme Casalegno, Supersonic Imagine)

Il en résulte un front d'onde de cisaillement dont la vitesse de propagation est réactualisée en temps réel par imagerie ultra-rapide. L'élastographie SSI est dans la catégorie d'élastographie dite « transitoire » car l 'émission des forces radiaires n'est pas continue mais la réactualisation des données en temps réel donne une impression d'élastographie « quasi-dynamique ».

# • Applications cliniques

Au moment de débuter nos travaux en 2013, très peu d'études avaient analysé l'intérêt clinique de cette technique récente dans les maladies chroniques du foie. Seules 2 études pilotes, l'une de 2012 avec des patients ayant une hépatite chronique virale C (29), et l'autre de 2013 sur l'hépatite chronique virale B (30), semblaient présager de performances satisfaisantes comparativement au FibroScan.

Auteurs	Patients	Pathologies	SSI			Fibroscan			
			≥F2	≥F3	F4	≥F2	≥F3	F4	
Ferraioli (2012) (29)	121	VHC	0.92	0.98	0.98	0.84	0.96	0.96	
Leung (2013) (30)	226	VHB	0.88	0.93	0.98	0.78	0.83	0.92	

Tableau 5 : études pilotes présentant les performances diagnostiques de l'élastographie SSI pour grader la fibrose hépatique en comparaison au FibroScan. En jaune lorsque la supériorité est significative.

## Synthèse des techniques actuellement disponibles et nouvelle nomenclature

Devant la multiplication des techniques d'élastographie ces dernières années, et leur dénomination plus basée sur des critères marketing que scientifiques, il est devenu nécessaire d'y voir plus clair et de définir une nouvelle nomenclature qui identifie clairement chaque technologie d'élastographie. Cela est d'autant plus vrai que le terme d'élastographie transitoire, caractérisant le FibroScan dans la littérature depuis sa conception, peut également s'appliquer à l'ARFI et au SSI. De même, aussi bien l'ARFI que le SSI sont basés sur une technologie ARFI, acronyme qui ne reflète que la technologie d'énission d'ondes radiaires par ultrasons. Depuis 2013, chaque société savante d'échographie a proposé des guidelines sur l'utilisation en pratique courante de l'élastographie hépatique, ainsi que sur les dénominations à utiliser (31-34). Il semblerait que la nomenclature proposée par l'EFSUMB (European Federation of Societies for Ultrasound in Medicine and Biology) en 2013 soit actuellement la plus utilisée (31).

Dans cette nomenclature, le FibroScan, qui a été la première technique développée, garde en anglais la dénomination de « transient elastography ». Aucune autre entreprise n'a reprise à son compte ou ne s'est associé à EchoSens pour la commercialisation de cette technologie. La technique d'élastographie initialement appelée ARFI et développée par Siemens (Siemens Medical Solutions, Mountain View, Calif) sous le terme de Virtual Touch Quantification® (VTIQ ou VTQ), est dorénavant dénommée point Shear Wave Elastography (pSWE) car basée sur la mesure de la vitesse de propagation d'une onde de cisaillement sur une petite zone de quelques millimètres (pouvant grossièrement s'apparenter à un point). Initialement implémentée sur l'échographe Acuson S2000, le pSWE a été ensuite implémentée sur les autres échographes Siemens. Cette technologie a par la suite équipée des échographes de marque Philips où elle était commercialisée sous le terme ElastPQ®. Ces 2 techniques de pSWE ont montré des résultats très reproductibles (35-37). Finalement, la technologie SSI, aperçue aussi sous le terme de shear wave

elastography (SWE), est renommée dans ces guidelines sous le terme de 2D-SWE ou real-time 2D-SWE. En effet, cette technique est également basée sur la mesure de la vitesse de propagation d'une onde de cisaillement mais cette mesure s'effectue sur une zone en 2 dimensions, bien plus importante qu'en pSWE, et avec une réactualisation en temps réel comme précédemment décrit. Dans ce manuscrit, nous garderons le terme de SSI car cette nomenclature était encore peu usitée au moment de la réalisation de nos travaux.

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#### **ETUDE 1**

# Non-invasive assessment of liver fibrosis with impulse elastography: comparison of Supersonic Shear Imaging with ARFI and Fibroscan

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## ABSTRACT

**BACKGROUND AND AIMS.** Non-invasive assessment of liver fibrosis by elastography is a rapidly developing field with frequent technological innovations. The aim of this study was to assess the diagnostic performances of Supersonic Shear Imaging (SSI) for the diagnosis of liver fibrosis in chronic liver disease.

**METHODS.** A total of 349 consecutive patients with chronic liver diseases who underwent liver biopsy from November 2011 to October 2013 were prospectively enrolled. For each patient, liver stiffness was assessed by SSI, ARFI, FibroScan® (M probe for patients with BMI <30 kg/m(2), and XL probe for patients with BMI  $\geq$ 30 kg/m(2)), performed within two weeks of liver biopsy. Areas under the receiver operating curves (AUROCs) were performed and compared for each degree of liver fibrosis.

**RESULTS.** SSI, FibroScan®, and ARFI correlated significantly with histological fibrosis score (r=0.79, p<0.00001; r=0.70, p<0.00001; r=0.64, p<0.00001, respectively). AUROCs of SSI, FibroScan®, and ARFI were 0.89, 0.86, and 0.84 for the diagnosis of mild fibrosis; 0.88, 0.84, and 0.81 for the diagnosis of significant fibrosis; 0.93, 0.87, and 0.89, for the diagnosis of severe fibrosis; 0.93, 0.90, and 0.90 for the diagnosis of cirrhosis, respectively. SSI had a higher accuracy than FibroScan® for the diagnosis of severe fibrosis ( $\geq$ F3) (p=0.0016), and a higher accuracy than ARFI for the diagnosis of significant fibrosis ( $\geq$ F2) (p=0.0003). No significant difference was observed for the diagnosis of mild fibrosis and cirrhosis.

**CONCLUSIONS.** SSI is an efficient method for the assessment of liver fibrosis in chronic liver diseases, comparing favourably to FibroScan® and ARFI.

#### INTRODUCTION

Noninvasive assessment of liver fibrosis by elastography is a rapidly developing field with frequent technological innovations. Fibroscan (Echosens; Paris, France) was the first tool developed to quantify liver fibrosis by measuring mechanical shear wave propagation through the hepatic parenchyma. Many studies have confirmed its efficiency and reproducibility for the diagnosis of liver fibrosis and for the prognosis of patients with chronic liver diseases [1-4]. Since 2009, Acoustic radiation force impulse (ARFI) elastography was developed and applied for the assessment of liver fibrosis. Unlike Fibroscan, the elastography system is directly integrated on a standard ultrasonography device (Acuson S2000, Siemens Medical Solutions, Mountain view, CA), and the shear wave is localized, allowing selecting by the operator the site of measurement on a real-time B-mode ultrasound image. The limitations and advantages of ARFI are well known. Several studies evaluated performances of this new method, which appears as Fibroscan to be a good diagnostic method for the diagnosis of severe fibrosis or cirrhosis [5-7].

Recently, a new technological development in the field of liver elastography has been proposed. The supersonic shear imaging (SSI), also named Shear Wave<sup>™</sup> elastography, is also based on the measurement of the velocity of a local shear wave through soft tissues. As ARFI and conversely to Fibroscan, this method is built on an ultrasound device (Aixplorer, Supersonic Imagine, Aix-en-Provence, France), and requires no external vibrator to produce the shear wave. Whereas the ARFI or Fibroscan methods in which a single shear wave is emitted temporarily at a single frequency for each measurement, in SSI the ultrasound transducer emits a plurality of pulse wave beams at increasing depths, using a very wide frequency band ranging from 60 to 600 Hz, allowing the synchronous evaluation of the velocity of several shear wave fronts over a wide frequency range [8-10]. By generating a real-time color mapping of the elasticity encoded pixel by pixel in an image superimposed on the standard B-mode, SSI allows a quantitative imaging of the tissue elasticity. By placing a region of interest (ROI) in the center of the color mapping, the calculated value is the average of many values within the region of interest.

Despite this new method seems promising, data evaluating its effectiveness in the field of chronic liver diseases are lacking. The aim of this prospective study was to evaluate the diagnostic performances of SSI, in comparison with Fibroscan and ARFI elastography for the staging of liver fibrosis in chronic liver disease.

#### PATIENTS AND METHODS

#### Patients

We prospectively enrolled all consecutive patients with chronic liver disease who underwent liver biopsy in our institution from November 2011 to October 2013 (Figure 1). For each patient, liver stiffness measurement (LSM) evaluated by SSI, ARFI, and Fibroscan (using M probe for patients with body mass index (BMI) <30 kg/m<sup>2</sup> and XL probe for patients with BMI≥30kg/m<sup>2</sup>) [11] were performed within two weeks of liver biopsy. All physicians who performed LSM were experimented and blinded to the results of other non-invasive methods and liver biopsy. Our ethics committee approved the study design and a written informed consent was obtained for all patients.

#### Liver histological examination

Histopathological staging of liver fibrosis served as the reference standard. The liver biopsy was fixed in formalin and embedded in paraffin. All biopsy specimens were analysed by two pathologists specialized in liver diseases and blinded to the non-invasive test results. Patients with biopsy specimens that do not meet the following quality criteria were excluded: biopsy length under 10mm and/or less than 6 portal spaces, and/or more than 2 fragments except for cirrhosis.

Fibrosis and necroinflammatory activity were staged according to the METAVIR scoring system for patients with viral hepatitis or the BRUNT score for nonalcoholic fatty liver diseases (NAFLD) [12, 13]. For viral hepatitis, fibrosis was staged on a scale from 0 to 4 as follows: F0: no fibrosis; F1: portal fibrosis without septa; F2: portal fibrosis and few septa; F3: numerous septa without cirrhosis; and F4: cirrhosis. For NAFLD, fibrosis was staged as follows: stage 0: absence of fibrosis; stage 1: perisinusoidal or portal; stage 2: perisinusoidal and portal/periportal; stage 3: septal or bridging fibrosis; stage 4: cirrhosis. Activity was graded from 0 to 3: grade 0: none; grade 1: mild; grade 2: moderate; and grade 3: severe. Grade of steatosis was defined according to Kleiner *et al.*: 0: steatosis<5%; 1: steatosis 5-33%; 2: steatosis 33-66%; and 3: steatosis >66% [14].

#### Morphological and biological parameters

For all patients, the following parameters were determined at the time of liver stiffness measurement. Clinical parameters included age, sex, BMI, waist circumference, history of diabetes, and hypertension. A blood sample was obtained in order to quantify the platelet count, prothrombin time (PT), total bilirubin levels, gamma-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, and cholesterol levels. These parameters were then used to calculate the aspartate-to-platelet ratio index (APRI) [15], the aspartate aminotransferase/alanine aminotransferase ratio index (AST/ALT) [16], the Forns index (Forns) [17], and the Fib-4 [18], according to the published formulas. FibroTest score was computed on the Biopredictive Web site (www.biopredictive.com) [19], by combining the dosage in the blood of five indirect markers of fibrosis (alpha-2-Macroglobulin, Haptoglobin, Apolipoprotein A-1, total bilirubin level, and GGT), with adjustment for age and sex of the patient.

#### SSI

SSI examinations were performed by four experienced abdominal imaging radiologists, each of them had performed more than 100 SSI examinations prior to the study. SSI is integrated into a conventional ultrasound device (Aixplorer<sup>™</sup>, Supersonic Imagine, Aix-en-Provence, France). Using radiation force and ultrafast ultrasound imaging, the supersonic shear imaging technique allows one to remotely generate and follow a transient plane shear wave propagating in vivo in real time. The radial forces that generate the shear wave are focused at increasing depths causing a shear wave front which propagates in the area scanned. Unlike ARFI or Fibroscan where a single shear wave is emitted temporarily at a fixed frequency, the ultrasound transducer emits here a plurality of pulse wave beams at increasing depths, using a very wide frequency band ranging from 60 to 600 Hz. The abdominal convex probe may issue up to three parallel shear wave fronts. The same emitting probe receives at high frequency the propagation speed data of the shear waves, allowing updating in real time the received data, each sequence of the 3 shear wave fronts lasting about one second. The tissue shear modulus, i.e., its stiffness, can be estimated from the shear wave local velocity. The ultrasound system then generates a real-time color mapping of the elasticity encoded pixel by pixel in an image superimposed on the standard Bmode [8, 9].

SSI examinations were conducted in accordance with the manufacturer's instructions. LSM was performed with the patient lying in dorsal decubitus with the right arm in maximal abduction, on the right lobe of the liver, through intercostal spaces. The operator, assisted by a real-time B-mode ultrasound image, targeted a right liver portion at least 6-cm thick and free of large vascular structures and 15mm depth below the liver capsule. A ROI of 15mm of diameter was positioned in the center of a color mapping with complete and homogeneous fulfilling, obtained in a patient with apnea. Three measurements were performed on each patient. The mean
value of the 3 SSI measurements (with their standard deviation), expressed in kiloPascals (kPa) was used as the representative measurement [20]. Measurements were classified as failed when no or little signal was obtained in the SSI box for all acquisitions.

#### ARFI

ARFI imaging was performed by one of the four radiologists mentioned above who did not perform the SSI examination. Each investigator had more than 2 years of experience in ARFI elastography. Details of the technique and the examination procedure have been described in previous reports [21, 22]. ARFI elastography is integrated into a conventional ultrasound device (Acuson S2000, Siemens Medical Solutions, Mountain view, CA). ARFI was performed under the same conditions than SSI but with a patient with apnea or with quiet breathing. Ten valid measurements were performed on each patient. The median value was determined as the representative measurement. Unreliable results were defined as an interquartile range (IQR) /LSM greater than 30%.

#### Fibroscan

Transient elastography was performed with FibroScan (Echosens, Paris, France) using the M probe (also named the standard probe) in patients with BMI<30kg/m<sup>2</sup> and the XL probe (dedicated to overweighed patients) for patients with BMI $\ge$ 30kg/m<sup>2</sup> [11]. Characteristics of the M and XL probes were described in previous reports [23]. Examinations were performed by 2 trained nurses (with an experience of more than 5000 LSM performed) blinded to SWE, ARFI, and biological results. The objective was to obtain a total of 10 valid measurements (defining a successful liver stiffness measurement examination), with the maximum number of attempts set at 20. Liver stiffness evaluation was considered as unreliable when IQR/LSM was >0.30 in patients with LSM  $\ge$ 7.1 kPa [24].

#### Statistical analysis

Statistical analysis was performed using NCSS 9 (NCSS software, Kaysville, Utah). Data were expressed as mean ± standard deviation or median (interquartile range) as appropriate. Quantitative data were compared using either the two-sample t-test or the Wilcoxon on the ranks test, according to data distribution. Percentages were compared using the Khi-2 test or Fisher's exact test. The correlation between non-invasive tests and liver biopsy was tested using the non-parametric Spearman's

correlation coefficient. The influence of different factors on LSM failures with SSI was analysed using logistic regression. The influence of clinical, biological and histological factors on SSI results was analysed using stepwise forward multiple regression. As independent variables, we selected those that influenced each dependent variable studied according to the univariate analysis with a p value of <.05.

Receiver operating characteristics curves for each non-invasive test and of the main serum fibrosis markers were built. Areas under the receiver operating characteristics curves (AUROC) and the 95% confidence intervals (CI) of the AUROC values were calculated for detection of histological fibrosis stage 2 or more (F≥2), fibrosis stage of 3 or more (F≥3), and cirrhosis (F4) in the entire study population. Cut-off values were determined for SSI, ARFI, and Fibroscan to predict mild fibrosis, significant fibrosis, severe fibrosis, and cirrhosis by using an optimization step that maximized the Youden index.

Finally, AUROCs of non-invasive tests were compared using the paired method by Zhou *et al.* [25], excluding patients with missing data or failures of measurements. As multiple pairwise tests were performed on a single set of data, a Bonferroni correction has been used to reduce the risks of false-positive results. A Bonferroniadjusted significance level of 0.0167 was calculated for the comparison of AUROCs of SSI, Fibroscan, and ARFI. Except for the multiple pairwise comparison of AUROCs (pvalue<0.0167), p-values <0.05 were considered significant.

#### RESULTS

#### **Patients and biopsies**

A total of 349 patients were enrolled. The patient's characteristics are summarized in Table 1. Indications of liver biopsies were as follows: to make a decision regarding therapy (n=71) or in case of discordance between Fibroscan and FibroTest (n=8) for chronic hepatitis C patients; to make a decision regarding therapy according to the EASL 2012 recommendations [26] for chronic hepatitis B patients (n=33); for the diagnosis of mixed viral hepatitis or chronic hepatitis E (n=7); for the diagnosis of viral reactivation post-liver transplantation (n=8); for the diagnosis of alcoholic and non-alcoholic steatohepatitis (NASH) (n=145) or the diagnosis of other chronic liver disease (hemochromatosis, n=3; drug-induced liver injury, n=13; sclerosing cholangitis, n=5; auto-immune hepatitis, n=16; primitive biliary cirrhosis, n=7; overlap syndrome, n=2) or unexplained chronic cytolysis (n=31). Mean interval time between liver biopsy and SSI-ARFI, between liver biopsy and Fibroscan and between SSI-ARFI and Fibroscan were 3.7 days (median: 0 day), 2.8 days (median: 0 day), and 3.2 days (median: 0 day), respectively. Median size of liver biopsy sampling was 24.5 mm ([10-60]). There were 33 patients who could not undergo LSM with the 3 elastographic methods because of unavailability of ultrasound devices (transient failure or maintenance) at the time of the measurements: missing data in 13 patients for SWE, 12 patients for ARFI and 8 patients for Fibroscan.

#### LSM characteristics

LSM ranged from 3.6 to 62 kPa with SSI, 3.2 to 75 kPa with Fibroscan, and 0.75 to 4.23 m/s with ARFI, respectively in the study population. LSM was performed using M probe in 235 patients with BMI<30kg/m<sup>2</sup>, and XL probe in 106 patients with BMI≥30kg/m<sup>2</sup>.

SSI, Fibroscan, and ARFI significantly correlated with histological fibrosis score (Spearman's correlation coefficient r=0.79, p<.00001; 0.70, p<.00001; and 0.64, p<.00001, respectively) (Table 2, Figure 2).

#### LSM failures

LSM failures occurred in 10.4% of cases (35 of 336) with SSI, 2.6% (9 of 341) with Fibroscan (6 of 235 patients with BMI<30kg/m<sup>2</sup> for M probe, and 3 of 106 patients with BMI≥30kg/m<sup>2</sup> for XL probe) and none with ARFI (0 of 337). Differences were significant between SSI vs Fibroscan (p=0.0002); SSI vs ARFI (p<.0001); and Fibroscan

vs ARFI (p=0.003). Unreliable results were observed in 5.9% of cases with Fibroscan (20 of 341 patients, 9 of 235 with the M probe, and 11 of 106 with the XL probe), and 16% of cases with ARFI (54 of 337 patients) (p=0.0002).

Using logistic regression, variables associated with SSI failures were intercostal wall thickness (p<.00001), BMI (p<.00001), diabetes (p=.0002), histological liver steatosis (p=.0006), ALP (p=.018), haptoglobin level (p=.03), and history of hypertension (p=.047). SSI failures were more frequent in obese patients: 28% versus 2.6% (29 of 103 patients with BMI $\geq$ 30kg/m<sup>2</sup> vs 6 of 233 patients with BMI<30kg/m<sup>2</sup>; p<.00001). An intercostal wall thickness superior than 25mm was associated with high rates of SSI failures (35.5%, 27 of 76 patients).

#### Factors influencing SSI measurements

By univariate analysis, SSI results were significantly associated with fibrosis stage, activity grade, sex, age, diabetes, hypertension, platelet count, PT, ALP, AST, ALT, total bilirubin, GGT, albumin, ferritin, and cholesterol levels (Supplementary data 1). After entering these variables into stepwise multiple regression, 6 independent variables were found to be associated with SSI results: fibrosis stage (R<sup>2</sup>=0.43); albumin (R<sup>2</sup>=0.07); PT (R<sup>2</sup>=0.04); total bilirubin (R<sup>2</sup>=0.02); platelet count (R<sup>2</sup>=0.01); and GGT (R<sup>2</sup>=0.01); with a total R<sup>2</sup> of 0.62.

#### Discordance between SSI, Fibroscan, ARFI and histologic fibrosis staging

Defined by a difference in at least two stages between the diagnostic test and liver histologic staging, the number of discordant results were 19.6% (59 of 301) for SSI, 24.1% (80 of 332) for Fibroscan and 27.9% (94 of 337) for ARFI elastography respectively, and these figures were not statistically different (p=0.32, 0.07, and 0.44 for SSI vs Fibroscan, SSI vs ARFI, and Fibroscan vs ARFI, respectively). Of the 59 patients with discordance between SSI and liver histologic staging, 36 patients were downstaged (i.e., SSI predicted a lower fibrosis stage of at least 2 stages than the liver biopsy) and 23 patients were upstaged (ie, SSI predicted a higher fibrosis stage than the liver biopsy). Among clinical, biological or histologic criteria, ALP was found to be associated with a downstaging (p=0.01). The fibrosis stage and the intercostal wall thickness were associated with an upstaging (p=0.02 and 0.02, respectively).

## Diagnostic performances of SSI, Fibroscan, ARFI, and serum fibrosis biological markers (Table 3 and 4)

AUROCs for the diagnosis of mild fibrosis ( $\geq$ F1), significant fibrosis ( $\geq$ F2), severe fibrosis ( $\geq$ F3), and cirrhosis (F4) were 0.89, 0.89, 0.92, and 0.92 for SSI; 0.84, 0.83, 0.86, and 0.90 for Fibroscan; 0.81, 0.81, 0.85, and 0.84 for ARFI, respectively (Table 3). Optimal cut-off values for SSI, Fibroscan and ARFI with optimization of the Youden's index are presented in Table 4. AUROCs for the diagnostic accuracy of SSI, Fibroscan, and ARFI according to the following subgroups of patients: chronic viral hepatitis, alcoholic/NASH disorders, and BMI<30kg/m<sup>2</sup>, are presented in supplementary data 2.

#### Comparison of the diagnostic performances of SSI, Fibroscan and ARFI (Figure 3)

Pairwise comparison of AUROCs values between SSI, Fibroscan, and ARFI was performed on 276 patients (40 patients were excluded from the analysis for failures of measurement with SSI or Fibroscan, and 33 for missing LSM with one of the 3 elastographic methods).

For the diagnosis of mild fibrosis ( $\geq$ F1), no significant difference appeared between the performances of SSI, Fibroscan and ARFI (AUROCs of 0.89, 0.86 and 0.84, respectively; p=0.34 for SSI vs Fibroscan; p=0.028 for SSI vs ARFI; and p=0.39 for Fibroscan vs ARFI).

For the diagnosis of significant fibrosis ( $\geq$ F2), SSI had significantly better diagnostic performances than ARFI (AUROCs of 0.88, 0.84, and 0.81 for SSI, Fibroscan and ARFI, respectively; p=0.072 for SSI vs Fibroscan; p=0.0003 for SSI vs ARFI; and p=0.15 for Fibroscan vs ARFI).

For the diagnosis of severe fibrosis ( $\geq$ F3), SSI had significantly better diagnostic performances than Fibroscan (AUROCs of 0.93, 0.87, and 0.89 for SSI, Fibroscan and ARFI, respectively; p=0.0016 for SSI vs Fibroscan; p=0.02 for SSI vs ARFI; and p=0.44 for Fibroscan vs ARFI).

For the diagnosis of cirrhosis (F4), no significant difference was observed between the performances of SSI, Fibroscan and ARFI (AUROCs of 0.93, 0.90 and 0.90, respectively; p=0.09 for SSI vs Fibroscan; p=0.048 for SSI vs ARFI; and p=0.84 for Fibroscan vs ARFI).

#### DISCUSSION

Our prospective study carried on a cohort of 349 patients outlines the diagnostic performances of a new available method, SSI, also named Shear Wave Elastography for the detection and the quantification of liver fibrosis. To the best of our knowledge, our study was the first comparing the diagnostic accuracy of the three available devices allowing quantitative measurement of liver stiffness (SSI, Fibroscan and ARFI) using liver biopsy as reference. Our results showed that SSI is a valuable diagnostic tool for the diagnosis of all stages of liver fibrosis with AUROCs greater than 89%. SSI compared favorably to Fibroscan and ARFI, showing a higher accuracy than Fibroscan in the diagnosis of severe fibrosis ( $\geq$ F3) (AUROC of 0.93 vs 0.87; p=0.0016, respectively) and a higher accuracy than ARFI in the diagnosis of significant fibrosis ( $\geq$ F2) (AUROCs 0.88 vs 0.81; p=0.0003, respectively). SSI also showed a tendency to have an higher diagnostic accuracy than ARFI in the diagnosis of mild fibrosis (p=.028), severe fibrosis (p=.02), and cirrhosis (p=.048).

Previous studies have showed high diagnostic accuracy of Fibroscan and ARFI for the diagnosis of advanced fibrosis stages (F3 or higher) but lower performances for the diagnosis of the earliest fibrosis stages [3, 5-7, 27]. The fact that SSI demonstrated good diagnostic performances for all stages of liver fibrosis even for the diagnosis of  $\geq$ F1 and  $\geq$ F2, respectively) should be emphasized. SSI is therefore an efficient tool that could be used as a first-line examination like Fibroscan or ARFI for the diagnosis of cirrhosis or severe fibrosis, but also for the diagnosis of lower stages of fibrosis. Our results are supporting the statement of Leung *et al.* [28] who have suggested that SSI could be used as a screening tool for the diagnosis of mild liver fibrosis. Moreover, as previously reported with Fibroscan [2, 29], SSI could be used as a prognostic factor in patients with chronic liver diseases.

Our results for SSI yielded slightly lower AUROCs than previous studies that compared SSI and Fibroscan performances for the diagnosis of fibrosis [28,30]. This difference could be first explained by the presence of multiple etiologies of chronic liver diseases in our study. Previous studies evaluated only HCV or HBV patients [28, 29]. Second, our study included a cohort of European patients with a high prevalence of overweight patients, a major technical limitation reported with impulsive elastographic methods [5, 11]. In our population, mean BMI was 27.4kg/m<sup>2</sup>, including 106 patients with BMI>30kg/m<sup>2</sup>, whereas mean BMI was 25.4kg/m<sup>2</sup> in the study published by Ferraioli *et al.* [30], and median BMI was 24.2kg/m<sup>2</sup> in the study published by Leung *et al.* [28]. Unlike these previous studies, for obese patients, we

used the XL probe of Fibroscan that probably leads to an improvement of the Fibroscan accuracy [31].

Our study increases the understanding of technical and patient factors that might affect SSI performance. Failures of LSM were around 10%, which is greater than reported in previous studies but the prevalence of obese patients was higher in our study. No failure of LSM was observed with ARFI. However, a clear improvement of ARFI performance for the diagnosis of cirrhosis and severe fibrosis was observed after exclusion of patients with failures of LSM with SSI or Fibroscan (needed for paired comparison of AUROCs). Therefore, the diagnostic performance of ARFI elastography was poor in patients in whom LSM with SSI or Fibroscan failed, i.e. especially obese patients. Indeed, as for Fibroscan, BMI and intercostal wall thickness are the main variables associated with failures of LSM with SSI, probably owing to a poorer transmission of the ultrasound shear wave when the thickness of soft tissue/fat increases. Success of LSM with SSI appears directly related to the quality of the US image. Thus, SSI is subject to the same limitations encountered with other ultrasoundbased elastography, such as the expertise of the operator and the patient body habitus.

Our study had several limitations. First, our study cohort was of mixed etiologies. Further studies assessing diagnostic performances and reliability of SSI in specific etiologies should be performed. However, this limitation should not affect our main goal which was to compare several non-invasive diagnostic tests eachothers on the same large patient population with the best « gold standard » available. Second, the patients were in charge in a tertiary center specialized in liver fibrosis investigation. Therefore, the prevalence of liver fibrosis or cirrhosis is higher than in the general population. Third, one of the indications of liver biopsy was the presence of a discrepancy between Fibroscan and fibrosis blood markers, which is a selection bias. However, it should be noted that the distribution of the different fibrosis stages in our study population was homogeneous. Finally, liver biopsy was used as the reference method to assess and quantify liver fibrosis. Its diagnostic efficiency is limited by variability in the representativeness of the samples and by inter- and intraobserver variability. To reduce these limitations, we excluded all patients with biopsy that do not meet specific quality criteria [32]. Moreover, we found that the main results of our study sustained when biopsies below 16 mm were excluded (data not shown).

In conclusion, SSI is an efficient diagnostic tool for the diagnosis of all stages of liver fibrosis. SSI provides more accurate performance of liver elasticity compared with Fibroscan in identification of stages  $\geq$  F3 and with ARFI in identification of stage  $\geq$  F2. With respect to Fibroscan and ARFI, SSI benefits from a real-time color mapping of the tissue stiffness in an image superimposed on the standard ultrasound B-mode, allowing performing LSM in regions with sufficient acoustic signal and without artifacts. Further prospective studies need to be carried out in order to confirm the applicability and efficiency of SSI according to the etiology of liver disease and to assess the prognostic value of SSI in patients with chronic liver diseases.

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### TABLES

## Table 1. Characteristics of the 349 included patients

Variable					
Gender – Male : n (%)	188 (54)				
Age (years)	54.8 ± 14 (15-85)				
BMI (kg/m <sup>2</sup> )	27.4 ± 6.4 (15.4-52)				
<25	141				
25 to 29.9	102				
≥30	106				
Waist circumference (cm)	97.4 ± 17.3 (58-154)				
Diabetes : n (%)	105 (30)				
Hypertension : n (%)	143 (41)				
AST (IU/L)	102 ± 183 (14-508)				
ALT (IU/L)	116 ± 170 (11-612)				
GGT (IU/L)	197 ± 253 (9-850)				
Total bilirubin (µmol/L)	29.3 ± 59 (5-105)				
Platelet count (x10 <sup>9</sup> /L)	185 ± 86 (21-508)				
Prothrombin Time (%)	96 ± 20 (21-139)				
Alkaline Phosphatase (IU/L)	115 ± 96 (26-755)				
Albumin (g/L)	40.5 ± 5 (13-50.2)				
Cholesterol (mmol/L)	5.3 ± 3.3 (1.2-11.8)				
Histologic fibrosis stage n (%)					
F0	48 (13.8)				
F1	86 (24.6)				
F2	70 (20)				
F3	51 (14.6)				
F4	93 (26.7)				
Activity grade n (%)					
0	63 (18)				
1	149 (42.7)				
2	105 (30)				
3	31 (8.9)				
Steatosis grade n (%)					
S0 (<5%)	101 (28.9)				
S1 (5-33%)	128 (36.7)				
S2 (34-66%)	64 (18.3)				
S3 (>66%)	56 (16)				

Unless otherwise indicated, results are expressed as mean ± standard deviation (range).

n, number; na, non-available; s.d., standard deviation; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase;

.

Table 2: SSI, Fibroscan and ARFI mean values according to the fibrosis stage in study population. Correlation between SSI, Fibroscan, ARFI, and the fibrosis stage was tested using the non-parametric Spearman's correlation coefficient.

	FO	F1	F2	F3	F4	Correlation
						coefficient
SSI (kPa)	5.7	7.4	8.7	11.9	22.2	0.79
	(±1)	(±3)	(±2.8)	(±3.7)	(±11.5)	(p<.00001)
Fibroscan	6.1	7.9	9.3	11.3	26.2	0.70
(kPa)						
	(±3.8)	(± 4.6)	$(\pm 4.8)$	(± 5.6)	(±16.8)	(p<.00001)
ARFI	1.16	1.28	1.51	1.77	2.24	0.64
(m/s)						
	(±0.60)	(±0.42)	(±0.70)	(±0.54)	(±0.69)	(p<.00001)

Except for the correlation coefficient, results are expressed as means ± standard deviations.

SSI, Supersonic Shear Imaging; ARFI, Acoustic Radiation Force Impulse; kPa, KiloPascals. Correlation coefficient between SSI results and histological fibrosis score was significantly superior than correlation coefficients between Fibroscan results and histological fibrosis score (0.79 vs 0.70; p<0.00001) and between ARFI results and histological score (0.79 vs 0.64; p<0.00001). Difference between Fibroscan and ARFI correlation coefficients was not significant (0.70 vs 0.64; p=0.068).

Table 3: Areas under the receiver operating characteristic curve (with 95% confidence interval) for the diagnostic accuracy of SSI, Fibroscan, ARFI, and serum fibrosis biological markers for the diagnosis of histologic fibrosis stage.

n=349	≥F1	≥F2	≥F3	F4
SSI	0.89	0.89	0.92	0.92
	(0.84-0.92)	(0.84-0.92)	(0.89-0.95)	(0.89-0.95)
Fibroscan	0.84	0.83	0.86	0.90
	(0.77-0.89)	(0.78-0.87)	(0.81-0.89)	(0.86-0.93)
ARFI	0.81	0.81	0.85	0.84
	(0.73-0.87)	(0.75-0.85)	(0.80-0.89)	(0.79-0.88)
Fibrotest	0.79	0.74	0.78	0.81
	(0.71-0.85)	(0.68-0.79)	(0.73-0.83)	(0.75-0.85)
FIB-4	0.77	0.75	0.77	0.82
	(0.70-0.83)	(0.70-0.80)	(0.72-0.82)	(0.76-0.86)
APRI	0.73	0.71	0.72	0.74
	(0.66-0.79)	(0.65-0.77)	(0.66-0.77)	(0.67-0.80)
Forns index	0.71	0.70	0.75	0.78
	(0.61-0.78)	(0.63-0.75)	(0.69-0.80)	(0.71-0.83)
AST/ALT	0.58	0.61	0.67	0.74
	(0.49-0.65)	(0.55-0.67)	(0.60-0.72)	(0.67-0.79)

A direct "head-to-head" comparison between the AUROCs of the 3 impulse elastographic tests and those of the Fibrotest and FIB-4 are presented in supplementary data 3.

**Table 4: Optimal cut-off values of liver stiffness measurements for the diagnosis of liver fibrosis according to the highest Youden's index (Sensitivity+Specificity-1).** Sensitivity, specificity, predictive positive (PPV) and negative value (NPV), true positive (TP) and negative (TN), false positive (FP) and negative (FN), and accuracy ((TP+TN)/(TP+FP+FN+TN)) were expressed for each cut-offs.

Variable	Aim	Cut-	Sensitivity	Specificity	PPV	NPV	ТР	FP	FN	TN	Accuracy
		off	(%)	(%)	(%)	(%)					(%)
SSI	≥F1	7.8	68	100	100	33	175	0	84	42	72
	≥F2	8	83	82	88	75	152	21	32	96	82
	≥F3	8.9	90	81	77	92	113	34	13	141	84
	F4	10.7	85	83	65	93	71	38	13	179	83
Fibroscan	≥F1	8	65	91	98	29	185	4	102	41	68
	≥F2	8.5	76	81	87	67	156	24	50	102	78
	≥F3	8.5	88	71	69	89	124	56	17	135	78
	F4	14.6	77	91	77	91	69	21	21	221	87
ARFI	≥F1	1.35	61	96	99	28	176	2	114	45	65
	≥F2	1.38	72	81	85	65	146	26	58	107	75
	≥F3	1.5	79	81	74	85	108	38	28	163	80
	F4	1.61	81	77	55	92	70	57	17	193	78

Cut-off values are expressed in kiloPascal (kPa) for SSI and Fibroscan and in meters/seconds (m/s) for ARFI.

#### Figure 1

#### Flowchart of study patients.

n, number of patients; NASH, non-alcoholic steato-hepatitis; SSI, Supersonic shear imaging; ARFI, acoustic radiation force impulse.



#### Figure 2:

**Box plots showing distribution of SSI (A), Fibroscan (B) and ARFI (C) values in study population according to histological fibrosis stage.** The top and bottom of the box are the 25<sup>th</sup> and 75<sup>th</sup> percentiles. The length of the box is the interquartile range and the median (50<sup>th</sup> percentile) is the line drawn through the box.



Figure 3:

ROC curves of SSI, Fibroscan, and ARFI for the diagnosis of mild fibrosis, significant fibrosis, severe fibrosis, and cirrhosis. No significant difference appeared for the diagnosis of mild fibrosis and cirrhosis. SSI had a higher accuracy than Fibroscan for the diagnosis of severe fibrosis ( $\geq$ F3) (p=0.0016), and a higher accuracy than ARFI for the diagnosis of significant fibrosis ( $\geq$ F2) (p=0.0003).



#### Supplementary data 1

Table: Spearman's correlation coefficient and p-values for all the variables included in univariate model analysis.

		Univariate model		Multivariate	model
		Correlation	p-value	<b>R</b> <sup>2</sup>	p-value
		Coefficient			
Gender*		0.19	0.0009		
Age*		0.16	0.005		
BMI		-0.06	0.3		
NAFLD disease		-0.09	0.1		
Viral Disease		0.07	0.3		
Parietal	wall	-0.09	0.1		
thickness					
Diabetes*		0.13	0.03		
Hypertension*		0.13	0.03		
AST*		0.45	< 0.00001		
ALT*		0.22	0.0002		
GGT*		0.34	< 0.00001	0.01	0.006
Total bilirubin*		0.34	< 0.00001	0.02	0.0008
Platelet count*		-0.46	< 0.00001	0.01	0.02
Prothrombin time	*	0.43	< 0.00001	0.04	0.0001
Alkaline		0.27	< 0.00001		
phosphatase*					
Albumin*		-0.32	< 0.00001	0.07	0.0004
Ferritin*		0.26	0.00001		
Cholesterol*		-0.21	0.0003		
Histologic fib:	rosis	0.79	< 0.00001	0.43	< 0.00001
stage*					
Activity grade*		0.42	< 0.00001		
Steatosis		0.08	0.17		

\* Variables included in the final multivariate model

BMI, body mass index; NAFLD, non-alcoholic fatty liver diseases; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase;

#### Supplementary data 2

Table: Areas under the receiver operating characteristic curve (with 95% confidence interval) for the diagnostic accuracy of SSI, Fibroscan, and ARFI according to the following subgroups of patients: chronic viral hepatitis, alcoholic/NASH disorders, and BMI<30kg/m<sup>2</sup>.

	Study population			Viral Hepatitis			Alcoholic/NASH			BMI<30 kg/m <sup>2</sup>			
							disorders						
	(n-349)			(n=127)						(n=243)			
							(n=145)						
	≥F2	≥F3	F4	≥F2	≥F3	F4	≥F2	≥F3	F4	≥F2	≥F3	F4	
SSI	0.89	0.92	0.92	0.86	0.91	0.90	0.91	0.94	0.94	0.90	0.93	0.93	
	(0.84-	(0.89-	(0.89-	(0.77	(0.85	(0.83	(0.84	(0.87	(0.88	(0.85-	(0.90-	(0.89-	
	0.92)	0.95)	0.95)	-	-	-	-	-	-	0.93)	0.96)	0.96)	
				0.92)	0.95)	0.94)	0.95)	0.97)	0.97)				
Fibroscan	0.83	0.86	0.90	0.87	0.87	0.89	0.79	0.83	0.90	0.88	0.90	0.91	
(XL probe	(0.78-	(0.81-	(0.86-	(0.78	(0.79	(0.81	(0.69	(0.74	(0.83	(0.83-	(0.85-	(0.86-	
when BMI	0.87)	0.89)	0.93)	-	-	-	-	-	-	0.92)	0.93)	0.95)	
≥30)				0.92)	0.92)	0.94)	0.86)	0.88)	0.95)				
ARFI	0.81	0.85	0.84	0.80	0.88	0.85	0.80	0.79	0.82	0.82	0.89	0.90	
	(0.75-	(0.80-	(0.79-	(0.70	(0.80	(0.75	(0.69	(0.70	(0.74	(0.76-	(0.84-	(0.85-	
	0.85)	0.89)	0.88)	-	-	-	-	-	-	0.87)	0.93)	0.94)	
				0.86)	0.93)	0.91)	0.87)	0.85)	0.88)				

#### Supplementary data 3:

Table: p-values of direct comparisons between the AUROCs of the 3 impulse elastographic tests (SSI, Fibroscan, and ARFI) and the AUROCs values of FibroTest and FIB-4. AUROCs values of each test in study population are noted in parenthesis according to each stage of fibrosis. For paired comparison, these AUROCs values can slightly vary as patients with missing data or failures of measurements are excluded.

	SSI	SI Fibroscan				ARFI			
	≥F2	≥F3	F4	≥F2	≥F3	F4	≥F2	≥F3	F4
	(0.89)	(0.92)	(0.92)	(0.83)	(0.86)	(0.90)	(0.81)	(0.85)	(0.84)
≥F1 (0.79)									
≥F2	< 0.0001			0.003			0.01*		
(0.74)	*			*					
≥F3		<0.0001			0.005			0.05	
(0.78)		*			*				
F4			<0.0001			0.0002			0.43
(0.81)			*			*			
≥F1									
(0.77)									
≥F2	< 0.0001			0.03*			0.04*		
(0.75)	*								
≥F3		<0.0001			0.004			0.03*	
(0.77)		*			*				
F4 (0.82)			<0.0001 *			0.002*			0.53
	≥F1 (0.79) ≥F2 (0.74) ≥F3 (0.78) F4 (0.81) ≥F1 (0.77) ≥F2 (0.75) ≥F3 (0.77) F4 (0.82)	SSI $\geq$ F2         (0.89) $\geq$ F1         (0.79) $\geq$ F2 $\diamond$ (0.0001 $\geq$ F2         (0.74) $\geq$ F3         (0.78)         F4         (0.78) $\geq$ F1         (0.78) $\geq$ F1         (0.77) $\geq$ F2         (0.77) $\geq$ F3         (0.77) $\geq$ F3         (0.77) $\in$ F3         (0.77)         F4         (0.77) $\in$ F3         (0.77) $\in$ F3         (0.77)         F4         (0.82)	SSI $\geq$ F2 $\geq$ F3 $(0.89)$ $(0.92)$ $\geq$ F1 $(0.79)$ $\geq$ F2 $<0.0001$ $\geq$ F2 $<0.0001$ $\geq$ F3 $<0.0001$ $<$ SI $<0.0001$ $<$ CO.0001 $*$ $<$ SF3 $<0.0001$ $<$ F4 $<$ $<$ O.770 $<$ $<$ SF3 $<$ O.0001 $<$ SF3 $<$ $<$ SF3 $<$ $<$ SF3 $<$ $<$ O.0001 $*$ $<$ SF3 $<$	SSI $\geq$ F2 $\geq$ F3F4(0.89)(0.92)(0.92) $\geq$ F1(0.70)(0.92) $\geq$ F2<0.0001	SSIFibroson $\geq$ F2 $\geq$ F3F4 $\geq$ F2(0.89)(0.92)(0.92)(0.83) $\geq$ F1 $(0.92)$ (0.92)(0.83) $\geq$ F1 $(0.0001$ $(0.003)$ $(0.74)$ $(0.0001)$ $\geq$ F3 $<0.0001$ $(0.78)$ $(0.0001)$ $(0.001)$ $\{10,78)$ $(0.0001)$ $(0.0001)$ $(0.001)$ $\geq$ F1 $(0.0001)$ $(0.0001)$ $(0.03^{*})$ $\geq$ F1 $(0.0001)$ $(0.0001)$ $(0.03^{*})$ $\geq$ F3 $<0.0001$ $(0.001)$ $(0.03^{*})$ $\geq$ F3 $<0.0001$ $(0.0001)$ $(0.001)$ $\geq$ F3 $<0.0001$ $(0.0001)$ $(0.0001)$ $\{0.020)$ $(0.0001)$ $(0.0001)$ $(0.0001)$ $\{0.020)$ $(0.0001)$ $(0.0001)$ $(0.0001)$ $\{0.020)$ $(0.0001)$ $(0.0001)$ $(0.0001)$ $\{0.020)$ $(0.0001)$ $(0.0001)$ $(0.0001)$ $(0.020)$ $(0.0001)$ $(0.0001)$ $(0.020)$ $(0.0001)$ $(0.0001)$ $(0.020)$ $(0.0001)$ $(0.0001)$ $(0.020)$ $(0.0001)$ $(0.0001)$ $(0.020)$ $(0.0001)$ $(0.0001)$ $(0.02)$ $(0.0001)$ $(0.0001)$ $(0.02)$ $(0.0001)$ $(0.0001)$ $(0.02)$ $(0.0001)$ $(0.0001)$ $(0.02)$ $(0.0001)$ $(0.0001)$ $(0.02)$ $(0.0001)$ $(0.0001)$ $(0.02)$ $(0.0001)$ $(0.0001)$	SSIFibrosculation $\geq F2$ $\geq F3$ F4 $\geq F2$ $\geq F3$ $(0.89)$ $(0.92)$ $(0.92)$ $(0.83)$ $(0.86)$ $\geq F1$ $$	SSIFibroscurve $\geq F2$ $\geq F3$ $F4$ $\geq F2$ $\geq F3$ $F4$ $(0.89)$ $(0.92)$ $(0.83)$ $(0.86)$ $(0.90)$ $\geq F1$ $(0.79)$ $\geq F2$ $<0.001$ $0.033$ $(0.74)$ $<$ $0.001$ $\geq F3$ $<0.0001$ $10.005$ $(0.78)$ $<0.0001$ $10.005$ $(0.78)$ $<0.0001$ $10.002$ $0.002$ $(0.81)$ $<0.001$ $10.024$ $(0.77)$ $\geq F2<0.0001(0.77)\geq F3(0.77)\geq F3\geq F3\geq F1\geq F2\geq F3$	SSIFibros	SSIFibrosoccessionARFI2F2>F3F4>F3>F4>F3>F3(0.89)(0.92)(0.83)(0.80)(0.90)(0.81)(0.90)(0.81)2F1 </td

\* Significant differences

SSI demonstrated significant better diagnostic performances than Fibrotest and FIB-4 for each level of fibrosis.

#### Pièce jointe n°2

# Reply to: "New imaging assisted methods for liver fibrosis quantification: Is it really favorable to classical transient elastography?

Cassinotto C, de Ledinghen V.

*Journal of Hepatology* 2015 Sep;63(3):767. doi: 10.1016/j.jhep.2015.05.002. Epub 2015 May 14.

To the editor:

We read with great interest comments by Dr. Lanthier et al. [1] regarding our article published in JOURNAL OF HEPATOLOGY [2]. We thank the authors for their kind interest in our work and welcome the opportunity to clarify points they raised.

First, in our study we did not apply nor even mention reliability criteria regarding the Supersonic Shear Imaging (SSI) technique. As Dr Lanthier said, SSI is a quite recent technique, and to date, no study focused on the description or definition of reliability criteria of this technique. In our study, interquartile range (IQR)/ liver stiffness measurement (LSM) was < 0.10 in 207 patients (69%), between 0.10 and 0.30 in 91 (30%), and > 0.30 in 3 patients (1%). So, it is quite obvious that the reliability criteria of Fibroscan<sup>®</sup> are probably not applicable to SSI, but we believe that the definition of such criteria requires a large multicentre study and should not be performed too hastily in a single centre study.

Second, we'd like to clarify the key point that the failure rate of Fibroscan<sup>®</sup> in our study is low, around 2.6%, because the XL probe was used for obese patients (i.e. with body mass index  $\geq$ 30 kg/m<sup>2</sup>) (30% of patients of our study). In our experience, the failure rate of SSI is very close to that of the M probe of Fibroscan<sup>®</sup>. Indeed, we can notice that the failure rate of SSI in our study (10.4% in the whole population; 28% for obese patients but only 2.6% for others) is really close to the failure rate of the M probe described in the study by de Lédinghen et al. [3] among a patient population quite representative of our study population although with a slightly lower BMI (8.4% in the whole population; 26% for obese patients but only 2.5% for others). Unfortunately, as for ARFI and Fibroscan<sup>®</sup>, the reliability of liver stiffness measurement with SSI is dependent on the quality of the shear wave transmission, which rapidly decreases as the overweight increases. This is likely to be evident in SSI and ARFI techniques where operators can notice the strong relationship between the reliability of the LSM and the

quality of the spatial resolution on the B-mode US image.

Finally, as we've discussed, we agree that further studies are needed assessing the diagnostic performances and limits of SSI in specific aetiologies of chronic liver diseases. However, there is mounting evidence that SSI should be considered as an accurate and promising elastography technique in the field of chronic liver diseases.

To conclude, we are living in an era of great and frequent technological innovations, which is particularly exciting, but we have the duty to analyse, criticize and finally validate as quick as possible each new progress in order to ultimately provide the best diagnosis and treatment for all patients.

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#### **ETUDE 2**

## Liver and spleen elastography using Supersonic Shear Imaging for the non-invasive diagnosis of cirrhosis severity and esophageal varices

<u>Cassinotto C</u>, Charrié A, Mouries A, Lapuyade B, Hiriart JB, Vergniol J, Gaye D, Hocquelet A, Charbonnier M, Foucher J, Laurent F, Chermak F, Montaudon M, De Ledinghen V.

#### Digestive and Liver Disease

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#### ABSTRACT

**BACKGROUND.** Elastography is a promising non-invasive approach for assessing liver fibrosis. We assessed diagnostic performances of liver and spleen stiffness using supersonic shear imaging for diagnosing cirrhosis severity and oesophageal varices.

**METHODS.** 401 consecutive cirrhotic patients were prospectively enrolled from November 2012 to March 2014. All patients underwent liver and spleen stiffness measurement with supersonic shear imaging and Fibroscan.

**RESULTS.** Failures of measurement were 6.2% and 29.2% for liver and spleen stiffness (supersonic shear imaging), and 18.4% for liver stiffness (Fibroscan). Liver and spleen stiffness were correlated with severity of cirrhosis, with values increasing according to Child–Pugh subclasses and presence of complications. With a negative predictive value  $\geq$ 90%, liver stiffness cut-offs for high-risk oesophageal varices, history of ascites, Child–Pugh B/C, variceal bleeding and clinical decompensation were 12.8, 19, 21.4, 30.5, and 39.4 kPa, respectively. Areas under the curve of spleen and liver stiffness (supersonic shear imaging), and liver stiffness (Fibroscan) were 0.80, 0.77 and 0.73 respectively for detection of oesophageal varices.

**CONCLUSION.** Liver stiffness using supersonic shear imaging is a relevant diagnostic tool for assessing cirrhosis severity and its complications. Spleen stiffness shows promising results for the detection of oesophageal varices but is not yet sufficiently robust for clinical practice owing to high failure rates.

#### INTRODUCTION

Liver elastography is one of the most promising techniques to have emerged in the past years in the field of chronic liver diseases. Liver stiffness measurement (LSM) using transient elastography (FibroScan<sup>®</sup>) is a widely accepted method to predict the severity and prognosis of liver disease (1-3). LSM is efficient for diagnosing cirrhosis and its severity, with values increasing according to the presence of clinical complications such as portal hypertension (4). In cirrhotic patients, LSM can also be used to predict the presence of clinically significant portal hypertension (hepatic venous pressure gradient (HVPG)≥10 mmHg) or severe portal hypertension (HVPG≥12 mmHg) (5-7). However, it is not considered sufficiently accurate for the prediction or the grading of esophageal varices (EVs) in clinical practice (8).

The ability of a non-invasive approach to predict accurately the presence and size of EVs in cirrhotic patients is of great clinical interest, especially for selecting a target population that would benefit from endoscopic screening programs and/or prophylactic therapy (8-10). The assessment of portal pressure and varices in patients with liver cirrhosis using spleen stiffness measurement (SSM) has been proposed in recent studies (11-13). The increase in spleen stiffness is likely due to spleen congestion that leads to increased organ stiffness. Thus, several authors have reported an excellent correlation of SSM using either Fibroscan (11) or Acoustic Radiation Force Impulse (ARFI) (12-13) with the presence of CSPH and EVs, exploring the feasibility of using elastographic techniques as non-invasive diagnostic and prognostic tools in cirrhotic patients.

Recently, a new technology in the field of liver elastography was proposed. Supersonic shear imaging (SSI), also named Shear Wave<sup>TM</sup> elastography, is based on an ultrasound device (Aixplorer, Supersonic Imagine, Aix-en-Provence, France). Unlike the ARFI or Fibroscan methods in which a single shear wave is emitted temporarily at a single frequency for each measurement, the ultrasound transducer in SSI emits many pulse wave beams at increasing depths, allowing the synchronous evaluation of the velocity of several shear wave fronts over a wide frequency range (14-15). By generating a real-time color mapping of the elasticity encoded pixel by pixel in an image superimposed on the standard B-mode, SSI allows quantitative imaging of the tissue elasticity. Preliminary results on this novel method are promising, showing a higher diagnostic accuracy than Fibroscan or ARFI for the non-invasive assessment of liver fibrosis (16-18). However, no study has ever evaluated the clinical impact of SSI among cirrhotic patients for the non-invasive diagnosis of cirrhosis severity and the detection of EVs.

In the present study, we aimed to assess the diagnostic performances of LSM and SSM using SSI for the non-invasive diagnosis of cirrhosis severity and the detection of EVs.

#### MATERIALS AND METHODS

#### Patients

Between November 2012 and March 2014, all consecutive patients with cirrhosis referred for liver ultrasound (US) examination at our radiology department were prospectively included. Inclusion criterion was cirrhosis either biopsy-proven or diagnosed on combined physical, biological, and radiological evidence. Exclusion criteria were as follows: idiopathic portal vein thrombosis, presence of trans-jugular intra-hepatic porto-cave shunt, cardiac congestive liver, regenerative nodular hyperplasia, hepatocellular carcinoma (HCC) graded as Barcelona Clinic Liver Cancer (BCLC) B or C.

For each patient, LSM and SSM were performed using SSI (LSM-SSI and SSM-SSI, respectively). LSM with Fibroscan (LSM-Fibroscan) was performed the same day as the SSI examination using the Fibroscan M probe (Echosens, Paris, France). All physicians who performed the LSM examinations were blinded to the results of other non-invasive tests. An ethics committee approved the study design and written informed consent was obtained for all patients.

#### SSI technique

SSI was integrated in a conventional ultrasound device (Aixplorer<sup>TM</sup>, Supersonic Imagine, Aix-en-Provence, France). Elastography was performed with the convex probe routinely used for abdominal US examination. Details of the technique and the examination procedure have been described in previous reports (14-18).

#### US examination and stiffness measurements

After overnight fasting, patients underwent a complete upper abdomen US examination by one of four experienced abdominal imaging radiologists. The following criteria were recorded and analyzed: left and right liver lobe diameter, large and small splenic axis and portal vein diameter in millimeters, portal vein velocity in cm/s, presence of steatosis (defined as an increased visual hepatic/renal echogenicity ratio), and presence of ascites. Immediately after, LSM and SSM were performed with the same probe, the patient lying in dorsal decubitus with the arms in maximal abduction, on the right lobe of the liver for LSM and on the inferior pole of the spleen for SSM, through the intercostal spaces. The operator, who was assisted by a real-time B-mode ultrasound image, targeted in a patient with apnea, a region with a good spatial resolution on B-mode US image. In both LSM-SSI and SSM-SSI, a color

mapping with complete and homogeneous fulfilling was obtained in this zone. Then, a region of interest of 15mm of diameter was positioned in the center of the color mapping, in a zone free of large vascular structures and 15mm below the capsule. The mean value of the 5 LSM-SSI and 5 SSM-SSI measurements (with their standard deviation) expressed in kiloPascals (kPa) was used as the representative measurement. Measurements were classified as failed when no or little signal was obtained in the SSI box for all acquisitions.

#### Fibroscan

LSM-Fibroscan was performed by two trained nurses with more than 5000 LSM experience who were blinded to clinical, biological and SSI results. The objective was to obtain a total of 10 valid measurements (defining a successful liver stiffness measurement examination), with the maximum number of attempts set at 20. LSM-Fibroscan was considered as unreliable when IQR/LSM was >0.30 in patients with LSM  $\geq$ 7.1 kPa (19).

#### Morphological and biological parameters

The following parameters were determined for all patients at the time of US examination. Clinical parameters included age, gender, body mass index (BMI), history of diabetes and hypertension, past history of ascites or variceal bleeding, presence of ascites and hepatocellular carcinoma. These parameters were used to classify patients according to their clinical status: compensated cirrhosis, compensated cirrhosis with past history of decompensation, and decompensated cirrhosis. Decompensated cirrhosis was defined as the presence of clinically significant ascites, variceal bleeding, jaundice, or hepatic encephalopathy. Biological parameters included platelet count (Plt), prothrombin time (PT), total bilirubin levels, gamma-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, hyaluronic acid levels, and renal function. These parameters were then used to calculate the FIB-4 score (20), the aspartate aminotransferase/alanine aminotransferase ratio index (AST/ALT) [21], the aspartate-to-platelet ratio index (APRI) (22), and the platelet count/spleen diameter ratio (Plt/Spleen diameter) (23). The liver stiffness\*spleen diameter/platelet count ratio score (LSPS) was calculated as previously described by Kim and colleagues as: LSM-Fibroscan X spleen diameter/platelet ratio (LSPS-Fibroscan) (24). LSPS was also calculated according to the same formula but using LSM-SSI instead of LSM-Fibroscan (LSPS-SSI). Child-Pugh score and model for end-stage liver disease (MELD) score were calculated according to the published formulae (25,26).

#### Upper endoscopic examination

For statistical analysis, we included endoscopic examinations performed within 3 months of SSI examinations for patients with EVs and within 6 months for patients without EVs (n=305 patients). Endoscopic examinations were performed by experienced operators in the endoscopy unit of our hospital, which performs more than 2000 upper endoscopies per year. Varices were graded as follows: grade 0, absence of EVs, grade I, varices were flattened by insufflation; grade II, non-confluent varices protruding in the lumen despite insufflation; grade III, confluent varices were not flattened by insufflation. According to the Baveno V criteria, EVs with high risk of rupture (high-risk EVs) were defined as (i) grade II or III EVs, or (ii) grade I EVs with red signs or Child-Pugh class C (27).

#### Statistical analysis

Inter- and intra-observer agreements for LSM-SSI and SSM-SSI were assessed using intra-class correlation coefficient (ICC) and Bland-Altman analysis on 25 subjects no longer included in the study population. Categorical data were expressed as numbers (percentages) and compared using the Khi-2 test or Fisher's exact test, as appropriate. Quantitative data were expressed as mean ± standard deviation or median ± interquartile range according to data distribution, and compared using either two-sample t-tests or Wilcoxon rank-sum tests. The influence of clinical and biological parameters on LSM and SSM failures with SSI was analyzed using logistic regressions. Nonparametric Kruskal-Wallis rank-sum tests with the multiple comparison Z test were performed to compare non-invasive diagnostic tests amongst the different Child-Pugh classes (A, B, or C) and amongst the different clinical statuses (compensated cirrhosis, compensated cirrhosis with past history of decompensation, or decompensated cirrhosis). Correlations between non-invasive tests and Child-Pugh or MELD scores were assessed using the Pearson's correlation coefficient. Comparison of the different non-invasive tests between patients with and without EVs and patients with and without high-risk EVs were performed using the Wilcoxon rank-sum test. Receiver operating characteristics curves were built for non-invasive tests related to the presence of EVs or high-risk EVs. Areas under the receiver operating characteristics curves (AUC) and their 95% confidence intervals (CI) were calculated in both, the entire study population and amongst patients with compensated cirrhosis only, and were compared using the paired method of Zhou and colleagues (28). Noninvasive tests related to the presence of EVs or high-risk EVs were then included in a stepwise forward multivariate analysis. Finally, the usefulness of SSI was assessed by determining optimal cut-off values in order to predict the absence of complications of cirrhosis with a negative predictive value (NPV) of more than 90%. All statistical analysis was performed using NCSS 9 (NCSS software, Kaysville, Utah), except agreement analysis (Microsoft Excel). Results were considered significant at a p-value of less than 0.05.

#### RESULTS

#### Patients

A total of 401 patients were enrolled (Supplementary Figure 1). Their characteristics at the time of SSI examination are summarized in Table 1. Cirrhosis was biopsy-proven in 176 patients (43.9%) or diagnosed on results of combined physical, biological, radiological and Fibroscan findings in the other 225 patients (56.1%). Etiologies of cirrhosis were: chronic hepatitis C (n=146, 36.4%), chronic hepatitis B (n=18, 4.5%), mixed viral hepatitis (n=23, 5.7%), alcohol-related cirrhosis (n=121, 30.2%), non-alcoholic steatohepatitis (n=63, 15.7%) and other chronic liver diseases (n=30, 7.5%).

The prevalence of decompensated cirrhosis was 18% (72/401 patients). Eightysix patients (21.4%) had HCC diagnosed on biopsy or radiological features (all BCLC stage A; mean diameter=29.4mm±17). Among the 305 patients with upper endoscopic examination, 114 patients had no EVs (37.3%), 57 patients had low-risk EVs (18.7%) and 134 patients had high-risk EVs (43.9%).

#### Agreement of LSM and SSM using SSI

In a preliminary study, agreements regarding the repeatability of LSM-SSI and SSM-SSI were excellent with intra-class correlation coefficients of 0.95 and 0.96 for the intra-observer agreement and 0.94 and 0.87 for the inter-observer agreement, respectively.

#### Failure of measurements

LSM-SSI, LSM-Fibroscan and SSM-SSI ranged from 4.8 to 66.4 kPa, 3.3 to 75 kPa, and 11.8 to 68.1 kPa, respectively. LSM-SSI, LSM-Fibroscan and SSM-SSI failures occurred in 6.2% of cases (25 of 401), 18.4% of cases (74 of 401) and 29.2% of cases (117 of 401), respectively. SSM-SSI failures occurred more frequently than LSM-Fibroscan or LSM-SSI failures (p<0.00001), while LSM-Fibroscan failures occurred more frequently than LSM-SSI failures (p<0.00001).

Variables associated with LSM-SSI and SSM-SSI failures are shown in Supplementary Table 1. Most of these variables were related to the presence of overweight and metabolic syndrome (BMI, intercostal wall thickness, US liver steatosis, hypertension, and cholesterol levels). The percentages of LSM-SSI and SSM-SSI failures in normalweight (BMI<25kg/m<sup>2</sup>), overweight (25≤BMI<30kg/m<sup>2</sup>), and obese patients (BMI≥30kg/m<sup>2</sup>) were 0.6% (1/158) and 18.4% (29/158), 4.6% (6/131) and

25.2% (33/131), 16.1% (19/112) and 49.1% (55/112), respectively. SSM-SSI failures were also more frequent in the absence of splenomegaly: 49.3% (74 of 150 patients with large splenic axis <12cm) vs 17.1% (43 of 251 patients with large splenic axis  $\geq$  12cm) (p<0.00001).

#### Relationships between non-invasive diagnostic tests and severity of cirrhosis

Comparisons of non-invasive tests according to Child-Pugh classes and clinical status are shown in Table 2 and Figure 1. LSM-SSI and LSPS-SSI demonstrated significant differences when comparing all pairs of either Child-Pugh subclasses or clinical status subgroups. Examples of liver and spleen stiffness measurements performed in cirrhotic patients are showed in Supplementary Figure 2.

As shown in Supplementary Table 2, all non-invasive tests demonstrated a significant correlation with Child-Pugh score and MELD score. In particular and apart from PT, bilirubin and albumin levels, which are components of the Child-Pugh score, LSM-SSI, LSPS-SSI, LSPS-Fibroscan, and hyaluronic acid had Pearson's coefficients greater than 0.40 and 0.30 when correlating with Child-Pugh score and MELD score, respectively.

#### Detection of EVs and high-risk EVs

Among compensated cirrhotic patients, LSM-SSI, LSPS-SSI, LSM-Fibroscan, LSPS-Fibroscan, SSM-SSI, hyaluronic acid levels, FIB-4 and Plt/Spleen diameter ratio were significantly higher in patients with EVs and high-risk EVs than in those without EVs or without high-risk EVs, respectively (Supplementary Table 3). AUC of the tests significantly related to EVs or high-risk EVs in both the whole population of our study and patients presenting with compensated cirrhosis are described in Table 3.

Among compensated cirrhotic patients, SSM-SSI, LSM-SSI, LSM-Fibroscan, LSPS-SSI and LSPS-Fibroscan showed better AUC for predicting EVs than hyaluronic acid levels, FIB-4, or Plt/Spleen diameter ratio (p-values<0.01). Furthermore, SSM-SSI, LSPS-Fibroscan and LSPS-SSI demonstrated better AUC for predicting high-risk EVs than hyaluronic acid levels, FIB-4 or Plt/Spleen diameter ratio (P-values<0.01). However, no significant differences were observed between AUC of SSM-SSI, LSM-SSI, LSM-Fibroscan, LSPS-SSI and LSPS-Fibroscan for predicting EVs or high-risk EVS (Supplementary Figure 3).

When using multiple regression, SSM-SSI was the only independent variable significantly associated with the presence of EVs or high-risk EVs:  $R^2$ =0.29 for a total  $R^2$ =0.36 and  $R^2$ =0.26 for a total  $R^2$ =0.32.

#### Diagnostic accuracy of LSM and SSM using SSI in clinical practice

With an NPV of more than 90%, the cut-offs of LSM-SSI in the study population for the presence of cirrhosis complications are represented in Table 4. Among compensated cirrhotic patients, the cut-off with an NPV of more than 90% of SSM-SSI, LSM-SSI and LSM-Fibroscan for the presence of high-risk EVs was 25.6 kPa (AUC of 0.75), 12.8 kPa (AUC of 0.70, and 12.5 kPa (AUC of 0.72), respectively. In view of these NPV, the usefulness of LSM-SSI in clinical practice is represented in Figure 2.

#### DISCUSSION

This prospective cross-sectional study performed in a large cohort of cirrhotic patients shows that SSI could be of great clinical value in the management of cirrhotic patients. Liver elastography using SSI is a valuable diagnostic tool that provides good diagnostic accuracy for assessing cirrhosis severity, regardless of the etiology. LSM-SSI exhibited significant differences when comparing. LSM-SSI demonstrated a significant correlation with Child-Pugh score and MELD score, and significant differences were observed when comparing the different Child-Pugh classes and cirrhosis clinical stages, and patients with or without EVs or high-risk EVs. We also proposed the cutoff values for cirrhosis complications, with an NPV of more than 90%. Thus, more than 90% of patients with LSM-SSI values below 20 kPa did not have a past history of ascites and were not Child-Pugh classes B or C, those below 30.5 kPa did not experience variceal bleeding, and those below around 38/39 kPa did not have ascites at US examination or clinical decompensation. Obviously, the clinical value of SSI technology and other elastography methods is not to diagnose the presence of ascites or clinical decompensation as these symptoms are clinically evident. However, there's an urgent need for new diagnostic methods to simply assess the severity of cirrhosis more accurately than the clinical and biological scores. In clinical practice, such diagnostic methods as SSI should be of major relevance and have many implications for the staging, the prognosis and the follow-up of cirrhotic patients.

Our results show that SSI is as useful as Fibroscan for the non-invasive assessment of cirrhosis and complications. A major advantage of SSI is that it can be performed with an ultrasound device and therefore during a conventional US and Doppler liver examination. This advantage is very relevant in the context of cirrhosis as liver US examination is mandatory during cirrhosis, firstly during episodes of decompensation and secondly every 6 months for the screening of HCC in all cirrhotic patients. Further studies assessing the value of LSM-SSI as an additional diagnostic tool for longitudinal follow-up of patients with cirrhosis would be of great clinical interest. This technical advantage also means that SSI is technically more feasible than Fibroscan in cirrhotic patients. Unlike the latter, it is applicable in patients with ascites and is more readily usable in patients with HCC, since the region of interest where liver stiffness is measured can be chosen. In the current study, LSM using Fibroscan exhibited higher failure rates than usually reported in literature owing in particular to the inclusion of patients with ascites (28 failures of measurement using LSM-Fibroscan of 55 patients with ascites). Thus, our findings should open the way for further studies and improvements in the management of cirrhotic patients. In fact, the potential

clinical applications are such that we recommend the systematic implementation of LSM for each liver US examination performed with an SSI device in cirrhotic patients.

Our findings on the accuracy of SSM for the diagnosis or grading of EVs were less striking than the results of some previous studies that assessed the accuracy of SSM obtained with Fibroscan or ARFI (11-13,29,30). However, our data confirm the conclusions of a recent meta-analysis that showed that the accuracy of SSM is currently limited for its widespread clinical use in the prediction of EVs (31). The SSI technique is indeed not yet ready for SSM as evidenced by the nearly 30% of failures of measurements, despite the expertise of the operators. In our experience, the assessment of SSM with SSI requires high-quality US B-mode images, a thin parietal wall, an adequately thick spleen parenchyma and the absence of movement. However, the quality of imaging of the spleen during US examination is traditionally not as good as for the liver, owing to the thinness and the superior position of the splenic parenchyma, even in many cirrhotic patients, and to the presence of movements due to the proximity of the left cardiac ventricle. These limitations should also be encountered with other tests using a physical approach to assess elastography, such as ARFI and Fibroscan, where the parietal wall thickness and the quality of imaging are of great importance for the reliability of measurements (32). Accordingly, the assessment of spleen stiffness calls for more robust techniques than those normally used to assess liver stiffness.

Another important issue is to compare the diagnostic performances of LSM-SSI with other non-invasive diagnostic tests. In our large cohort of cirrhotic patients with various etiologies, the diagnostic accuracy of LSM-SSI for assessing cirrhosis severity or predicting EVs was at least as good as that of other non-invasive tests such as FIB-4, APRI, AST/ALT, and Plt/Spleen diameter ratio. Moreover, the diagnostic accuracy of combined scores using liver stiffness (LSPS either with Fibroscan or SSI) was not superior to LSM-SSI alone. This finding is relevant as simple diagnostic methods are required that do not involve calculations or other combinations of criteria. Furthermore, the range of LSM-SSI values and cut-offs for diagnosing fibrosis and assessing cirrhosis severity is quite close to those of Fibroscan although in general a little lower (18), which is convenient for its wide dissemination among hepatologists already familiar with the range of Fibroscan values.

Our study has several limitations. First, it was a single-center study. As SSI is quite a novel technique, our results need external validation. Second, the patients were managed in a tertiary center specialized in liver investigation. Therefore, the prevalence of high-stage cirrhosis and EVs was higher than in the general population of cirrhotic patients. Third, no correlation between LSM and SSM using SSI and portal venous pressure assessed with HVPG was performed. Finally, our study cohort was somewhat heterogeneous because it comprised cirrhotic patients of mixed etiologies who potentially had small HCC. However, this limitation did not affect our main goal, which was to assess the accuracy of SSI in a cohort of cirrhotic patients best reflecting patient populations managed by clinicians and hepatologists in clinical practice.

In conclusion, our results show that SSI could be of great clinical value in the management of cirrhotic patients. Liver elastography using SSI is a valuable diagnostic tool that provides good diagnostic accuracy for assessing cirrhosis severity. It is performed during a conventional liver US examination, thereby providing relevant additional information to morphological and Doppler liver findings. This advantage is crucial in patients with cirrhosis as US examination is mandatory every 6 months for the screening of liver nodules, thus providing more accurate longitudinal follow-up. Therefore, we believe that liver stiffness should be performed during each liver US examination performed with an SSI device in cirrhotic patients. However, SSI has technical limitations for performing reliable SSM, with a failure rate of SSM around 30%. Further studies are needed in order to develop more robust techniques for investigating spleen stiffness.
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# TABLES

Table 1: Baseline characteristics of patients at SSI examination

	Study population	
	(n=401)	-
Gender: male	291 (72.6)	
Age (y)	60 ±11.6	18-86
BMI (kg/m <sup>2</sup> )	27.1 ±5.3	16-48.4
Diabetes	128 (31.8)	
Hypertension	171 (42.5)	
AST (UI/1)	95.6 ±150	12- 1977
ALT (UI/1)	82.7 ±102.7	9- 996
GGT (UI/l)	206 ±248	16-1709
Total bilirubin (µmol/l)	27.4 ±33.6	5-382
Platelet count (x10 <sup>9</sup> /L)	129 ±70	7.3- 688
Prothrombin Time (%)	84 ±19.6	23-126
Alkaline Phosphatase (IU/L)	128.3 ±74.3	26-649
Albumin (g/l)	38.2 ±6.3	18.3-60
Creatinin (µmol/L)	74.8 ±45.7	29- 718
Hyaluronic acid (µg/L)	$255 \pm 228$	20-700
LSM-FibroScan (kPa)	27.3 ±20.5	3.3-75
Hepatocellular carcinoma	86 (21.4)	
Child-Pugh class		
Α	294 (73.3)	
В	80 (20)	
С	27 (6.7)	
MELD	6.41 ±2.3	1.98- 14.53
US examination findings		
liver left lobe (cm)	9.6 ±2.8	4-21
liver right lobe (cm)	14.4 ±3	7-30
Portal vein diameter (mm)	12 ±2.9	6-24
Portal vein velocity (cm/sec)	15.4 ±6.2	-19- 36
Steatosis	106 (26.4)	
Large splenic axis (mm)	132 ±28	69-250
Small splenic axis (mm)	56 ±15	22-104
Ascites	55 (13.7)	
LSM-SSI (kPa)	21.7 ±12	4.8-66.4
SSM-SSI (kPa)	33.1 ±9.7	11.8-68.1

Results are expressed as number (percentages) for categorical variables and mean  $\pm$  standard deviation or median  $\pm$  interquartile range for quantitative variables as appropriate.

BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; LSM, liver stiffness measurement; MELD, model for end-stage liver disease; US, ultrasonographic; SSM, spleen stiffness measurement.

	Child A (n=294)	Child B (n=80)	Child C (n=27)	p- values	Compensated cirrhosis (n=265)	Compensated with previous decompensation	Decompensated cirrhosis (n=72)	p- values
						(n=64)		
LSM-SSI	16.2	24.9	40.8	< 0.00001	15.2	25.2	35.9	< 0.00001
(kPa)	±14	±20.4	±11.1	< 0.00001	±12.2	±12.1	±17.7	< 0.00001
				0.0004				< 0.00001
SSM-SSI	30.6	36	37.8	0.002	28.5	37.3	37.7	< 0.00001
(kPa)	±14.2	±13.5	±7.6	0.0004	±12.6	±10.4	±11.8	< 0.00001
				0.1				0.62
LSM-	16.9	27.7	41.8	0.00003	15.9	29.9	39.3	0.00001
Fibroscan	±20.4	±30.9	±27.5	0.0004	±17.2	±31.5	±27.7	< 0.00001
(kPa)				0.12				0.1
Hyaluronic	113	374	700	< 0.00001	107	222	435	0.002
acid (µg/L)	±223	±529	±402	< 0.00001	±220	±390	±532	< 0.00001
				0.02				0.01
FIB-4	3.84	6.91	9.54	< 0.00001	3.89	4.8	8.24	0.07
	±3.9	±7.1	±11	< 0.00001	±4.3	±4.6	±9.5	< 0.00001
				0.05				0.002
AST/ALT	1.06	1.49	1.85	< 0.00001	1.02	1.36	1.62	0.00003
	±0.62	±0.75	±1.22	< 0.00001	±0.61	±0.61	±0.94	< 0.00001
				0.01				0.003
APRI	1.18	1.85	2.66	0.0006	1.24	1.33	2.28	0.77
	±1.64	±2.75	±5.6	0.0003	±1.82	±1.44	±3.36	0.0002
				0.05				0.005
Plt/Spleen	1022	648	524	0.00001	1022	700	620	0.0003
diameter	±911	±527	±667	0.0003	±931	±657	±586	< 0.00001
ratio				0.35				0.29
LSPS-	1.78	5.28	9.47	< 0.00001	1.6	4.38	7.77	< 0.00001
Fibroscan	±3.26	±6.14	±14.1	0.00006	±2.88	±7.25	±7.37	< 0.00001
				0.08				0.07
LSPS-SSI	1.73	4.31	6.36	< 0.00001	1.44	3.62	5.21	< 0.00001
	±2.89	±4.14	±8.46	< 0.00001	±2.63	±3.33	±6.01	< 0.00001
				0.04				0.003

Table 2: Comparison of non-invasive diagnostic tests according to Child-Pugh classes and clinical status

Data are medians (± interquartile range). Comparison of values between the different subgroups was performed using non-parametric Kruskal-Wallis Z test. The p-values refer to the comparison between the first and second subgroups (i.e. for example between Child-Pugh A and B); between the first and third subgroups (i.e. between

Child-Pugh A and C); and between the second and third subgroups (i.e. between Child-Pugh B and C), respectively.

Abbreviations: LSM, liver stiffness measurement; SSI, supersonic shear imaging; kPa, kiloPascals; SSM, spleen stiffness measurement; AST/ALT, aspartate aminotransferase/alanine aminotransferase ratio index; APRI, AST/platelet ratio index; Plt, Platelet count; LSPS, liver stiffness\*spleen diameter/platelet count ratio score.

	Cut offs	AUC	Se	Sp	PPV	NPV	+ LR	- LR
Study population								
No hepatocellular carcinoma	LSM-SSI ≤ 14.6 kPa	0.62	84%	38%	27%	90%	1.35	0.42
No past history of ascites	LSM-SSI ≤ 19 kPa	0.81	65%	85%	55%	90%	4.33	0.41
No Child-Pugh B or C	LSM-SSI ≤ 21.4 kPa	0.78	77%	68%	44%	90%	2.37	0.34
No variceal bleeding	LSM-SSI ≤ 30.5 kPa	0.76	52%	82%	36%	90%	2.87	0.59
No US ascites	LSM-SSI≤37.7 kPa	0.78	46%	90%	31%	95%	4.71	0.6
No clinical decompensation	LSM-SSI ≤ 39.4 kPa	0.85	46%	97%	73%	90%	13	0.56
Compensated cirrhotic patients only								
No high-risk EVs	LSM-SSI ≤ 12.8 kPa	0.70	92%	36%	44%	90%	1.44	0.22
	SSM-SSI ≤ 25.6 kPa	0.75	94%	36%	50%	90%	1.47	0.17

Table 3: Diagnostic accuracy of LSM-SSI and SSM-SSI for cut-off values with NPV of more than 90%

Abbreviations: AUC, area under receiver operating characteristic curve; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio; LSM-SSI, liver stiffness measurement using Supersonic Shear Imaging; US, ultrasound; EVs, esophageal varices.

Variables		LSM	-SSI	SSM-SSI	
		Odds	95% CI	Odds	95% CI
		Ratios		Ratios	
BMI		1.22	1.13-1.32	1.17	1.11-1.23
Right intercostal	wall	1.21	1.13-1.30		
thickness					
Left intercostal	wall			1.24	1.17-1.31
thickness					
Albumin		0.92	0.87-0.98	1.04	1.002-1.08
Male gender		0.38	0.17-0.87	0.52	0.33-0.85
Splenic large axis				0.96	0.95-0.97
Splenic small axis				0.94	0.92-0.96
<b>US</b> liver steatosis				2.46	1.76-3.42
Hypertension				2.59	1.61-4.17
Prothrombin time				1.02	1.01-1.03
LSM-SSI failure				4.42	1.92-10.18
SSM-SSI failure		4.42	1.92-10.18		
MELD score				0.87	0.78-0.97
Cholesterol levels				1.32	1.10-1.57

Supplementary Table 1: Variables associated with LSM-SSI and SSM-SSI failures.

Only significant results of the binomial logistic regression were reported.

Abbreviations: LSM, liver stiffness measurement; SSI, supersonic shear imaging; SSM, spleen stiffness measurement; CI, confidence intervals; BMI, Body mass index; MELD, model for end-stage liver disease.

Supplementary Table 2: Correlation of different parameters with Child-Pugh and MELD scores

Variables	Child-Pu	igh score	MELD score		
	(ranging from	m A5 to C15)			
	Correlation	95% CI	Correlation	95% CI	
	coefficient		coefficient		
Non-invasive diagnostic te	sts				
LSM-SSI	0.53	0.46-0.60	0.32	0.23-0.41	
SSM-SSI	0.24	0.14-0.36	0.17	0.05-0.27	
LSM-Fibroscan	0.36	0.26-0.45	0.28	0.17-0.37	
Hyaluronic acid	0.51	0.43-0.58	0.36	0.26-0.44	
FIB-4	0.31	0.22-0.40	0.22	0.12-0.32	
AST/ALT	0.33	0.23-0.41	0.20	0.10-0.29	
APRI	0.15	0.05-0.25	0.11	0.006-0.20	
Plt/Spleen diameter	-0.27	-0.170.36	-0.31	-0.220.40	
LSPS-Fibroscan	0.42	0.32-0.50	0.31	0.21-0.41	
LSPS-SSI	0.48	0.39-0.55	0.34	0.25-0.43	
Other variables					
Right liver lobe diameter	-0.23	-0.120.32	-0.15	-0.040.25	
Portal vein velocity	-0.18	-0.80.28	-0.08	0.020.18	
Splenic large axis	0.24	0.15-0.33	0.34	0.25-0.43	
Splenic small axis	0.27	0.17-0.36	0.32	0.23-0.40	
Plt	-0.23	-0.130.32	-0.22	-0.130.32	
Prothrombin time	-0.68	-0.620.73	-0.67	-0.610.72	
Albumin levels	-0.67	-0.610.72	-0.37	-0.280.45	
Alkaline phosphatase	0.33	0.25-0.52	0.24	0.14-0.33	
Bilirubin levels	0.58	0.51-0.64	0.50	0.42-0.57	
Creatinin levels	0.03	-0.07- 0.13	0.49	0.41-0.56	

Abbreviations: MELD, model for end-stage liver disease; CI, confidence intervals; LSM, liver stiffness measurement; SSI, supersonic shear imaging; SSM, spleen stiffness measurement; AST/ALT, aspartate aminotransferase/alanine aminotransferase ratio index; APRI, AST-to-Platelet ratio index; Plt, Platelet count; LSPS, liver stiffness\*spleen diameter/platelet count ratio score; ns, non-significant.

# Supplementary Table 3: Comparison of non-invasive diagnostic tests according to presence or not of EVs and high-risk EVs in compensated cirrhotic patients (n=237)

	No EVs	EVs	p-values	No EVs or	High-risk	p-values
	(n=103)	(n=134)		low-risk	EVs	
				EVs	(n=84)	
				(n=153)		
LSM-SSI (kPa)	14.4 ±9.1	21.5 ±15.1	< 0.00001	15.9 ±11.6	$21.5 \pm 14.5$	< 0.00001
SSM-SSI (kPa)	$25.9 \pm 10.6$	36.9 ±11.5	< 0.00001	$28.5 \pm 13$	$37.9 \pm 10$	< 0.00001
LSM-Fibroscan (kPa)	13.7 ±14.7	26.3 ±22.2	< 0.00001	16 ±17.3	$26.6 \pm 26.2$	< 0.00001
Hyaluronic acid (µg/L)	97 ±185	$188 \pm 328$	0.002	107 ±221	229 ±382	0.0003
FIB-4	$3.22 \pm 4.04$	$4.97 \pm 4.62$	0.001	$3.63 \pm 4.21$	5.17 ±4.97	0.003
AST/ALT	$1.05 \pm 0.68$	$1.2 \pm 0.68$	0.06	$1.03 \pm 0.66$	$1.27 \pm 0.72$	0.0008
APRI	$1 \pm 1.74$	$1.62 \pm 1.62$	0.004	$1.24 \pm 1.89$	$1.53 \pm 1.54$	0.18
Plt/Spleen diameter	1161 ±988	$650 \pm 753$	< 0.00001	1113 ±928	703 ±588	< 0.00001
ratio						
LSPS-Fibroscan	$1.24 \pm 1.85$	$3.56 \pm 4.23$	< 0.00001	$1.7 \pm 2.44$	$4.48 \pm 4.53$	< 0.00001
LSPS-SSI	1.17 ±1.69	3.1 ±3.1	< 0.00001	$1.5 \pm 2.32$	$3.5 \pm 2.8$	< 0.00001

Data are medians  $\pm$  interquartile range.

Abbreviations: LSM, liver stiffness measurement; SSI, supersonic shear imaging; kPa, kiloPascals; SSM, spleen stiffness measurement; AST/ALT, aspartate aminotransferase/alanine aminotransferase ratio index; APRI, AST/platelet ratio index; Plt, Platelet count; LSPS, liver stiffness\*spleen diameter/platelet count ratio score.

Supplementary Table 4: Diagnostic performances expressed as AUC values (95%CI) of non-invasive diagnostic tests for the prediction of EVs and high-risk EVs in study population (n=305) and in compensated cirrhotic patients only (n=237).

	Presen	ice of EVs	Presence of "H	High-risk" EVs
	Study	Compensated	Study	Compensated
	population	cirrhosis	population	cirrhosis
LSM-SSI	0.77	0.74	0.75	0.70
	(0.71-0.82)	(0.67-0.80)	(0.68-0.80)	(0.62-0.76)
SSM-SSI	0.80	0.79	0.78	0.75
	(0.73-0.86)	(0.71-0.85)	(0.71-0.83)	(0.67-0.82)
LSM-Fibroscan	0.73	0.74	0.71	0.72
	(0.65-0.78)	(0.66-0.80)	(0.64-0.77)	(0.64-0.78)
Hyaluronic acid	0.67	0.63	0.68	0.65
	(0.60-0.73)	(0.54-0.70)	(0.61-0.74)	(0.56-0.72)
FIB-4	0.65	0.63	0.65	0.62
	(0.58-0.71)	(0.55-0.70)	(0.58-0.71)	(0.54-0.70)
Plt/Spleen diameter	0.68	0.70	0.68	0.71
ratio	(0.62-0.74)	(0.62-0.76)	(0.62-0.74)	(0.63-0.77)
LSPS-Fibroscan	0.74	0.75	0.74	0.76
	(0.67-0.80)	(0.68-0.82)	(0.67-0.80)	(0.68-0.82)
LSPS-SSI	0.75	0.75	0.75	0.74
	(0.68-0.81)	(0.67-0.81)	(0.68-0.80)	(0.66- 0.80)

Abbreviations: AUC, area under receiver operating characteristic curve; 95% C.I., 95% confidence interval; EVs, esophageal varices; LSM, liver stiffness measurement; SSI, supersonic shear imaging; SSM, spleen stiffness measurement; Plt, platelet count; LSPS, liver stiffness\*spleen diameter/platelet count ratio score.

Figure 1: Distribution of liver stiffness measurements using supersonic shear imaging. Box plots showing distribution of liver stiffness measurements using supersonic shear imaging according to Child-Pugh classes (A) and clinical status of cirrhosis (B) (0=compensated cirrhosis; 1=compensated cirrhosis with past history of decompensation; 2=decompensated cirrhosis)

LSM= liver stiffness measurements; SSI= supersonic shear imaging



Figure 2: Usefulness of liver stiffness measurements with supersonic shear imaging in clinical practice (cut-off values with negative predictive value of more than 90%)



# Supplementary Figure S1 Flow chart of the studied patients



# **Supplementary Figure S2**

Examples of liver and spleen stiffness measurements obtained with Supersonic Shear Imaging in cirrhotic patients.

Liver stiffness value of 9.3 kPa (Figure S2A) and spleen stiffness value of 18.9 kPa (Figure S2B) in a 53 years old male patient with previous history of chronic hepatitis C (genotype 1b) diagnosed in 2000. Cirrhosis was biopsy-proven in 2007 (METAVIR score: Activity 2 / Fibrosis 4). After 10 years of treatment failures, hepatitis C was cured in 2011/2012 using a combination of Peginterferon alfa-2a, Ribavirin, and Telaprevir. Cirrhosis is well compensated (Child-Pugh score A5) without previous history of decompensation, and the patient has no clinical symptoms related to cirrhosis or portal hypertension. This case illustrates the difficulties to obtain a measurement of quality for spleen stiffness in a patient with a normal-sized spleen. Liver stiffness value of 46.6 kPa (Figure S2C) and spleen stiffness value of 41.2 kPa (Figure S2D) in a 42 years old male patient with an alcoholic and chronic hepatitis C cirrhosis waiting for liver transplantation. Cirrhosis is decompensated with ascites and jaundice (Child-Pugh score C11); MELD score is 21; and upper endoscopy examination revealed large esophageal varices (grade 2).



**Supplementary Figure S3:** 

Receiver operating characteristic curves of liver and spleen stiffness with supersonic shear imaging, and liver stiffness with Fibroscan for the diagnosis of esophageal varices (Figure S3A) and « high-risk » esophageal varices (Figure S3B). No significant difference appeared between liver and spleen stiffness with supersonic shear imaging, and Fibroscan for the diagnosis of esophageal varices (areas under the curves of 0.77, 0.82, and 0.79, respectively; all p-values>0.2) or « high-risk » esophageal varices (areas under the curves of 0.71, 0.79, and 0.70, respectively; all p-values>0.1)



# **ETUDE 3**

# Liver stiffness in nonalcoholic fatty liver disease: a comparison of Supersonic Shear Imaging, FibroScan and ARFI with liver biopsy

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# Hepatology

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# ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) has become a major public health issue. The goal of this study was to assess the clinical use of liver stiffness measurement (LSM) evaluated by supersonic shear imaging (SSI), FibroScan, and acoustic radiation force impulse (ARFI) in a cohort of NAFLD patients who underwent liver biopsy. A total of 291 NAFLD patients were prospectively enrolled from November 2011 to February 2015 at 2 French university hospitals. LSM was assessed by SSI, FibroScan (M probe), and ARFI within two weeks prior to liver biopsy. Calculations of the area under the receiver operating curve (AUROC) were performed and compared for the staging of liver fibrosis. AUROC for SSI, FibroScan, and ARFI were 0.86, 0.82, and 0.77 for diagnoses of ≥F2; 0.89, 0.86, and 0.84 for ≥F3; and 0.88, 0.87, and 0.84 for F4, respectively. SSI had a higher accuracy than ARFI for diagnoses of significant fibrosis ( $\geq$ F2) (P = 0.004). Clinical factors related to obesity such as body mass index  $\geq$  30 kg/m(2) , waist circumference  $\geq$ 102 cm or increased parietal wall thickness were associated with LSM failures when using SSI or FibroScan and with unreliable results when using ARFI. In univariate analysis, FibroScan values were slightly correlated with NAFLD activity score and steatosis (R = 0.28 and 0.22, respectively), whereas SSI and ARFI were not; however, these components of NAFLD did not affect LSM results in multivariate analysis. The cutoff values for SSI and FibroScan for staging fibrosis with a sensitivity  $\ge 90\%$  were very close: 6.3/6.2 kPa for  $\ge F2$ , 8.3/8.2 kPa for  $\ge F3$ , and 10.5/9.5 kPa for F4.

**CONCLUSION.** Although obesity is associated with an increase in LSM failure, the studied techniques and especially SSI provide high value for the diagnosis of liver fibrosis in NAFLD patients.

# INTRODUCTION

As a result of the growing obesity epidemic, nonalcoholic fatty liver disease (NAFLD) has become a major public health issue. NAFLD currently represents the leading cause of chronic liver disease in Western countries and the second leading etiology among adults awaiting liver transplantation in the United States.<sup>1-3</sup> Liver-related morbidity and mortality in NAFLD patients are linked to the development of nonalcoholic steatohepatitis (NASH), which can progress to fibrosis and cirrhosis lesions.<sup>4</sup> NASH is also associated with an increased risk of hepatocellular carcinoma development and an increased risk of cardiovascular mortality and type 2 diabetes mellitus.<sup>5</sup> Liver biopsy is considered the gold standard for the evaluation and quantification of liver fibrosis in NAFLD patients. However, this diagnostic method is invasive and often painful and can be associated with rare but severe complications.<sup>6</sup>

The development of non-invasive diagnostic tools to assess liver fibrosis has been an increasingly studied focus of research. In recent years, several non-invasive diagnostic tools, based on liver stiffness measurements (LSM) such as FibroScan® (Echosens, Paris, France), acoustic radiation force impulse (ARFI, Siemens Medical Solutions, Mountain View, CA) elastography, and Supersonic Shear Imaging (SSI, Supersonic Shear Imagine, Aix-en-Provence, France) have been developed to quantify liver fibrosis through the measurement of mechanical or ultrasound shear wave propagation through the hepatic parenchyma. Numerous studies have confirmed the efficiency and reproducibility of these techniques in the diagnosis of severe fibrosis and cirrhosis in patients affected by a chronic viral hepatitis.7-12 NAFLD is a challenge for these non-invasive techniques based on a physical approach, as the increased soft tissue/fat in obese patients lead to a poorer transmission of the ultrasound or mechanical beam, leading to failures in measurements or unreliable results. For FibroScan, failures of measurement or non-interpretable results occur in 5% and 15% of cases, respectively, with the main limiting factor being obesity.<sup>13</sup> ARFI elastography demonstrates a decrease in its diagnostic performances that increases with the extent of overweight.<sup>14</sup> Regarding the SSI technique, no study has focused on the effects of overweight on its diagnostic performance for assessing liver fibrosis. As overweight and NAFLD are intimately linked, it is important to question how this new technique behaves in NAFLD/NASH patients. Moreover, the impact on liver stiffness of major components of NAFLD such as steatosis, inflammation and hepatocyte ballooning remains unclear.

The aim of this study was to prospectively compare the diagnostic accuracy of SSI, FibroScan, and ARFI for the staging of fibrosis in NAFLD-related chronic liver disease using liver histology as the reference standard.

#### **METHODS**

#### Patients

We prospectively enrolled consecutive adult patients with NAFLD who had undergone liver biopsy from November 2011 to February 2015. Patients were recruited from 2 French academic centers, Angers and Bordeaux university hospitals, which are tertiary centers for the management of liver diseases (108 patients recruited from the university hospital of Bordeaux had been included in a previous study comparing several non-invasive tests in patients with chronic liver diseases of mixed etiologies).<sup>12</sup> Included were patients who had LSM performed using SSI, ARFI, and FibroScan within the two weeks prior to liver biopsy. Exclusion criteria were high alcohol consumption (i.e., >21 drinks, on average, per week in men and >14 drinks, on average, per week in women),<sup>4,15</sup> associated causes of liver disease (alcoholic, viral, or other causes of liver injury), and a liver biopsy length of less than 10 mm and/or fewer than 6 portal spaces and/or more than 2 fragments, except for in cases of cirrhosis. All physicians who performed LSM experiments were blinded to the results of other noninvasive methods and liver biopsies. An ethics committee approved the design of this cross-sectional study, and a written informed consent was obtained from all patients.

# Liver histological examination

Histopathological staging for liver fibrosis served as the reference standard. Liver biopsies were fixed in formalin and embedded in paraffin. All biopsy specimens were analyzed by one of 3 pathologists who specialized in liver diseases and were blinded to the non-invasive test results. Fibrosis was staged according to NASH CRN scoring system <sup>16</sup> as follows: stage 0 (F0), absence of fibrosis; stage 1 (F1), perisinusoidal or portal; stage 2 (F2), perisinusoidal and portal/periportal; stage 3 (F3), septal or bridging fibrosis; stage 4 (F4), cirrhosis. The grade of steatosis was also defined according to Kleiner et al.: S0, <5%; S1: 5-33%, mild; S2: 34-66%, moderate; and S3: >67%, marked. Disease activity was quantified using the NAFLD activity score (NAS), which is based on the severity of steatosis (0-3), inflammation (0-3), and hepatocellular ballooning (0-2).<sup>16</sup>

## Morphological and biological parameters

For all patients, the following parameters were determined at the time of liver stiffness measurement. Clinical parameters included age, sex, body mass index (BMI), waist circumference, history of diabetes, and hypertension. A blood sample was obtained to quantify the platelet count, prothrombin time, total bilirubin levels, and gamma-glutamyl transpeptidase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, albumin, cholesterol, triglyceride, and HDLcholesterol levels. Intercostal wall thickness in millimeters was also measured during liver ultrasound examination.

#### SSI

SSI is integrated into a conventional ultrasound device (Aixplorer<sup>™</sup>, Supersonic Imagine, Aix-en-Provence, France). Using radiation force and ultrafast ultrasound imaging, the supersonic shear imaging technique allows one to remotely generate and follow a transient plane shear wave that is propagating in vivo in real time. The radial forces that generate the shear wave are focused at increasing depths, causing a shear wave front that propagates in the area scanned. The same emitting probe receives, at high frequency, the propagation speed data from the shear waves, allowing for the updating, in real time, of the received data. Each sequence of 3 shear wave fronts lasts approximately one second. The tissue shear modulus, representing its stiffness, can be estimated from the shear wave local velocity. The ultrasound system then generates a real-time color map of the elasticity encoded pixel by pixel in an image superimposed on the standard B-mode.<sup>17,18</sup>

One of 6 experienced abdominal imaging radiologists performed SSI examinations in accordance with the manufacturer's instructions. LSM was performed through intercostal spaces while the patient was lying in dorsal decubitus with the right arm in maximal abduction. The operator, who was assisted by a real-time Bmode ultrasound image, targeted under apnea, a region of the right lobe of the liver with good spatial resolution for B-mode US imaging. Once a color map with complete and homogeneous filling was obtained in the zone, a region of interest 15 mm in diameter was positioned in the center of the color map in a zone free of large vascular structures and at least 15 mm below the capsule. As described in literature and in accordance with manufacturer's recommendations, less than 10 values were used for SSI technique.<sup>11,19</sup> Five measurements were performed on each patient. The median value of the 5 SSI measurements (with their interquartile range), expressed in kilopascals (kPa) was used as the representative measurement. Measurements were classified as failed when no or little signal was obtained in the SSI box for all acquisitions. As no reliability criteria have been validated for SSI or ARFI techniques, we applied the reliability criteria of FibroScan for the 3 techniques used in this study.<sup>20</sup> SSI results were then considered as unreliable when the interquartile range (IQR)/LSM was >0.30 in patients with LSM  $\geq$ 7.1 kPa.

#### FibroScan

Transient elastography was performed with FibroScan (Echosens, Paris, France) using the standard probe (also named the M probe). Examinations were performed by one of 3 trained nurses (with experience performing more than 5000 LSM) blinded to SSI, ARFI, and biological results. The objective was to obtain a total of 10 valid measurements (defined as a successful liver stiffness measurement examination), with the maximum number of attempts set at 20. Liver stiffness evaluation was considered as unreliable when IQR/LSM was >0.30 in patients with LSM  $\geq$ 7.1 kPa.<sup>20</sup>

## ARFI

ARFI imaging was performed by one of the six radiologists noted above who did not perform the SSI examination. Each investigator had more than 2 years of experience in ARFI elastography. Details of the technique and the examination procedure have been described in previous reports.<sup>21</sup> ARFI elastography is integrated into a conventional ultrasound device (Acuson S2000, Siemens Medical Solutions, Mountain View, CA). ARFI was performed under the same conditions as SSI but in patients with apnea or with quiet breathing. Ten valid measurements were performed on each patient. The median value was determined as the representative measurement. In extension of the reliability rules applied for FibroScan, unreliable results were defined as an IQR/LSM >30% in patients with LSM ≥1.54 m/s.

#### Statistical analysis

Statistical analysis was performed using NCSS 9 (NCSS software, Kaysville, Utah). Data were expressed as the mean ± standard deviation or the median (IQR) and compared using either the two-sample t-test or the Wilcoxon signed-rank test, according to data distribution. Percentages were compared using the Chi<sup>2</sup> test or Fisher's exact test as appropriate. The influence of different factors on LSM failures or unreliable results was analyzed using logistic regression. The influence of clinical, biological and histological factors on LSM was analyzed using stepwise forward multiple regression. For independent variables, we selected those that influenced each dependent variable that was studied according to the univariate analysis with a p value <0.1. The diagnostic performances of non-invasive tests were assessed in a perprotocol strategy (i.e., with exclusion of failed measurements and unreliable results) by correlating tests results and histological fibrosis stage using the non-parametric Spearman correlation coefficient, which were compared to each other according to Guilford et al.,<sup>22</sup> and by building receiver operating characteristics curves. Areas under

the receiver operating characteristics curves (AUROCs) and the 95% confidence intervals (CI) of the AUROC values were calculated for the detection of histological fibrosis stage 2 or higher (F≥2), fibrosis stage 3 or higher (F≥3), and cirrhosis (F4). Optimal cut-off values were determined for SSI, FibroScan, and ARFI to predict significant fibrosis, severe fibrosis, and cirrhosis according to at least 90% sensitivity, at least 90% specificity, the Youden index (maximum sum of sensitivity and specificity), and the best diagnostic accuracy (i.e., the maximum sum of true positives and true negatives over the total number of patients). Finally, AUROC values for noninvasive tests were compared using the paired method by Zhou et al.<sup>23</sup> As multiple pairwise tests were performed on a single set of data, a Bonferroni correction was used to reduce the risks of false-positive results. A Bonferroni-adjusted significance level of 0.0167 was calculated for the comparison of AUROC values for SSI, FibroScan, and ARFI.

#### RESULTS

From November 2011 to February 2015, 328 NAFLD patients underwent liver biopsy and LSM. Fourteen patients were excluded due to a liver biopsy length less than 10 mm, and 23 patients were excluded because one of the 3 elastographic methods was not available at the time of the measurements due to the unavailability of ultrasound devices (defective device or maintenance). Finally, 291 patients having had LSM with SSI, M probe of FibroScan, and ARFI were included for analysis. Patient characteristics are summarized in Table 1. The number of patients with cirrhosis was lower in the Angers cohort than in the Bordeaux cohort (6% vs. 24%, respectively; p<0.0001). Otherwise, both groups had quite close rates of obesity, type 2 diabetes, hypertension, and metabolic syndrome. With the exception of the SSI median stiffness value for diagnoses of F3 or greater disease, there were no significant differences between both centers in median levels of LSM using SSI, FibroScan or ARFI, adjusted for fibrosis stage, or for the correlation between LSM and fibrosis stage (Supplementary Table S1), allowing results from both centers to be pooled together.

#### Failures of measurements or unreliable results

LSM failures occurred in 13% of cases (38 of 291) with SSI, 14.4% (42 of 291) with FibroScan and 0.7% with ARFI (2 of 291) (Table 2 and Figure 1). Unreliable results were observed in 7.2% of cases with SSI (21 of 291 patients), 8.9% of cases with FibroScan (26 of 291), and 18.2% of cases with ARFI (53 of 291 patients). Failure rates were lower for ARFI than for SSI or FibroScan (both p<0.0001), whereas unreliable results were higher for ARFI than for SSI or FibroScan (p=0.0001 and p=0.001, respectively). Very reliable results (as defined for FibroScan, i.e. IQR/LSM<0.10) were more frequent with SSI than for FibroScan or ARFI (both p<0.0001). In all, there were no differences in the reliability of results, with 79.7% reliable results produced by SSI (232 of 291), 76.6% by FibroScan (223 of 291), and 81% by ARFI (236 of 291) (p=0.4 for SSI vs. FibroScan, p=0.7 for SSI vs. ARFI, and p=0.2 for FibroScan vs. ARFI).

## Factors associated with LSM failures or unreliable results

Using logistic regression, we determined that the variables associated with failure of measurement or unreliable results with at least 2 of the 3 techniques studied included: waist circumference (R<sup>2</sup>=0.19 for SSI, 0.16 for FibroScan, and 0.18 for ARFI), BMI (R<sup>2</sup>=0.10 for SSI, 0.08 for FibroScan, and 0.16 for ARFI), intercostal wall thickness (R<sup>2</sup>=0.08 for SSI, 0.05 for FibroScan, and 0.08 for ARFI), diabetes (R<sup>2</sup>=0.04 for SSI and 0.05 for FibroScan and ARFI), presence of metabolic syndrome (R<sup>2</sup>=0.07 for FibroScan

and 0.10 for ARFI), and ALT (R<sup>2</sup>=0.03 for FibroScan and 0.02 for ARFI). For each of the 3 elastography techniques, proportions of reliable results were lower in patients with a waist circumference  $\geq$ 102 cm (Table 2): 74.6% versus 91.9% for SSI (p<0.00001), 71.7% versus 88.4% for FibroScan (p<0.00001), and 74.6% versus 96.5% for ARFI (p<0.00001). Similarly, proportions of reliable results were lower in patients with BMI  $\geq$ 30 kg/m<sup>2</sup>: 72.6% versus 90.5% for SSI (p<0.00001), 70.9% versus 85.3% for FibroScan (p<0.00001), and 72% versus 94.8% for ARFI (p<0.00001). However, no significant differences appeared among the 3 elastography techniques regarding the proportions of reliable results among patients with a waist circumference  $\geq$ 102 cm or BMI  $\geq$ 30 kg/m<sup>2</sup>.

## Confounding factors of liver stiffness in NAFLD patients (Supplementary Table S2)

By univariate analysis, among many factors associated with liver stiffness, those associated with all 3 techniques were: fibrosis stage (Spearman's correlation coefficient from 0.55 to 0.68), aspartate aminotransferase (0.18 to 0.34), gamma-glutamyl transpeptidase (0.17 to 0.30), albumin (-0.22 to -0.31), age (0.16 to 0.25), platelet count (-0.20 to -0.29), diabetes (0.23 to 0.29), metabolic score (0.23 to 0.28), waist circumference (0.18 to 0.26), hypertension (0.18 to 0.26), alkaline phosphatase (0.13 to 0.26), prothrombin time (-0.12 to -0.25), and cholesterol (-0.14 to -0.28). In stepwise multiple regression, 4 independent variables were found to be associated with LSM results when using any of the 3 techniques: fibrosis stage, alkaline phosphatase, albumin, and waist circumference. It is notable that the presence of inflammation and hepatocyte ballooning (assessed via the NAS score), and steatosis were slightly correlated in the univariate analysis with FibroScan results (Spearman's correlation coefficient of 0.28 and 0.22, respectively), but not with SSI or ARFI results. However, neither NAS nor steatosis affected the LSM results of FibroScan in the multivariate analysis.

# Diagnostic performances of elastography techniques

The median values of LSM for each stage of fibrosis are presented in Table 3. SSI, FibroScan, and ARFI results demonstrated a strong correlation with histological fibrosis stage (Supplementary Figure S1). However, the correlation between SSI results and fibrosis stage was significantly superior to the correlation between ARFI results and fibrosis stage ( $r^2=0.72$  vs. 0.59, p=0.0003; respectively), whereas the difference between SSI and FibroScan correlation coefficients was not significant ( $r^2=0.72$  vs. 0.67; p=0.1) as well as the difference between FibroScan and ARFI correlation coefficients ( $r^2=0.67$  vs. 0.59; p=0.08). Measurements by SSI, FibroScan, and ARFI correlated well with each other ( $r^2=0.70$  for SSI and FibroScan, 0.70 for SSI and ARFI, and 0.56 for M and ARFI). Diagnostic performance according to the AUROC values for the diagnosis of significant fibrosis, severe fibrosis and cirrhosis was good for SSI, which ranged

from 0.86 to 0.89; good for FibroScan, which ranged from 0.82 to 0.87; and fair or good for ARFI, which ranged from 0.77 to 0.84 (Table 4). The AUROC values for diagnosing severe fibrosis or cirrhosis were particularly good, between 0.86 and 0.89, for SSI or FibroScan.

## Pairwise comparison of diagnostic performances

Pairwise comparisons of AUROC values between SSI, FibroScan, and ARFI (Figure 2) were performed on 231 patients (60 patients were excluded from the analysis due to failure of measurement either with SSI, FibroScan, or ARFI). For the diagnosis of significant fibrosis ( $\geq$ F2), SSI had a significantly better diagnostic performance than ARFI (AUROC of 0.85, 0.83, and 0.76 for SSI, FibroScan and ARFI, respectively; p=0.5 for SSI vs. FibroScan; p=0.004 for SSI vs. ARFI; and p=0.07 for FibroScan vs. ARFI). For the diagnosis of severe fibrosis ( $\geq$ F3), no significant differences were observed between SSI, FibroScan, and ARFI (AUROC of 0.87, 0.86, and 0.84, respectively; p=0.5 for SSI vs. FibroScan; p=0.2 for SSI vs. ARFI; and p=0.5 for FibroScan vs. ARFI). Similarly, no significant differences were observed for the diagnosis of cirrhosis (F4) between SSI, FibroScan and ARFI (AUROC of 0.88, 0.86 and 0.84, respectively; p=0.5 for SSI vs. FibroScan; p=0.07 for SSI vs. ARFI; and p=0.5 for FibroScan vs. ARFI).

# **Optimal liver stiffness cut-off values**

The optimal LSM cut-off values, according to a sensitivity of at least 90% and a specificity of at least 90% for the diagnosis of F2 or greater, F3 or greater, and F4 diseases, are described in Table 4. It is notable that the cut-off values for SSI and FibroScan for ruling out diseases with at least 90% sensitivity were very close: 6.3 kPa and 6.2 kPa for the diagnosis of F2 or greater, 8.3 kPa and 8.2 kPa for the diagnosis of severe fibrosis, and 10.5 kPa and 9.5 kPa for the diagnosis of cirrhosis, respectively (Figure 3). The LSM cut-off values for SSI and FibroScan, according to the Youden index and the best diagnostic accuracy, were also very close (Supplementary Table S3). However, SSI generated lower stiffness values than FibroScan for ruling in diseases with a specificity of at least 90%: 8.7 kPa and 9.8 kPa for the diagnosis of F2 or greater, 10.7 kPa and 12.5 kPa for the diagnosis of severe fibrosis, and 16.1 kPa for the diagnosis of cirrhosis, respectively.

#### DISCUSSION

In this prospective cohort of NAFLD patients who received liver biopsies, SSI, FibroScan and ARFI performed well to diagnose different stages of liver fibrosis. These techniques achieved good diagnostic performance, especially SSI with AUROC values ranging from 0.86 to 0.89 and FibroScan with AUROC values from 0.82 to 0.87, whereas ARFI AUROC values ranged from 0.77 to 0.84. The diagnostic accuracy of SSI was greater than that of ARFI in the diagnosis of significant fibrosis or greater disease (AUROC of 0.85 vs. 0.76, p=0.004). Another important finding of our study is that several cut-off values for SSI and FibroScan were very close, which can be of great utility in routine daily practice.

Though several validation studies have previously confirmed the accuracy of testing LSM using FibroScan or ARFI in NAFLD, our study is the first to assess the accuracy of SSI and to compare these 3 techniques in this field. The diagnostic performance of the M probe for FibroScan in our patient population was consistent with previous prospective studies.<sup>24,25</sup> Conversely, our results concerning ARFI accuracy were less striking than those in previous studies. In a preliminary study by Yoneda et al.,<sup>26</sup> analysis was performed on 54 NAFLD patients, including few patients with severe fibrosis or cirrhosis (F3, n=4; and F4, n=6). Palmeri et al.<sup>27</sup> found that the best accuracy for ARFI in NAFLD patients occurred when distinguishing between patients with no or moderate fibrosis (F0 to F2) and those with severe fibrosis or cirrhosis (F3-F4). Unfortunately, in our study, the improved accuracy of ARFI for diagnosing F2 or greater disease was not confirmed.

Indeed, the major challenge when measuring LSM in NAFLD patients is the lower success rate in obese patients. Consistent with previous results,<sup>28,29</sup> obesity was, in our study, the main feature associated with a significant increase in the failure rate when using FibroScan. We showed that failed, unreliable and reliable LSM rates when using SSI were very close to those of FibroScan, with a great impact of obesity on LSM failure rates. Reliable results when using SSI were obtained in around 90% of patients with a BMI<30 kg/m<sup>2</sup> (around 85% for FibroScan) but only in nearly 73% of patients with a BMI $\geq$ 30 kg/m<sup>2</sup> (71% for FibroScan). The interference by obesity on the ARFI technique was less conspicuous, with a failure rate that was not significantly increased in obese patients. However, the impact of obesity on ARFI diagnostic accuracy and reliability should not be underappreciated, as a much higher number of patients had unreliable LSM in obese patients.

In multivariate analysis, LSM was mainly associated with fibrotic stage. Multivariate analysis also showed that steatosis, inflammation or hepatocyte ballooning (indirectly assessed via the NAS score) didn't play a significant role on LSM results. This result can be considered as positive because the impact of different confounding factors is frequently a limitation for most diagnostic techniques. However, the lack of a link between these factors and LSM can also be considered to be negative when diagnosing NAFLD patients because some NASH patients may have no fibrosis but do present with liver injury lesions with inflammation or hepatocyte ballooning. Therefore, LSM may not aid in the diagnosis of early-stage NASH without fibrosis. Moreover, it is interesting to note that in univariate analysis, FibroScan results had a slight correlation with steatosis and NAFLD activity score (R= 0.22 and 0.28) whereas SSI and ARFI did not have (with close correlation coefficients: R=0.10 and 0.07 for NAS, and R=0.03 and 0.0002 for steatosis, respectively). We can suppose that these differences are possibly based on technical differences between the technique of FibroScan and those of SSI and ARFI, as the velocity of the mechanical wave generated by the probe of FibroScan is recorded on about 4 cm into the liver, whereas the shear wave propagation issued from the focused ultrasonic beams of ARFI and SSI are recorded on a very low amplitude.

Our study has several limitations. First, liver biopsy was used as the reference standard to assess and quantify liver fibrosis. The diagnostic efficiency of biopsy is normally limited by the variability in the representativeness of the samples and by inter- and intraobserver variability. To reduce these limitations, we excluded all patients with biopsies that did not meet specific quality criteria. Moreover, the main results of our study were sustained when biopsies between 10 to 15 mm were excluded (data not shown). A second limitation of our study is the lack of use of the XL probe used for FibroScan. The XL probe, which is used for overweight patients, has been shown to significantly increase the rate of reliable results, especially in obese patients, with an accuracy similar to that of the M probe.<sup>30</sup> It would have been interesting to analyze the added value of the XL probe when SSI and the M probe were also available; unfortunately, the XL probe was not available at the time of LSM for a part of our study population. Third, our study focused on LSM techniques and did not analyze the impact or clinical value of the biological fibrosis tests that are usually performed in the field of NAFLD. Further studies are needed to assess the ways that LSM and biological tests could be used together to provide the best diagnostic performance for NAFLD patients.

# CONCLUSION

This prospective study confirmed that SSI, FibroScan, and ARFI are valuable diagnostic tools for the staging of liver fibrosis in NAFLD patients. However, the diagnostic accuracy of SSI was superior to that of ARFI for the diagnosis of F2 or greater disease states. Most of the cut-off values for SSI for the diagnosis of different stages of liver disease were quite close to those for FibroScan, which could be of great importance for the applicability of this technique and its wide dissemination among radiologists and hepatologists in their daily practice. However, as for the M probe of FibroScan, the SSI technique remains limited by a significant increase in its failure rate in cases of obesity, whereas ARFI presents with a great increase of unreliable results.

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# Table 1. Clinical characteristics of NAFLD patients.

Variable	All (n=291)	Bordeaux cohort (n=174)	Angers cohort (n=117)	Р
Gender – Male	172 (59.1%)	93 (53.4%)	79 (67.5%)	0.02*
Age (years)	56.7 ± 12 (18-80)	57 ± 12 (18-80)	56.3 ± 12 (21-79)	0.5
Body mass index (kg/m <sup>2</sup> )	32.1 ± 6 (21.3-57.4)	31.8 ± 6 (21.3-52)	32.5 ± 6 (22-57.4)	0.4
<25	28 (9.6%)	20 (11.5%)	8 (6.8%)	0.2
25 to 29.9	88 (30.2%)	55 (31.6%)	33 (28.2%)	0.5
≥30	175 (60.1%)	99 (56.9%)	76 (65%)	0.2
Waist circumference (cm)	110 ± 16 (71-193)	107 ± 16 (71-193)	113.5 ± 16 (84-168)	0.002*
Intercostal wall thickness (mm)	24.8 ± 7.4 (11-64)	24.9 ± 6.7 (11-64)	24.8 ± 8 (11-61)	1
Type 2 Diabetes	153 (52.6%)	93 (53.4%)	60 (51.3%)	0.7
Hypertension	167 (57.4%)	101 (58%)	66 (56.4%)	0.8
Metabolic syndrome	181 (62.2%)	104 (59.8%)	77 (65.8%)	0.3
ALT (IU/L)	71.2 ± 50.7 (12-332)	74.3 ± 57 (12-332)	66.7 ± 38.7 (15-229)	0.2
Alkaline Phosphatase	79.2 ± 42.3 (26-578)	83.5 ± 41 (26-578)	74.5 ± 32 (32-221)	0.04*
Cholesterol (mmol/L)	5 ± 1.4 (1.3-12.7)	5 ± 1.4 (1.3-8.9)	5 ± 1.4 (2.6-12.7)	1
Length of liver biopsy (mm)	26.8 ± 11 (10-70)	25.7 ± 11 (10-70)	28.4 ± 9 (10-60)	0.03*
Histologic fibrosis stage				
F0	25 (8.6%)	11 (6%)	14 (12%)	0.09
F1	60 (20.6%)	31 (18%)	29 (25%)	0.2
F2	80 (27.5%)	46 (26%)	34 (29%)	0.6
F3	77 (26.5%)	44 (25%)	33 (28%)	0.6
F4	49 (16.8%)	42 (24%)	7 (6%)	<0.0001 *
NAFLD Activity score				
0/1/2/3/4/5/6/7/8	6/23/23/44/56/85/41/ 11/2 2%/8%/8%/15%/19% /29%/14%/4%/1%	1/12/13/18/30/59/2 9/10/2 1%/7%/7%/10%/1 7%/34%/17%/6%/ 1%	5/11/10/26/26/26/12/1/0 4%/9%/9%/22%/22%/22 %/10%/1%/0%	
Steatosis grade				
S0 (<5%)	10 (3%)	5 (3%)	5 (4%)	0.5
S1 (5-33%)	109 (38%)	44 (25%)	65 (56%)	< 0.0001
S2 (34-66%)	93 (32%)	62 (36%)	31 (27%)	*
S3 (>66%)	79 (27%)	63 (36%)	16 (14%)	0.1 <0.0001 *

Unless otherwise indicated, results are expressed as mean ± standard deviation (range) or number (percentage).

n, number; ALT, alanine aminotransferase; NAFLD, Non-alcoholic fatty liver disease.

\* significant p-values <0.05

Table 2. Success and reliability of Supersonic Shear Imaging, M probe of Fibroscan, and ARFI.

n=291	SSI	Fibroscan	ARFI	P-value
Failure (0 valid	13%	14.4%	0.7%	0.6
measurements)	(38)	(42)	(2)	< 0.00001*
				< 0.00001*
Unreliable results	7.2%	8.9%	18.2%	0.5
(IQR/M>0.3 with LSM≥7.1)	(21)	(26)	(53)	0.0001*
				0.001*
Reliable results	79.7%	76.6%	81%	0.4
(IQR/M≤0.30 or >0.3 with	(232)	(223)	(236)	0.7
LSM<7.1)				0.2
Waist circumference <102 cm	( <i>n=86</i> )			
Failure	3.5%	3.5%	0%	1
	(3/86)	(3/86)	(0/86)	0.08
				0.08
Unreliable results	4.7%	8.1%	3.5%	0.4
	(4/86)	(7/86)	(3/86)	0.7
				0.2
Reliable results	91.9%	88.4%	96.5%	0.4
	(79/86)	(76/86)	(83/86)	0.2
				0.04*
Waist circumference ≥102 cm	( <i>n</i> =205)			
Failure	17.1%	19%	1%	0.6
	(35/205)	(39/205)	(2/205)	< 0.00001*
				<0.00001*
Unreliable results	8.3%	9.3%	24.4%	0.7
	(17/205)	(19/205)	(50/205)	<0.00001*
				<0.00001*

Body mass index < 30 kg/m <sup>2</sup> (n=116)						
Failure	5.2%	3.4%	0%	0.5		

71.7%

(147/205)

74.6%

(153/208)

0.5

1 0.5

74.6%

(153/205)

Reliable results

	(6/116)	(4/116)	(0/116)	0.01*
				0.04*
Unreliable results	4.3%	11.2%	5.2%	0.05*
	(5/116)	(13/116)	(6/116)	0.8
				0.09
Reliable results	90.5%	85.3%	94.8%	0.2
	(105/116)	(99/116)	(110/116)	0.2
				0.02*
Body mass index $\geq$ 30 kg/m <sup>2</sup>	( <i>n</i> =175)			
Failure	18.3%	21.7%	1.1%	0.4
	(32/175)	(38/175)	(2/175)	<0.00001*
				<0.00001*
Unreliable results	9.1%	7.4%	26.9%	0.6
	(16/175)	(13/175)	(47/175)	<0.00001*
				< 0.00001*
Reliable results	72.6%	70.9%	72%	0.7
	(127/175)	(124/175)	(126/175)	0.9
				0.8

Data are numbers (± percentages). Comparison of proportions was performed using the Khi-2 test or Fisher's exact test as appropriate. The first p-value refers to the comparison between SSI and Fibroscan; the second p-value refers to SSI versus ARFI; and the third to Fibroscan versus ARFI. In the subgroup analysis according to waist circumference and body mass index, reliable results refer to very reliable results and reliable results together (i.e. all patients without failure or unreliable results according to Boursier et al. definition).<sup>20</sup>

Abbreviations: SSI, supersonic shear imaging; ARFI, acoustic radiation force impulse imaging.
Table 3. Supersonic shear imaging, Fibroscan and ARFI median values according to the fibrosis stage in study population. Correlation between Supersonic shear imaging, Fibroscan, ARFI, and the fibrosis stage was tested using the non-parametric Spearman's correlation coefficient.

	FO	F1	F2	F3	F4	Correlation
						coefficient
SSI (kPa)	6	6.3	8	12	17	0.72
	(±1.9)	(±2.7)	(±3.4)	(±5)	(±13.6)	(p<.00001)
Fibroscan	6	6.7	8.4	12	23.6	0.67
(kPa)	(±2)	(± 3.4)	(± 4.9)	(± 5.7)	(±23.6)	(p<.00001)
ARFI	1.02	1.02	1.13	1.45	1.88	0.59
(m/s)	(±0.33)	(±0.27)	(±0.45)	(±0.7)	(±1.37)	(p<.00001)

Except for the correlation coefficient, results are expressed as medians ± interquartile range.

Abbreviations: SSI, Supersonic Shear Imaging; ARFI, Acoustic Radiation Force Impulse; kPa, KiloPascals.

Variable	Fibrosis	AUROC	Best	Aim	Cut-	Sensitivity	Specificity
	stage	(95% CI)	accuracy		off		
SSI	≥F2	0.86	80%	Sensitivity≥90%	6.3	90% (148/164)	50% (34/68)
(kPa)		(0.79-0.90)	(185/232)	Specificity≥90%	8.7	71% (116/164)	90% (61/68)
	≥F3	0.89	85%	Sensitivity≥90%	8.3	91% (91/100)	71% (93/132)
		(0.83-0.92)	(196/232)	Specificity≥90%	10.7	71% (71/100)	90% (119/132)
	F4	0.88	87%	Sensitivity≥90%	10.5	90% (34/38)	72% (139/194)
		(0.82-0.92)	(202/232)	Specificity≥90%	14.4	58% (22/38)	90% (174/194)
Fibroscan	≥F2	0.82	77%	Sensitivity≥90%	6.2	90% (141/156)	45% (30/67)
(kPa)		(0.76-0.87)	(172/223)	Specificity≥90%	9.8	60% (93/156)	90% (60/67)
	≥F3	0.86	79%	Sensitivity≥90%	8.2	90% (83/92)	61% (80/131)
		(0.80-0.90)	(175/223)	Specificity≥90%	12.5	57% (52/92)	90% (118/131)
	F4	0.87	89%	Sensitivity≥90%	9.5	92% (34/37)	62% (116/186)
		(0.79-0.92)	(198/223)	Specificity≥90%	16.1	65% (24/37)	90% (168/186)
ARFI	≥F2	0.77	74%	Sensitivity≥90%	0.95	90% (144/160)	36% (31/76)
(m/s)		(0.70-0.83)	(175/236)	Specificity≥90%	1.32	56% (90/160)	91% (69/76)
	≥F3	0.84	79%	Sensitivity≥90%	1.15	90% (84/93)	63% (90/143)
		(0.78-0.89)	(186/236)	Specificity≥90%	1.53	59% (55/93)	90% (128/143)
	F4	0.84	84%	Sensitivity≥90%	1.3	90% (35/39)	67% (132/197)
		(0.78-0.89)	(199/236)	Specificity≥90%	2.04	44% (17/39)	90% (177/197)

# Table 4. Diagnostic performances of Supersonic shear imaging, M probe of Fibroscan, and ARFI.

Abbreviations: AUROC, areas under the receiver operating characteristic curves; CI, confidence intervals; SSI, Supersonic shear imaging.

	Study site	FO	F1	F2	F3	F4	Correlation coefficient
SSI (kPa)	Bordeaux	6 (±2)	6.1 (±2.2)	8 (±3.8)	10.5 (±5.7)	17 (±13.6)	0.74
	Angers	6.6 (±1.9)	6.8 (±3)	8.7 (±4.3)	13.3 (±5.8)	23.5 (±9.7)	0.63
	p-value	0.1	0.2	0.3	0.0008*	0.3	0.1
Fibroscan (kPa)	Bordeaux	6.4 (±3)	7.5 (± 3.2)	8.8 (± 5.2)	12 (± 5.5)	22.7	0.65
(KI U)	Angers	6 (±1.9)	6.2 (±3.2)	8.1 (±4.7)	13.2 (±7.1)	38.2 (±54.8)	0.66
	p-value	0.9	0.2	0.6	0.3	0.3	0.9
ARFI (m/s)	Bordeaux	1.03 (±0.36)	1.01 (±0.24)	1.24 (±0.46)	1.54 (±0.7)	1.92 (±1.32)	0.58
	Angers	1.15 (±0.25)	1.06 (±0.31)	1.13 (±0.44)	1.85 (±1)	2.91 (±1.74)	0.50
	p-value	0.5	0.4	0.3	0.09	0.06	0.3

Supplementary Table S1. Supersonic shear imaging, Fibroscan and ARFI median values according to the fibrosis stage in Bordeaux and Angers cohort.

Except for the correlation coefficient (Spearman's correlation coefficient), data are expressed as medians (± interquartile range). Comparison of values between the different subgroups was performed using non-parametric Wilcoxon rank-sum test, and Spearman's correlation coefficients were compared each other according to Guilford et al.<sup>22</sup>

Abbreviations: SSI, Supersonic Shear Imaging; ARFI, Acoustic Radiation Force Impulse; kPa, KiloPascals.

Supplementary Table S2. Factors influencing liver stiffness measurements. Univariate analysis was performed using Spearman's correlation coefficient (Q) and multivariate analysis using stepwise forward regression (R<sup>2</sup>).

	Supersonic Shear			FibroScan			ARFI		
		Imaging							
	Q	p-value	<b>R</b> <sup>2</sup>	Q	p-value	R <sup>2</sup>	Q	p-value	<b>R</b> <sup>2</sup>
Gender	-	0.7		-	0.6		-	0.01	
	0.03			0.03			0.15		
Age	0.25	0.00008		0.20	0.002		0.16	0.006	
Body mass index	0.09	0.2		0.15	0.02		0.21	0.0003	
Parietal wall	-	0.5		0.09	0.2		0.04	0.5	
thickness	0.04						~ ~ ~		
Metabolic score (0- 5)	0.28	0.000009		0.23	0.0002		0.23	0.0009	
Metabolic	0.24	0.0001		0.16	0.01		0.17	0.004	
syndrome									
Waist	0.18	0.004	0.03	0.19	0.002	0.02	0.26	0.000006	0.07
circumference									
Diabetes	0.29	0.000003		0.29	0.000005		0.23	0.0001	
Hypertension	0.26	0.00003		0.25	0.0001		0.18	0.002	
AST	0.29	< 0.000001	0.03	0.34	< 0.000001		0.18	0.003	
ALT	-	0.9		0.05	0.4		-	0.4	
	0.01						0.05		
GGT	0.26	0.00004		0.30	0.000002	0.01	0.17	0.004	
Total bilirubin	-	0.7		-	0.3		-	0.9	
	0.02			0.07			0.01		
Platelet count	-	0.000003		-	0.002	0.02	-	0.0006	
	0.29			0.20			0.20		
Prothrombin time	-	0.00008	0.01	-	0.006		-	0.04	
4 11 11	0.25	0.02	0.04	0.17	0.02	0.00	0.12	0.000000	0.04
Alkaline	0.13	0.03	0.04	0.14	0.03	0.09	0.26	0.000009	0.04
phosphatase		0.000001	0.00		0.000	0.04		0.000001	
Albumin	- 0.31	<0.000001	0.09	- 0.22	0.0007	0.01	- 0.29	0.000001	0.02
Ferritin	-	0.4		-	0.6		-	0.02	
	0.05	5.2		0.04			0.14	5.0-	
Cholesterol	-	0.006		-	0.009		-	0.02	
	0.18			0.17			0.14		

Histologic	fibrosis	0.68	< 0.000001	0.19	0.67	< 0.000001	0.33	0.55	< 0.000001	0.22
stage										
NAFLD	activity	0.10	0.1		0.28	0.000007		0.07	0.3	
score										
Steatosis		0.03	0.7		0.22	0.0006		0.00	1	
								2		
Total R <sup>2</sup>				0.40			0.48			0.36

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; NAFLD, Non-alcoholic fatty liver disease.

# Supplementary Table S3. Cut-off values of Supersonic shear imaging, Fibroscan, and ARFI according to the Younden index, and the best diagnostic accuracy.

Variable	Fibrosis	AUROC	Aim	Cut-	Sensitivity	Specificity	Accuracy
	stage	(95%CI)		off			
SSI	≥F2	0.86	Younden	8.9	68% (111/164)	94% (64/68)	75% (175/232)
(kPa)		(0.79-0.90)	Best accuracy	6.2	93% (152/164)	49% (33/68)	80% (185/232)
	>F2	0.89	Voundan	03	84% (84/100)	83% (110/132)	81% (191/232)
	215	(0.83-0.92)	Rest accuracy	9.5 10.2	79% (79/100)	89% (117/132)	85% (196/232)
		(0.00 0.02)	Deer weenney	1012	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	0,00 (11,7102)	00 /0 (190,202)
	F4	0.88	Younden	10	95% (36/38)	69% (133/194)	73% (169/232)
		(0.82-0.92)	Best accuracy	20.3	42% (16/38)	96% (186/194)	87% (202/232)
Fibroscan	≥F2	0.82	Younden	8.5	72% (113/156)	79% (53/67)	74% (166/223)
(kPa)		(0.76-0.87)	Best accuracy	6.3	89% (139/172)	49% (33/67)	77% (172/223)
	≥F3	0.86	Younden	9.3	82% (75/92)	75% (98/131)	78% (173/223)
		(0.80-0.90)	Best accuracy	10.9	72% (66/92)	83% (109/131)	79% (175/223)
	F4	0.87	Younden	10.2	89% (33/37)	68% (126/186)	71% (159/223)
		(0.79-0.92)	Best accuracy	20	60% (22/37)	95% (176/186)	89% (198/223)
ARFI	>F2	0.77	Vounden	1 28	59% (95/160)	90% (68/76)	69% (163/236)
(m/s)		(0.70-0.83)	Best accuracy	1.07	80% (128/160)	62% (47/76)	74% (175/236)
· ·			5		· · · /	· · /	( · · )
	N T 2	0.04	24 1	1.00	010/ (75/00)	<b>F</b> 00/ (111/140)	
	2F3	(0.84)	I OUNAEN Best accuracy	1.20	81% (75/93) 81% (75/93)	78% (111/143) 78% (111/143)	79% (186/236) 79% (186/236)
		(0.70-0.03)	Desi accuracy	1.20	0170 (75/55)	7070 (111/143)	7778 (100/200)
	F4	0.84	Younden	1.39	85% (33/39)	74% (145/197)	75% (178/236)
		(0.78-0.89)	Best accuracy	2.51	41% (16/39)	93% (183/197)	84% (199/236)

Abbreviations: AUROC, areas under the receiver operating characteristic curves; CI, confidence intervals; SSI, Supersonic shear imaging.

Figure 1. Success and reliability of liver stiffness measurements.



Figure 2. ROC curves for Supersonic shear imaging, FibroScan, and ARFI for the diagnosis of significant fibrosis (F2 or greater) (A), severe fibrosis (F3 or greater) (B), and cirrhosis (F4) (C).

SSI had a higher accuracy than ARFI for the diagnosis of significant fibrosis (≥F2) (p=0.004). No significant differences were observed among the 3 elastography techniques for the diagnosis of severe fibrosis and cirrhosis.



Figure 3. Optimal liver stiffness cut-off values for Supersonic Shear Imaging and FibroScan.



Supplementary Figure S1. Box plots showing the distribution of Supersonic Shear Imaging (A), FibroScan (B) and ARFI (C) values in the study population according to histological fibrosis stage. The top and bottom of the box are the 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively. The length of the box is the interquartile range, and the median (50<sup>th</sup> percentile) is the line drawn through the box.





#### **DISCUSSION ET PERSPECTIVES**

#### Questions techniques

#### o variabilité

Avec la multiplication des techniques d'élastographie devenues accessibles sur le marché en peu de temps, la question de la variabilité entre ces différents systèmes est déjà cruciale. Des consensus devront être trouvés pour homogénéiser et harmoniser la technique d'acquisition, ainsi que le rendu et l'interprétation des résultats. Il est probable que la fréquence d'émission de l'onde mécanique ou acoustique soit en grande partie responsable des variabilités inter-constructeurs. Arrivera-t-on à s'en affranchir ou faudra-t-il au contraire trouver des moyens de correspondance ou de les différentes mesures? En 2008, la société de radiologie conversion entre d'Amérique du Nord (RSNA) a créé l'alliance des biomarqueurs en imagerie quantitative (QIBA) avec pour but de développer l'imagerie quantitative en pratique clinique et dans les essais. Le comité en charge des ultrasons au sein de cette alliance a réalisé une étude inter-laboratoire en 2012 évaluant la vitesse de propagation des ondes de cisaillement sur fantômes (1). L'étude montrait de faibles différences entre les opérateurs dans la réalisation des mesures mais des différences plus importantes liées au système d'élastographie utilisé et à la profondeur de la mesure. Le but de ces travaux à terme est d'obtenir une correspondance des mesures quelque soient les systèmes utilisés (2). Par chance possiblement ou pour des raisons techniques, nous avons pu constater dans nos différents travaux que les résultats et valeurs-seuils obtenues avec le SSI étaient souvent très proches de celles du FibroScan. Il sera intéressant de confirmer cette hypothèse en analysant l'applicabilité des valeurs-seuils du FibroScan au SSI.

#### o Techniques de mesure, fiabilité, et facteurs confondants

De nombreuses questions doivent être approfondies concernant la technique de réalisation des mesures d'élastographie hépatique avec le SSI, ainsi que les critères de fiabilité des mesures. Combien de mesures doivent être réalisées, 10, 5, 3, voire qu'une seule (3,4) ? Doit-on changer de zone d'acquisition à chaque mesure ou rester au même endroit ? Quels critères de fiabilité peut-on appliquer (5,6) ? Dans le même ordre d'idée, il faudra considérer les moyens permettant de diminuer l'impact des facteurs confondants, et notamment les principaux que sont l'hépatite aiguë et la congestion hépatique d'origine cardiaque. Pour ces 2 facteurs, des critères cliniques ou biologiques peuvent aider mais ne sont pas toujours suffisants, surtout pour la congestion d'origine cardiaque. L'apport de l'échographie morphologique pourrait être d'utilité en recherchant systématiquement d'éventuels signes indirects d'insuffisance cardiaque droite comme la dilatation de la veine cave inférieure ou des veines sus-hépatiques.

#### Expérience et formation des opérateurs

L'expérience de l'opérateur est un facteur important à prendre à compte. Même si la technique SSI est reconnue comme hautement reproductible (7), dans notre expérience, la fiabilité de la mesure en SSI est directement corrélée à la qualité du remplissage couleur de l'aire d'analyse, et donc à la qualité de la fenêtre échographique. Une période d'apprentissage est nécessaire pour réaliser des mesures de qualité correctes, et acquérir le bon tempo pour réaliser les mesures chez un patient en très courte apnée. Cependant, nous avons pu constater que les opérateurs qui avaient déjà une bonne expérience de l'échographie hépatique en général, avec une bonne prise en main de la sonde et l'habitude d'appuyer assez fortement en intercostal pour obtenir une image de bonne qualité, n'avaient aucune difficulté à réaliser des mesures d'élastographie correctes après juste quelques patients. Cette notion est d'autant plus cruciale que déjà maintenant mais encore plus à l'avenir, les patients en surcharge pondérale qui sont difficiles à imager constitueront une grande partie des indications d'élastographie. Dans cette optique, il faudra peut-être considérer un encadrement plus étroit de la formation à l'élastographie hépatique, voire une accréditation sur un modèle proche de ce qui a été réalisé dans le cadre du dépistage du cancer du sein par mammographie, d'autant plus que le dépistage du carcinome hépato-cellulaire qui est réalisé par échographie de façon semestrielle chez les patients cirrhotique reste lui aussi perfectible.

#### Questions cliniques

#### Champs d'application

L'élastographie hépatique a montré son intérêt dans une multitude d'applications cliniques : dépister, diagnostiquer et grader la fibrose hépatique, évaluer la gravité de la cirrhose et l'hypertension portale, monitorer la progression de la maladie, évaluer la réponse au traitement, et enfin évaluer le prognostic des patients. Chacun de ces champs d'application a été étudié avec le FibroScan, et logiquement, les mêmes résultats ou la même validation scientifique commencent à arriver avec les techniques d'élastographie échographiques comme le SSI ou l'ARFI. Il est peut-être un domaine où les techniques d'élastographie échographiques auront un rôle à jouer plus décisif que le FibroScan ; il s'agit du dépistage de la fibrose (8). Contrairement au FibroScan qui est entre les mains des hépatologues dans l'immense majorité des cas, et qui est donc appliqué à des patients déjà ciblés avec une hépatopathie chronique, les techniques d'élastographie échographiques bénéficient d'une diffusion beaucoup plus large au sein du réseau radiologique qu'il soit public ou libéral. Imaginons qu'une élastographie hépatique soit réalisée pour chaque patient avec une indication d'échographie abdominale et présentant un ou des facteurs de risque d'hépatopathie chronique comme une surcharge pondérale, un diabète, ou une consommation alcoolique. Un dépistage de la fibrose hépatique pourrait être appliqué à une grande

partie de la population. Avant cela, il faudra bien évidemment répondre à une multitude de questions touchant au coût d'un tel dépistage, avec entre autre la masse d'examens complémentaires que cela va engendrer, et le bénéfice réel sur la santé des patients sur une grande échelle.

#### • Quelles hépatopathies chroniques ?

Le développement de l'élastographie hépatique dans les années à venir sera intimement lié à l'évolution et aux progrès de l'hépatologie. Avec les nouveaux traitements antiviraux, l'hépatite C est amenée à disparaître dans un futur proche. L'hépatite B étant déjà peu développée en occident, les hépatopathies alcooliques et surtout les stéatopathies métaboliques vont occuper une place prépondérante dans l'activité des hépatologues. Or, l'obésité est le principal facteur limitant des techniques actuelles d'élastographie transitoire. Nous avons vu que le SSI était performant chez les patients en surcharge pondérale mais reste perfectible avec des taux d'échec encore élevés et une fiabilité moindre. Comme l'a déjà anticipé le FibroScan avec le développement de la sonde XL, les techniques d'élastographie échographiques devront elles aussi développer des outils plus robustes, permettant d'accroître leurs performances chez les patients en surcharge pondérale.

#### • Quantification de la stéatose et place de l'IRM

Pour ces mêmes raisons, la quantification et le monitorage de la stéatose hépatique va rapidement devenir indispensable. L'échographie morphologique hépatique permet d'apprécier qualitativement la surcharge stéatosique mais ne permet pas de la quantifier et donc de surveiller son évolution dans le temps. La société Echosens a développé un outil, le CAP (9), permettant de quantifier la stéatose hépatique, et équipant les nouvelles générations de FibroScan. Cette estimation reste pour l'instant grossière mais sera probablement sujette à amélioration dans les années à venir. Cette situation où les stéatopathies métaboliques constituent l'enjeu des années à venir soulève la question de la place que va occuper l'IRM et notamment l'élastographie par IRM dans le futur. L'IRM est en théorie l'examen idéal. Elle est le gold standard dans la quantification de la stéatose hépatique (10), meilleure que la PBH ; elle permet de monitorer de façon étroite les variations de cette stéatose (11), de quantifier d'autres surcharges hépatiques potentielles comme celle du fer (12); de fournir une analyse morphologique détaillée du parenchyme hépatique et de l'hypertension portale associée ; de diagnostiquer le carcinome hépato-cellulaire ; et maintenant de quantifier également de façon fiable la fibrose hépatique avec l'élastoMR sans limitation liée à la surcharge pondérale (13,14). Restent cependant les problèmes de son accessibilité, de son coût, et là aussi de la variabilité inter-constructeur (15).

#### Inflammation

L'inflammation est un élément important à considérer dans la prise en charge d'une hépatopathie chronique. Pour l'instant, seule l'analyse anatomo-pathologique permet de la rechercher et la quantifier. Les valeurs d'élastographie hépatique peuvent être soumises à de discrètes variations selon le niveau d'inflammation mais pas suffisamment pour être exploitées à visée diagnostique. De même, l'IRM hépatique n'a pas encore trouvé les clés permettant de la diagnostiquer ou la quantifier.

#### • Quelles valeurs-seuils pour quels critères de jugement ?

Au chapitre de l'interprétation des résultats, la question s'est longtemps posée de savoir quelles valeurs-seuils appliquer pour diagnostiquer le stade de fibrose. Des valeurs-seuils ont donc été appliquées pour le FibroScan selon les stades de fibrose F de la classification METAVIR. Raisonner en terme de stade F a cependant ses limites et ouvre finalement assez peu de perspectives. Comme nous l'avons déjà vu, l'élastographie hépatique est un reflet de la dureté du foie, qui est largement impactée, mais pas seulement, par la fibrose. D'autre part, l'interprétation anatomopathologique a aussi ses limites et la reproductibilité inter-observateur pour grader la fibrose est loin d'être excellente (16). L'avenir de l'interprétation des résultats en élastographie hépatique est plus certainement dans l'applicabilité clinique que dans le grade de fibrose. Par exemple, à partir de quelle valeur d'élastographie le risque de développer un carcinome hépato-cellulaire est-il significatif ? A partir de quelle valeur d'élastographie le risque de développer des symptômes de la cirrhose est-il significatif? et de développer des complications de l'hypertension portale? La réponse à cette dernière question a déjà été en partie résolue. Dans le dernier consensus Baveno VI, la valeur d'élastographie hépatique par FibroScan a été appliquée à l'hypertension portale et implémentée dans la pratique courante (17). Dans la conférence de consensus, les experts basés sur la littérature scientifique estiment que les patients ayant des valeurs d'élastographie de moins de 20 kPa et un taux de plaquettes supérieur à 150000 ont de très fortes chances de ne pas avoir de varices oesophagiennes et peuvent ne pas avoir d'endoscopie digestive haute de dépistage. Ce consensus permet également d'utiliser la valeur d'élastographie pour mieux identifier de façon précoce les patients à risque de développer une hypertension portale cliniquement significative. Le terme d'hépatopathie chronique compensée avancée (compensated advanced chronic liver disease, cACLD) décrit cette catégorie de patient et permet de mieux refléter le continuum pathologique de la fibrose sévère et de la cirrhose chez ces patients asymptomatiques. En pratique, les patients avec des valeurs d'élastographie de moins de 10 kPa sont à très faible risque de cACLD, alors que ceux à plus de 10, et encore plus si supérieur à 15 kPa sont suspects de cACLD et doivent consulter un spécialiste pour confirmation, examens complémentaires, suivi, et traitement. Nous ne sommes qu'au début de la considération de l'élastographie hépatique en tant qu'entité médicale à part entière, et d'autres travaux seront à mener pour continuer à accroître l'impact de l'élastographie dans la prise en charge des patients, en l'associant ou la combinant avec d'autres facteurs diagnostiques qu'ils soient cliniques, biologiques ou morphologiques.

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#### CONCLUSION

Le marché de l'élastographie a connu une expansion frénétique ces dernières années. A partir de 2008, date à laquelle le premier système d'élastographie a été incorporé sur un échographe, tous les constructeurs d'échographe ont eu à cœur d'équiper leurs machines d'une technique d'élastographie. Pour qu'une technique soulève un tel engouement, l'impact clinique est nécessairement de grande valeur, et c'était d'autant plus évident que les manufacturiers avaient eu vent du succès du FibroScan quelques années auparavant. Cette course à l'innovation technologique n'a d'ailleurs pas été sans rappeler celle qui a eu lieu au début des années 1990 pour le Doppler et celle du début des années 2000 pour l'échographie de contraste. C'est dans ce contexte que les médecins peuvent parfois être submergés de nouvelles évolutions technologiques qui sont certes prometteuses mais qui ont encore à prouver leur réelle plus-value et les bénéfices qu'elles peuvent apporter à la prise en charge des patients. Contrairement au marché du médicament où chaque produit est placé sur le marché une fois son efficacité démontrée, le marché des dispositifs médicaux implantables et celui des outils et produits diagnostiques est soumis à moins de contraintes et les innovations technologiques n'ont qu'à faire preuve de leur innocuité avant d'être mises à disposition. Dans un tel domaine sans cesse en progrès, il est indispensable d'être réactif afin d'analyser en détail et valider scientifiquement chaque nouvelle innovation technologique.

Nos travaux ont participé à la validation clinique de la technique d'élastographie Supersonic Shear Imaging, autrement appelée 2D-Shear Wave Elastography. Dans une première étude, nous avons démontré que cette technique d'élastographie était fiable, présentant des performances diagnostiques dans l'ensemble satisfaisantes à excellentes, meilleures que celles de l'ARFI pour le diagnostic de fibrose significative et que celles du FibroScan pour le diagnostic de fibrose sévère. Dans une seconde étude, nous avons confirmé que l'élastographie hépatique, y compris lorsque réalisée avec SSI, était un outil diagnostique utile pour apprécier la gravité des patients avec cirrhose, avec des valeurs corrélées au score de Child-Pugh et à la présence de complications dont celles liées à l'hypertension portale. Cette étude a également confirmé l'intérêt de l'élastographie de rate dans l'évaluation non-invasive de la sévérité de la cirrhose même si la technique d'élastographie SSI n'était pas encore assez robuste avec des taux d'échec encore élevés. Dans la troisième étude, nous avons centré notre analyse sur une population de patients avec stéatopathie métabolique. Nous avons confirmé les bonnes performances diagnostiques du SSI dans cette catégorie de patients malgré des taux d'échecs de mesure non négligeables, mais surtout nous avons participé à améliorer les connaissances sur les avantages et inconvénients des différentes techniques d'élastographie hépatique dans le contexte particulier de la stéatopathie métabolique.

Pour conclure, nos travaux ont démontré l'apport incontestable de l'élastographie hépatique, et notamment de la technique SSI, dans le diagnostic et la prise en charge des patients atteints de maladie chronique du foie. L'élastographie hépatique est une nouvelle entité médicale qui ne doit plus être limitée à l'évaluation d'un stade de fibrose mais qui doit continuer à se propager et accroître son impact dans la prise en charge de ces patients. Combinée aux autres outils diagnostiques usuels tels que le bilan biologique hépatique ou l'analyse morphologique hépatique par imagerie, il est probable qu'elle continue d'accroître sa place centrale dans l'optimisation du diagnostic, le suivi longitudinal, les décisions thérapeutiques, et d'une façon plus générale dans la totalité de la prise en charge des patients avec hépatopathie chronique.

**Titre :** Diagnostic et évaluation de la gravité des maladies chroniques du foie : Impact de l'élastographie par ondes de cisaillement « Supersonic Shear Imaging »

### Résumé :

Les maladies chroniques du foie constituent un problème majeur de santé publique. L'évaluation précise du degré de fibrose hépatique apporte au clinicien une estimation du pronostic dès la prise en charge initiale de ces patients, mais permet également la réalisation d'une surveillance évolutive, et intervient dans la décision et le choix d'un traitement. L'évaluation noninvasive de la fibrose hépatique par élastographie a permis de révolutionner la prise en charge des malades atteints d'une maladie chronique du foie. L'objectif de notre travail est d'évaluer les performances diagnostiques d'une nouvelle technique d'élastographie hépatique, appelée « Supersonic Shear Imaging » (SSI), et d'analyser sa plus-value dans l'évaluation non-invasive des maladies chroniques du foie.

Dans une première étude, nous avons prospectivement analysé et comparé les performances diagnostiques de l'élastographie SSI par rapport au FibroScan et l'ARFI pour le staging de la fibrose hépatique sur une série de 349 patients avec une maladie chronique du foie diagnostiquée et gradée par ponction-biopsie hépatique.

Dans une seconde étude, nous avons prospectivement étudié l'impact diagnostique de l'élastographie SSI sur le foie et la rate chez une population de 401 patients avec cirrhose pour l'évaluation de la gravité de la maladie cirrhotique.

Dans une troisième étude, nous avons réalisé une analyse prospective bicentrique (Angers et Bordeaux) de la performance diagnostique de l'élastographie SSI par rapport au FibroScan et l'ARFI dans l'évaluation noninvasive de la fibrose hépatique sur une série de 291 patients avec stéatopathie non-alcoolique et ponction-biopsie hépatique.

Mots clés : Elastographie par ondes de cisaillement, Fibrose hépatique, Diagnostic non-invasif

# **Title :** Impact of « Supersonic Shear Imaging » elastography in the noninvasive diagnosis of chronic liver diseases

### Abstract :

The management and the prognosis for chronic liver diseases are widely based on the presence and the development of a liver fibrosis. The progressive worsening of liver fibrosis leads in a certain number of patients to the development of cirrhosis and its complications. Thus, the development of non-invasive diagnostic tools for the diagnosis and the monitoring of the liver fibrosis is of crucial interest. Liver elastography is one of the most promising techniques that have recently emerged in the field of chronic liver diseases. In this study, we aim to assess the diagnostic accuracy of a new elastography technique, named "Supersonic Shear Imaging" (SSI), and to analyse its added value in the noninvasive diagnosis of chronic liver diseases.

In a first study, we prospectively analysed and compared the diagnostic performances of SSI elastography versus FibroScan and ARFI for the staging of liver fibrosis in a cohort of 349 patients with chronic liver diseases that consecutively underwent a liver biopsy.

In a second study, we prospectively analysed the impact of liver and spleen SSI elastography in a cohort of 401 cirrhotic patients for the noninvasive diagnosis of cirrhosis severity and oesophageal varices.

In a third study, we assessed the clinical use of liver stiffness measurement evaluated by SSI, FibroScan, and ARFI in a cohort of nonalcoholic fatty liver disease patients who underwent liver biopsy. A total of 291 NAFLD patients were prospectively enrolled at 2 French university hospitals (Angers and Bordeaux).

**Keywords**: Bidimensional Shear Wave Elastography, Liver fibrosis, Noninvasive diagnosis

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