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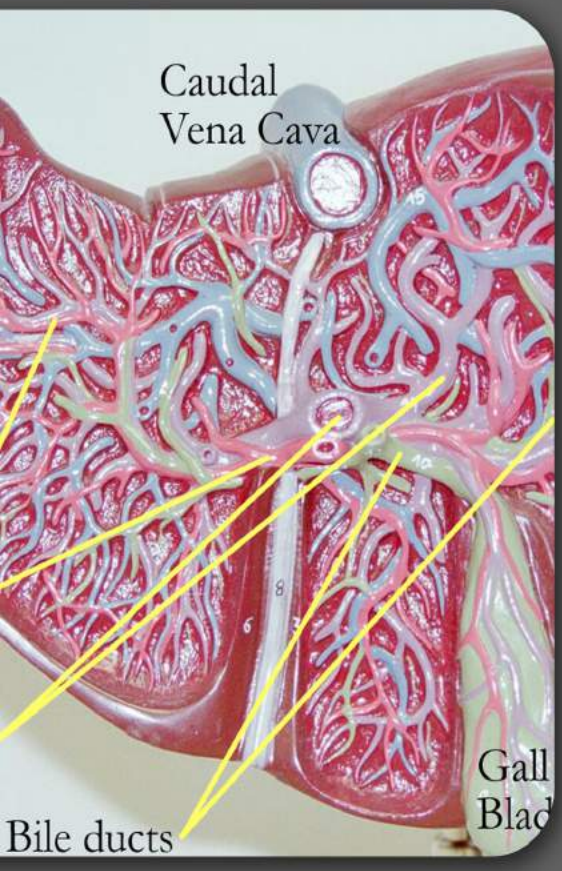
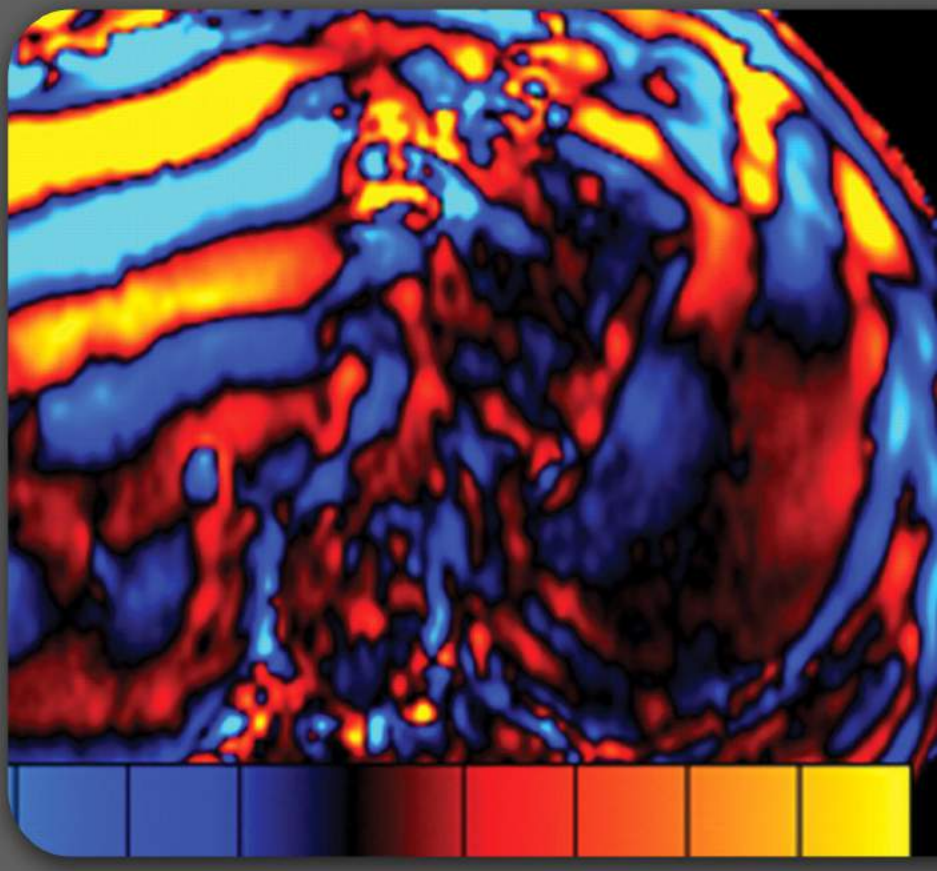
Hepatic Elastography Using Ultrasound Waves

Revised Edition of Volume 1





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Hepatic Elastography Using Ultrasound Waves

Revised Edition of Volume 1

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CONTENTS

FOREWORD

Chronic liver diseases are common worldwide, including chronic viral hepatitis B and C, alcohol related and non-alcoholic fatty liver disease (NAFLD) and many others. The use of ultrasound has significantly contributed to the evolution of hepatology.

B mode ultrasound is the most frequently used initial imaging modality to examine patients with acute and chronic liver diseases. Doppler ultrasound techniques provide morphological and functional information of the liver vascularity which is most important for the evaluation of portal hypertension and its complications. Contrast enhanced ultrasound has revolutionized liver imaging. More recently the ultrasound based elastography technology has introduced a new dimension of imaging. The introduction and widespread use of non-invasive elastography techniques have reduced the need for invasive liver biopsies (LB) in patients with chronic liver disease.

The revised edition of the eBook edited by Prof. Dr. Ioan Sporea and Dr. Roxana Şirli on liver elastography summarizes the current and up to date knowledge on the use of elastography in the evaluation of liver diseases. The ebook introduces an understanding of this novel technique through the lens of important clinical background information which is also discussed. The well-known Roumanian authors around Prof. Ioan Sporea have published not only this book but also evidence based National Guidelines and Practical Recommendations on liver elastography. This book and the “practical recommendations” are helpful for all doctors starting to use these methods.

The book describes the physical principles of elastography, referring to the elastography guidelines of the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) and World Federation on Ultrasound in Medicine and Biology (WFUMB).

Various elastography modalities are available, requiring different examination techniques and providing slightly different clinical information. The techniques described include transient elastography and acoustic radiation force impulse (ARFI) elastography, 2D shear Waves elastography and strain elastography amongst others. Importantly, examination technique, reproducibility and confounding factors are explained in detail.

A link to this book is available on the EFSUMB website (www.efsumb.org).

Congratulations to the authors who write with the benefit of their long standing clinical and research expertise in this field. I commend their valuable contributions to elastography.

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PREFACE

The incidence and prevalence of chronic liver diseases increases in everyday practice. The main etiologies are chronic hepatitis C or B, ethanol abuse (alcoholic steatohepatitis - ASH) or nonalcoholic steatohepatitis (NASH), while autoimmune hepatitis or primary biliary cirrhosis (PBC) are encountered more rarely, but are not negligible.

Staging liver fibrosis severity is essential in chronic liver diseases work-out for prognosis and for decision regarding treatment. Until a few years ago, fibrosis evaluation was made only by means of a liver biopsy (LB) - the "gold standard" technique for staging, but also for grading liver diseases [1].

After percutaneous LB was introduced in daily practice in hepatology (some decades ago), it became an indispensable tool for liver disease assessment. It evaluates the fibrosis stage and activity grade, but it also reveals fatty infiltration or specific markers for some hepatic diseases (such as the Mallory bodies in alcoholic steatohepatitis). The morphologic examination is considered the "gold standard" method for assessing lesions' severity in chronic hepatopathies and, until some years ago, was also considered mandatory for prognosis assessment.

An old problem of LB is that the specimen obtained is very small, only approximately 1/50,000 of the liver. Another issue is the uneven distribution of fibrosis in the liver. Also, an important problem is the specimen size. To be relevant, liver samples must be at least 2 to 4 cm long [2]. Other authors state that a specimen adequate for pathological examination should be longer than 25 mm and including more than 8 portal tracts [3] or, including at least 11 portal tracts [4]. Colloredo *et al.* [5] showed that the chance of underestimating fibrosis severity and necroinflammatory activity increases in parallel with the shortness of the liver sample. Bedossa *et al.* [6] imagined a mathematical model that predicted a 25% diagnostic error rate if the biopsy specimen was only 25 mm long. This model estimated that the optimal specimen hould be at least 40 mm long.

In daily practice, in many cases the liver specimen is suboptimal and can underestimate the fibrosis severity and necroinflammatory lesions. According to two multicentre studies performed in France, in up to 10-15% of cases the LB is uninterpretable due to the small size of the specimen [7]. In a previous multicentre Romanian study concerning the quality of liver sample obtained by percutaneous LB [8], only in half of the cases, the LB fragments were optimum for pathological interpretation, including more than 11 portal tracts, while in approximately two thirds of cases the fragments were only satisfactory (more than 8 portal tracts). In approximately 1/3 of cases, the tissue specimen was not good enough for a correct

staging of liver disease (less than 8 portal tracts).

In a systematic review on the quality of LB specimens [9], it was demonstrated that major and minor complications occur during the procedure in up to 6% of cases, while 0.04 to 0.11% of them can be life threatening. In this review including more than 8,700 patients, in more than half of cases the mean length and mean number of portal tracts of LB specimens was much lower than the published minimum sample size requirements [5, 6] (only 42% of liver samples included at least 10 more) [9].

Our group evaluated a cohort of more than 1000 percutaneous echo assisted LB performed with 1.4 mm and 1.6 mm Menghini modified needles, with 2 liver passages [10] in which the quality of liver specimen was evaluated. We divided the LBs into 4 groups (< 15 mm; 15- 24 mm; 25-39 mm; > 40 mm). We calculated the mean lengths for every group and using Bedossa's study [6] we analyzed the percentage of expected correctly classified biopsies. The overall mean length of liver specimen obtained in our cohort was 33±9 mm, with a mean number of portal tracts of 20±10 (indicative of a good quality specimen). 1% (10) of the LBS were included in the first group (< 15 mm) with a mean length of 9.8±2 mm, 13% (135) LBS were included in the second group (15- 24 mm) obtaining a mean length of 20±1.8 mm, 41% (418) of the LBs had between 25 and 39 mm with a mean length of 30±3 mm, 45% (449) of the LBs obtained specimens larger than 40 mm with mean length of 42±5 mm [10]. Using Bedossa's study and diagram referring to the sensitivity of LB for staging liver fibrosis according to the length of biopsy specimen, we obtained the following sensitivities: Group 1 (< 15 mm) 55%; Group 2 (15-24 mm) 70%; Group 3 (25-39 mm) 75%; Group 4 (> 40 mm) 83% and an overall sensitivity of LB of 80%. Thus, despite the fact that good liver specimens were obtained in our study using Menghini needles with 2 passages technique (mean length of liver specimen 33±9 mm, with a mean number of portal tracts of 20±10) the overall sensitivity of liver biopsy was only approximately 80% using Bedossa's criteria. The conclusion of the study was that the “gold standard’ method (LB) is not actually a very good “gold standard” [10]. This paper raised the question if similar (or better) results could not be obtained with other (non-invasive) methods?

Another problem when evaluating the LB results is the inter- and intraobserver concordance. A study on the interobserver agreement in assessing LBs from patients with chronic hepatitis C showed concordant opinion in assessing fibrosis of 0.78 and for necroinflammatory activity of 0.48 if Knodell score was used. For the Metavir score, the concordance for fibrosis assessment was 0.80, and 0.56 for necroinflammatory activity [7].

With regard to the patients’ perspective, we must ask ourselves why patients are afraid of LB. The first reason is pain and discomfort, but also the risk of complications, which is low, but not zero. A paper published in 2010 presented the results of a study regarding elective

percutaneous LBs performed using data collected by the National Health Service in England from 1998 to 2005 from 61,187 subjects [11]. Seven day mortality directly related to LB and bleeding episodes up to 7 days after biopsy were evaluated. The study revealed that death within 7 days, directly related to LB occurred, at most, in 1/10,000 biopsies, and that 6 episodes of major bleeding occurred per 1000 biopsies.

This risk of complications increases in patients with advanced fibrosis, as shown by the results of the HALT-C study [12]. In this study, from 2,740 liver biopsies, approximately 0.5% of patients with hepatitis C and advanced fibrosis experienced potentially serious bleeding after LB and the risk significantly increased in patients with a platelet count of 60,000/mm or less.

Thus, considering these limitations of LB in daily practice, maybe other methods can be used to evaluate the severity of liver lesions. Some years ago, hepatologists focused on non-invasive methods for the evaluation of liver diseases severity which could represent an alternative to LB. Some authors favor biological markers [13], some are in favor of elastographic methods [14, 15], while others consider that the combination of these methods can reduce the number of LBs [16, 17].

Indeed, the number of LBs performed across the world has decreased in the last years. In France, liver fibrosis in chronic hepatitis C patients can be assessed by means of LB or by non-invasive methods such as FibroTest or FibroScan®. In an American study from Beth Israel Medical Center New York which evaluated the last 15 years' experience regarding LB, the number of LBs performed for chronic hepatitis C peaked in 2003, followed by an annual decrease, while the number of annual biopsies for chronic hepatitis B increased during the same period [18]. On the other hand, nowadays, when very potent drugs are available for HCV chronic infection, with a cure rate of more than 90-95%, the patients can be treated to cure the infection and to stop disease progression, without much interest regarding the disease severity. Fibrosis severity evaluation is used (or can be used) only to prioritize treatment, considering its current high cost.

Schiano [19] wrote an interesting editorial in Clinical Gastroenterology and Hepatology, concerning the LB (in autoimmune hepatitis). The title is a very provocative one: "To B(iopsy) or Not to B(iopsy)...".

Thus, we can open the discussion concerning the future of LB — "Quo vadis" liver biopsy? The question is if there still is a place for LB in the evaluation of chronic hepatopathies? This is a very provocative question and long debates have been known to develop regarding this topic. If LB can be avoided (at least in the majority of cases), is this strategy applicable only for chronic hepatitis C, or is it also possible in chronic hepatitis B? But what should we do

regarding other chronic liver diseases, such as non-alcoholic steato-hepatitis (NASH), alcoholic liver disease (ALD), autoimmune hepatitis, cholestatic liver diseases, overlap syndrome or drug-induced liver injury (DILI) ?

The number of ***non-invasive methods for liver fibrosis assessment*** has increased in the last decade [20]. They can be: ***serum based tests*** (direct and indirect, the most frequently used in clinical practice being FibroTest) or ***imaging tests***. The latter, becoming more popular every day, based either on ultrasound (Ultrasound based liver Elastography) or on magnetic resonance imaging (MR Elastography-MRE), are used for liver stiffness (LS) assessment, as a marker of fibrosis. Serologic tests evaluate both the necroinflammatory activity (ActiTest) and fibrosis (FibroTest) and can give information concerning fat infiltration or alcohol abuse (Fibro Max) [20].

The first method used for LS evaluation using ultrasound waves was Transient Elastography (FibroScan®, Echosens® France). Other techniques have been latterly developed, such as Real Time Elastography (by Hitachi) or Acoustic Radiation Force Impulse (ARFI) Elastography. They are used more and more, in daily practice and many papers have been published, proving their value. 2D Shear Waves Elastography (2D SWE) has been developed more recently.

This book intends to be an overview regarding the value of different elastographic methods using ultrasound waves for LS assessment, in patients with chronic liver diseases. Our team's experience, together with published data from the latter years, offers the reader a perspective of the role that these methods play in the liver evaluation algorithm. Many papers concerning the value of different elastographic methods for LS evaluation have continued to be published, some considering LB as the reference method and others trying to demonstrate the non-inferiority of new elastographic methods, as compared to a validated method, such as Transient Elastography (FibroScan®).

At the end of this book, there is some information regarding the new development directions of elastography for the evaluation of focal liver lesions (FLL). The role of elastographic methods for FLL assessment has not yet been established, but some results have already been evidenced.

This e-book is the revised edition of Vol.1 of Hepatic Elastography Using Ultrasound Waves, presenting the most recent papers looking at the value of ultrasound based elastography for liver stiffness assessment. Rapid development in liver elastography, with new machines appearing in the market, made it imperative to produce this second edition, in which new guidelines and clinical recommendation have been included. We hope that readers of this book will gain enough practical information regarding all types of ultrasound based

liver elastography, that will permit them to work with these methods in clinical practice.

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CHAPTER 1

Transient Elastography (TE)

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Abstract: Transient Elastography (TE) is the first ultrasound-based method for fibrosis assessment, developed by Echosens®(France). In order to obtain reliable liver stiffness (LS) measurements by means of TE, the manufacturer recommended that at least 10 valid shots should be obtained. They should have a success rate (SR: the ratio of valid shots to the total number of shots) of at least 60% and an interquartile range (IQR, the difference between the 75th percentile and the 25th percentile, essentially the range of the middle 50% of the data) less than 30% of the median LS value. New quality criteria were proposed by Boursier in which only IQR is taken into consideration. TE fails if no valid shots can be obtained, and is unreliable if fewer than 10 valid shots are obtained. TE failure is correlated with the body mass index, increasing in obese patients. By using the XL probe, the success rate of TE measurements significantly improves. Also, unreliable results are obtained during aminotransferases flares that can lead to an overestimation of fibrosis. The LS upper limit in healthy subjects was estimated to be 5.3 kPa. Several meta-analyses assessed LS measurements by TE as a predictor of fibrosis, cut-offs for F≥2 ranging from 7.2-7.6 kPa and for F=4 from 12.5-17.3 kPa, according to the etiology of chronic liver disease. Several studies have been published regarding the value of TE for predicting the occurrence of cirrhosis complications. The AUROC's for predicting clinically significant portal hypertension were 0.945 - 0.99, for cut-off values between 13.6 - 21 kPa, while for predicting esophageal bleeding the best cut-offs ranged between 50.7 – 62.7kPa, with AUROC's 0.73-0.75. European Guidelines recognize TE as a reliable method to evaluate fibrosis.

Keywords: Cirrhosis, Esophageal varices, Liver fibrosis, Liver stiffness, Transient elastography.

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Ioan Sporea & Roxana Şirli (Eds.)

1. TE TECHNIQUE

Transient Elastography (TE) is a shear wave and ultrasound-based method, developed by Echosens® (France), initiating from the principles of Hooke's law, which characterizes a material's strain response to external stress [1]. A FibroScan® device is used (Fig. **1**), whose ultrasound transducer probe (Fig. **2**), mounted on the axis of a vibrator, transmits low-frequency vibrations into the liver. The transducer is placed in a right intercostal space and generates an elastic shear wave that propagates into the liver. A pulse-echo ultrasound acquisition is then used to detect shear waves propagation velocity, which is proportional to tissue stiffness; faster shear waves progression occurs through stiffer material. LS measurement is then performed and measured in kiloPascals (kPa) (values between 1.5kPa and 75 kPa are expected).

Fig. (1). The FibroScan® device.

Fig. (2). Pediatric (S), standard (M) and obese (XL) FibroScan® probes.

Using TE, liver stiffness measurements (LSMs) are performed in the right liver lobe through the intercostal spaces, while the patient lies in a dorsal decubitus position with the right arm in maximal abduction. The tip of the transducer is covered with coupling gel and placed in the 9th to 11th intercostal space, at the level where a liver biopsy would be performed. The operator, assisted by ultrasound A-mode images provided by the system, locates a portion of the liver at least 6 cm thick and free of large vascular structures. Once the area of measurement had been located, the operator presses the probe button to begin an acquisition. TE measures LS in a volume that approximates a cylinder 1 cm wide and 4 cm long, between 25 mm and 65 mm below the skin surface. Acquisitions that do not have a correct vibration shape or a correct follow-up of the vibration propagation are automatically rejected by the software [2 - 5]. Following each measurement, the measured value of LS is displayed (CS). Following 10 valid measurements, the median value of these values is displayed, as well as the IQR and the SR (Figs. **3**, **4**)

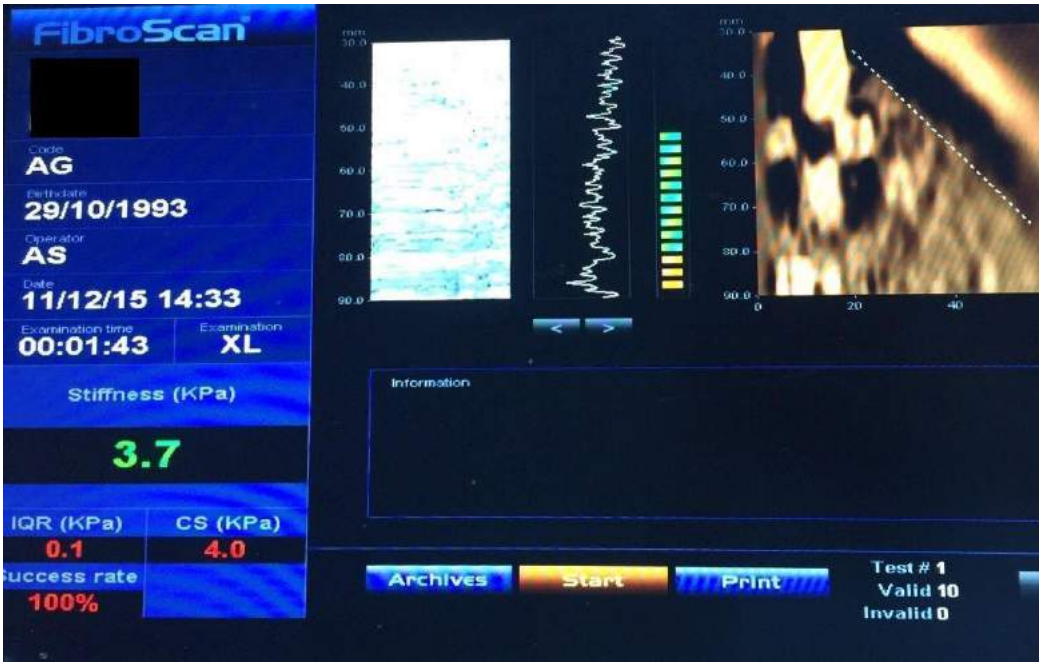

Fig. (3). Transient elastography measurement in a normal individual (median value of 10 measurements).

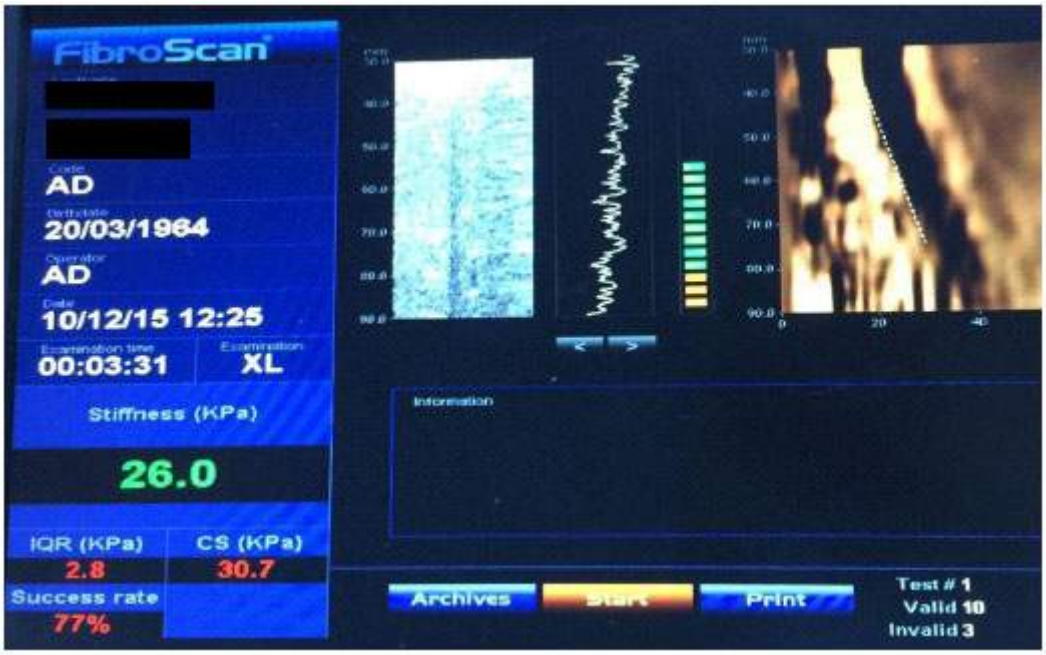

Fig. (4). Transient elastography measurement in a cirrhotic patient (median value of 10 measurements).

2. PITFALLS OF LS MEASUREMENTS BY MEANS OF TE

In order to obtain a reliable evaluation by means of TE, the manufacturer recommends that at least 10 valid measurements should be obtained. They should have a success rate (SR: the ratio of valid shots to the total number of shots) at least 60% and an interquartile range (IQR, the difference between the 75th percentile and the 25th percentile, essentially the range of the middle 50% of the data) less than 30% of the median LSM value. These recommendations have been included in most guidelines [2 - 5].

Thus, TE is considered ***failed*** if no valid shots can be obtained, and ***unreliable*** if fewer than 10 valid shots are obtained, with an IQR greater than 30%, and/or a SR less than 60% [2 - 6]. In a very large study published by Castera on more than 13,000 LSMs, the success rate of stiffness evaluation with TE was correlated with the body mass index (BMI), decreasing in obese patients (in which it is less than 80%) [6]. In a study from our group on 8218 patients, failed and unreliable LSMs were observed in 29.2% of cases. In univariant and multivariate analysis, the following risk factors were associated with failed and unreliable measurements:

age over 50 years (OR 2.04), female gender (OR 1.32), BMI>27.7kg/m^2 (OR 2.89), weight>77kg (OR 2.17) and height<162cm (OR 1.26). If all negative predictive factors were present (woman, older than 50 years, with BMI>27.7kg/m^2, heavier than 77 kg and shorter than 162 cm), the rate of failed and unreliable measurements was 58.5%. In obese patients (BMI≥30 kg/m^2), the rate of failed and unreliable measurements was 49.5% [7].

Regarding factors associated with failure, an earlier study performed by Kettaneh *et al.* [8] on 935 HCV patients, showed that the probability of valid measurements (correlated with the histological score) was higher if the operator was experienced (with more than 50 FibroScan® evaluations performed), if the patient was young (OR 0.96/year) and not obese (OR 0.19 if obese). Another study by Boursier *et al.* showed high measurement agreement between novices and expert operators, even during the first 10 cases [9], so that a formal session by a qualified trainer, followed by practice on 50 cases, should suffice for the training of most operators. Current guidelines state that TE can be performed with reliable results by a technician or nurse following 100 training examinations [2, 4, 5].

New quality criteria, which increase the rate of reliable measurements, without affecting the accuracy were proposed by Boursier, following a study that included 1165 patients evaluated by TE and LB. According to this study, SR should no longer be considered a quality parameter and measurements should be classified only based on IQR into: very reliable - those with IQR ≤ 10%, regardless of LS value; reliable - those with IQR = 11-30%, regardless of LS value or those with IQR/M > 30% if LS <7.1 kPa and poorly reliable those with IQR > 30% and LS ≥7.1 kPa [10]. These recommendations were accepted by the producer.

In a prospective study by Foucher *et al.* [11], the univariant analysis showed that failure was associated with: BMI>28 (OR 9.1), diabetes mellitus (OR 2.1), age >50 years (OR 4.0) and steatohepatitis (OR 3.4). Failure to obtain VM was not operator dependent and was not associated with the patient's gender, or with the aminotransferases level. In the multivariate analysis, the only factor associated with failure to obtain VM was BMI>28 (OR 10.0).

In a study published by our group [12] on 1461 patients, failure to obtain valid

LSM was observed in 6.9% of the patients. Female gender (OR=1.946), older age and higher BMI were significantly associated with failure to obtain a valid LSM.

Since in most studies BMI was the main factor associated with failed and unreliable measurements, a new probe was designed (XL probe), with lower frequency (2.5 MHz), and thus with deeper penetrability, aimed to be used in overweight and obese patients. De Ledinghen was the first to show that 10 valid measurements could be obtained in only 45% of the severely obese patients (BMI $\geq$ 40.5 kg/m^2), but using the XL probe, 76% of the subjects could be evaluated ($p<0.001$) (in 59% of cases that could not be evaluated by an M probe, valid measurements were obtained with the XL probe) [13].

A study that evaluated a cohort of overweight and obese patients, showed that failure to obtain values by TE by the XL probe was much lower than by the M probe (1.1% *vs.* 16%), as well as that of unreliable measurements (27% *vs.* 50%)($p < 0.00005$). On the other hand, by using the XL probe, 61% of patients in whom the M probe was unreliable could be evaluated [14]. In a study from our group that included 216 difficult to evaluate patients (mean BMI 30.1±4.1 kg/m^2), in which paired measurements were made with the M and XL probes, reliable measurements were obtained by the XL probe in 63% (80/127) patients that could not be evaluated by a M probe [15].

In a larger study from our group comprising 3235 patients, reliable LSMs by the M probe were obtained in 62.2% patients and by the XL probe in 80% (1011/1220) of those with unreliable measurements by the M probe; thus 93.5% of 3235 cases could be evaluated using both probes [16].

The XL probe also proved its utility in another study comprising 258 patients with BMI > 25 kg/m^2. In this study, the LSM by the XL probe was feasible in 94.6% cases and the diagnostic accuracy for severe fibrosis (F3, F4) was very good (AUROC= 0.955) [17]. In another European study, reliable LSM by the XL probe could be obtained in 93% (41/44) patients, in which reliable LSM could not be obtained by expert operators with the M probe [18].

There are other factors that can impair the correlation of LS values by TE with liver fibrosis. These factors are: increased aminotransferases level, liver

congestion due to heart failure and extrahepatic cholestasis [2 - 5].

In a study performed by Coco *et al.*, LS was evaluated considering the aminotransferases level, proving that another factor rather than fibrosis, independently associated with LS was ALT for patients with chronic hepatitis [19]. The LS dynamics profiles paralleled those of ALT, increasing 1.3 to 3 fold during ALT flares. This study also showed that LS remained unchanged in patients with a stable biochemical activity. In an Italian study on 12 patients with acute HBV hepatitis, repeatedly evaluated by TE and biological tests during a 24 weeks follow-up period, Vigano *et al.* concluded that the initial high values of LS mimicking LS cut-off of cirrhosis, likely reflect the liver cell inflammation, edema and swelling as they progressively taper down during hepatitis resolution [20]. In a study published in 2009, Chan *et al.* evaluated 161 patients with chronic HBV hepatitis and concluded that patients with the same fibrosis staging, but higher ALT levels, tend to have higher LSM, and the diagnostic performance for low stage fibrosis was most seriously affected when ALT was elevated [21]. All three studies confirmed previous results published by Arena and Sagir in 2008 [22, 23].

An initial observation of high LS values in a patient with cardiac failure, normalized following heart transplantation [24], was confirmed by Millonig *et al.* in an experimental model on landrace pigs. It showed that the stepwise increase of intravenous pressure to 36cm of water column (3.5kPa) linearly and reversibly increased LS to the upper detection limit of 75kPa [25]. The experimental data was confirmed in 10 patients with decompensated congestive heart failure, before and after recompensation. Initial LS was elevated in all patients, in 8 of them to values that suggested liver cirrhosis (median 40.7kPa). Upon recompensation with a median weight loss of 3.0kg, LS decreased in all 10 patients down to a median LS of 17.8kPa [25].

The same group of researchers evaluated LS in patients with obstructive jaundice, before and after drainage by endoscopic retrograde cholangio-pancreatography. After successful biliary drainage, LS decreased by 2.2 to 9.1 kPa, in correlation with bilirubin decrease [26]. This observation was confirmed in an animal model of bile duct ligation in landrace pigs, where liver stiffness increased from 4.6 kPa

to 8.8 kPa during 120 minutes of bile duct ligation and decreased to 6.1 kPa within 30 minutes after decompression [26].

A significant increase in liver stiffness was observed after food intake for up to 60 minutes, and the value normalized after 180 minutes. Even if the change was modest in most cases (mean change 1–2 kPa), it determined misclassifications in some [27]. Current guidelines recommend that LSM by TE should be performed in fasting patients [2 - 5].

Also, it was observed that LS values decrease in patients with heavy alcohol abuse, who stop drinking, suggesting that inflammation induced by heavy drinking plays a role in increasing LS values [28 - 30].

There are conflicting data regarding the influence of steatosis on LS measurements. Some studies state that the degree of hepatic steatosis does not appear to affect LS [27, 31], while in the study of Lupşor *et al.*, the univariant regression analysis demonstrated that fibrosis (R^2=0.610, p<0.0005), activity (R^2=0.145, p<0.0005) and steatosis (R^2=0.037, p<0.002) were correlated with LS. In multiple regression analysis, all three variable independently influenced LS: fibrosis (p<0.0005), activity (p=0.039) and steatosis (p=0.025) [32]. An Italian study on blood donors also proved that median LS values were higher in subjects with liver steatosis, than in those with a normal liver on US 5.3 kPa *vs.* 4.4 kPa, p<0.001 [33]. However, a population-based study from India in 437 healthy subjects showed that undernutrition and lower BMI increase liver stiffness values similar to obesity (6.05 kPa *vs.* 5.51 kPa *vs.* 6.60 kPa, p = 0.016 and 0.349, respectively) [34].

Several studies investigated ***TE reproducibility***. The intraobserver and interobserver agreements were good, with intraclass correlation coefficients generally above 90%; 0.98 in a study by Fraquelli *et al.* (both intraoperator and interoperator) [35], 0.96 in the Nobili study [36]. Reduced interobserver agreement was significantly associated with increased body mass index (BMI) (> 25 kg/m^2), steatosis (> 24%), and METAVIR stage < F2) [35]. A recently published American study also showed high intra and interobserver agreement for LS measurements by TE (0.98 and 9.96 respectively [37].

3. TE IN NORMAL SUBJECTS

In a study published by our group [38], 152 healthy subjects were evaluated. In 8 cases (5.3%), valid measurements (VM) could not be obtained. In the 144 subjects, in whom valid measurements were obtained, the mean LS value was 4.8±1.3 kPa, ranging from 2.3 to 8.8 kPa. The mean values of LS in each age group did not differ significantly (p=0.5263) (Table **1**). Also the mean LS in women was significantly lower than in men (4.6±1.2 kPa *vs.*5.1±1.2 kPa, p=0.0082).

Table 1. Mean liver stiffness values in each age subgroup.

Age group (years)	Nr. of patients with VM	Mean value of LS ± SD (kPa)	Minimum (kPa)	Maximum (kPa)
All patients	144	4.8±1.3	2.3	8.8
18-29	43	5±1.3	2.3	8.8
30-39	24	4.5±1.2	2.6	7.3
40-49	17	5±1.1	3.0	7.1
50-59	27	4.7±1.2	2.5	7.7
60-69	20	5±1.3	3.2	7.7
>70	13	4.7±1.4	3.0	7.1

In a study by Roulot performed on 429 consecutive apparently healthy subjects, the mean LS value was 5.49±1.59 kPa [39], while in a study performed by Corpechot *et al.* [40], a similar mean value (4.8 kPa) was obtained in a group of 71 healthy subjects. In an Italian study, the median LS value in healthy blood donors was 4.4 kPa [33]. In all three studies, *LS values were higher in men than in women*. Overall, the upper limit of normal LS was estimated to be 5.3 kPa [39, 41].

4. TE IN CHRONIC HEPATOPATHIES

a. TE in Chronic HCV Hepatitis

TE assessment of LS was used initially for the evaluation of chronic HCV hepatitis. Latterly published articles that will be discussed in the following pages,

proved the method's value in other chronic hepatopathies, such as chronic HBV hepatitis, hemochromatosis, primary biliary cirrhosis, human immunodeficiency virus (HIV)/HCV co-infection or non-alcoholic steatohepatitis (NASH).

In HCV viremic patients, if the LS is greater than 6.8–7.6 kPa (according to the results of several studies and meta-analysis) [42 - 46], there is a great probability of finding significant fibrosis on the liver biopsy (F2-F4) and subsequently the patient requires antiviral therapy. Probably, in these cases, LB is not required for a treatment decision.

In a multicentre French study coordinated by Beaugrand [47], performed on 494 HCV patients who were evaluated by means of percutaneous LB (with a significant fragment) and valid FibroScan® examination, a significant correlation was found ($p<0.001$) between the severity of fibrosis and the LS by TE ($r=0.57$). This study tried to establish cut-off values for LS that could differentiate between various stages of fibrosis. Thus, the cut-off value of 7.5 kPa differentiates F0-1 from F2-4 with 67% sensitivity, 87% specificity, 86% PPV and 68% NPV, with a diagnostic accuracy of 76%. Other studies [44 - 46] established cut-off values that differentiate F0-1 from F2-4 ranging from 6.8-7.3 kPa.

As a practical approach, viremic patients with LS lower than 7 kPa should undergo LB, in order to discover the ones with significant fibrosis underestimated by FibroScan® and who, otherwise, would not receive antiviral therapy. This strategy is already used in France, a country in which non-invasive evaluation of chronic C viral hepatitis is used more and more frequently.

TE is not accurate enough to differentiate among contiguous stages of fibrosis (especially 0, 1 and 2), but is sensitive enough to differentiate between the absence and mild fibrosis from significant fibrosis, essential for the decision regarding treatment. At the same time, in the future we must find exactly if histological activity, steatosis or biological activity (ALT) have an important role in the assessment of LS by means of FibroScan®, as shown in recent studies [19, 32].

In 324 consecutive patients with chronic HCV hepatitis, evaluated both by TE and LB in the same session, the LS values were strongly correlated with fibrosis

(r=0.759, p<0.0005), but also with steatosis (r=0.255, p<0.005), necroinflammatory activity (r=0.378, p<0.0005) and hepatic iron deposition (r=0.143, p=0.03). The conclusion of this study was that fibrosis is the main predictor of LS, but that it is also influenced by disease activity and steatosis [32].

In a study by our group that included 407 naive patients with HCV chronic hepatitis, in which LB and TE were performed in the same session, reliable LS measurements were obtained in 96.8% of the patients. A significant direct correlation of LS measurements with fibrosis was found: Spearman's r=0.605, *P*<0.0001. For a cut-off value of 6.8 kPa, LS had 58.9% sensitivity and 89.1 specificity (AUROC 0.760) for predicting significant fibrosis (at least F2 Metavir), while for a cut-off value of 12.6 kPa, the sensitivity was 92.1%, the specificity 91.6% (AUROC 0.953) for predicting cirrhosis [48].

Finally, several meta-analyses assessed LS measurements by TE as a predictor of significant fibrosis in patients with HCV hepatitis [42, 43, 49, 50]. In the Friedrich-Rust meta-analysis, based on 50 studies [43], the mean AUROC was 0.84, with a suggested optimal cut-off of 7.6 kPa. In the Tsochatzis meta-analysis, the pooled cut-off for F≥2 Metavir was also 7.6 kPa (range 5.1–10.1), with 0.78 pooled sensitivity and 0.89 pooled specificity [50].

Considering all these data, even if TE is not accurate enough to distinguish between

contiguous stages of fibrosis, it can differentiate absence and mild fibrosis from significant fibrosis and cirrhosis, which is more critical for decisions regarding treatment [2 - 5, 51]. By using cut-off values of 6.8–7.6 kPa, patients could be identified accurately enough to decide those who should be treated (F≥2 METAVIR) *versus* those who should not be treated in this moment (F<2 METAVIR), without performing a LB (Fig. **5**).

Combining FibroScan® with serum fibrosis markers can further improve the diagnostic accuracy of non-invasive liver fibrosis measurement [52 - 54] and different algorithms have been suggested.

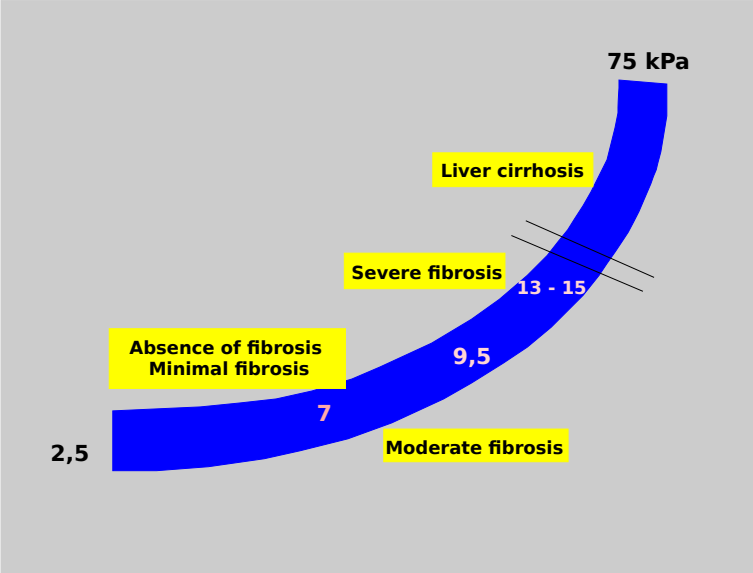

Fig. (5). Correlation between liver fibrosis and TE measurements.

Several studies suggested that TE may be used for the *evaluation of antiviral therapy results in HCV patients*. In a study published in 2011, Hezode *et al.* prospectively evaluated 91 patients with chronic HCV hepatitis during the antiviral therapy. LS was assessed by TE and compared with the virologic responses at weeks 4, 12, 24, end of treatment and 12 and 24 weeks after. A significant LS decrease was observed during therapy, which continued after treatment, only in patients who achieved a sustained virologic response (SVR). In this group, the median intra-patient decrease relative to baseline at the end of follow-up was - 3.4 kPa, *vs* - 1.8 kPa in the patients who did not achieve an SVR. In multivariate analysis, only the SVR was associated with long-term LS improvement (odds ratio: 3.10, p=0.019) [55].

A similar decrease in LS values by TE was observed in other studies performed in the European [56] and Asian population [57, 58]. All these data support the conclusion that fibrosis may be reversible in patients with HCV chronic hepatitis, which achieve SVR following antiviral therapy.

b. TE in Chronic HBV Hepatitis

Published studies concerning the value of LS measurement by means of TE in patients with HBV chronic hepatitis have shown conflicting results regarding the cut-off values for different stages of fibrosis (Table **2**).

Table 2. Mean LS values, according to fibrosis, in patients with HBV vs. HCV chronic hepatitis.

Category	HBV		HCV		*P*
	Nr. of cases	Mean values of LS (kPa)	Nr. of cases	Mean values of LS (kPa)	
Total cases	140	8.1±4.2	317	8.9±5.2	0.395 (NS)
F=0	1	7.4	5	5.2±0.7	-
F=1	32	6.5±1.9	34	5.8±2.1	0.0889 (NS)
F=2	67	7.1±2	146	6.9±2.5	0.3369 (NS)
F=3	33	9.1±3.6	93	9.9±5	0.7038 (NS)
F=4	7	19.8±8.6	39	17.3±6.1	0.6574 (NS)

In a study performed by Ogawa [59] on 68 patients with chronic HBV hepatitis, the mean LS values were 3.5 kPa for F0, 6.4 kPa for F1, 9.5 kPa for F2, 11.4 kPa for F3, and 15.4 kPa for F4 patients. The values were significantly correlated with fibrosis stage (r=0.559, P=0.0093).

In a prospective study by Marcellin *et al.*, on 202 patients with chronic HBV hepatitis, LS was significantly ($P<0.001$) correlated with METAVIR (r=0.65) fibrosis stage (0.65). The AUROCs for F≥2, F≥3 and F=4 were 0.81, 0.93 and 0.93 respectively. Optimal LS cut-off values were 7.2 and 11.0 kPa for F≥2 and F=4, respectively, by maximizing the sum of sensitivity and specificity, and 7.2 and 18.2 kPa by maximizing the diagnosis accuracy [60].

Several studies compared the LS values by TE in HCV and HBV patients. A previously published study of our group [61], performed on a large cohort of patients (140 subjects with HBV and 317 with HCV chronic hepatitis) showed that the mean LS values were similar in both groups, for the same stage of fibrosis (Table **2**). A significant direct correlation of LS measurements with fibrosis was found to exist in HCV patients (Spearman's correlation coefficient r=0.578,

$P<0.0001$), as well as in HBV patients ($r=0.408$, $P<0.0001$). The correlation was stronger in HCV than in HBV patients (Fisher's Z-test, $Z=2.210$, $P=0.0271$).

In this cohort of 140 chronic HBV infected patients, the mean values for F1, F2, F3 and F4 were: 6.5 kPa, 7.1 kPa, 9.1 kPa and 19.8 kPa, respectively, similar to those obtained in the study performed by Marcellin' s group [60].

A study published in 2012 by Cardoso *et al.* [62] on 202 HBV patients and 363 HCV subjects, revealed that TE exhibited comparable accuracies, sensitivities, specificities, predictive values and likelihood ratios in HBV and HCV groups. Contrary to studies in the Asian population [19 - 22], AUROC analysis showed no influence of ALT levels on the performance of TE in HBV individuals. ALT-specific cut-off values did not exhibit significantly higher diagnostic performances for predicting fibrosis in HBV patients with elevated ALT.

In another Asian study, that compared TE performance in HBV *vs.* HCV patients, the conclusion was that discrepancies between LS values and histological fibrosis are due to the degree of serum ALT levels, rather than to the cause of hepatitis itself [63].

The results of these studies, showing a weaker correlation of LS with histological fibrosis in HBV than in HCV patients, can be explained in part, by the fact that high levels of aminotransferases influence the LS values obtained by means of TE [19 - 22]. Thus, LS measurements have to be interpreted in a biochemical context; otherwise, there is a risk of overestimating the severity of fibrosis. Also this is why LS measurements are not performed in acute hepatitis or during alanine aminotransferase (ALT) flares in HBV chronic hepatitis [19, 64].

In order to minimize the risk of overestimating fibrosis during ALT flares, Chan *et al.* [21] calculated LS cut-off values for various stages of fibrosis considering also the aminotransferases levels. In this study, the LS cut-off value for F3 was 9 kPa in patients with normal ALT and 12 kPa in patients with ALT higher (than 5 times the upper limit of normal). The cut-offs for cirrhosis were 12 kPa in patients with normal ALT and 13.4 kPa in those with elevated ALT.

A study published in 2013 on 357 HBV patients showed a significant correlation

between LSM and histological fibrosis ($r = 0.58$, $p < 0.001$), with areas under ROC curve of LSM for significant fibrosis (F≥2), bridging fibrosis (F≥3), and cirrhosis (F4) of 0.84, 0.94, and 0.93 respectively. LSM < 6 kPa and > 9 kPa matched with histological fibrosis in 227/250 (91%) patients [65].

The Tsochatzis ***meta-analysis*** also assessed the predictive value of LS assessed by TE in HBV patients. The pooled cut-off for F≥2 Metavir was 7 kPa (range 6.9–7.2, lower than in HCV patients), with 0.84 pooled sensitivity and 0.78 pooled specificity [50]. The Chon ***meta-analysis*** comprised 2,772 HBV patients. The mean AUROCs for the diagnosis of significant fibrosis (F2), severe fibrosis (F3), and cirrhosis (F4) were 0.859, 0.887, and 0.929, respectively. The estimated cut-off for F2 was 7.9 kPa (Se 74.3% and Sp 78.3%), for F3 it was 8.8 (Se 74.0% and Sp 63.8%), while for F4 it was 11.7 kPa (Se 84.6% and Sp 81.5%) [66].

In 2015, two ***meta-analyses*** were published regarding the performance of TE in staging fibrosis in HBV patients. The Xu ***meta-analysis*** showed different AUROCs of TE for diagnosing F≥2 and F4 in European *vs.* Asian populations: 0.803 and 0.905 *vs.* 0.871 and 0.914, respectively. The pooled diagnostic odds ratios of TE for F≥2 and F4 were 11.19 and 26.87, respectively [67]. In the Li ***meta-analysis***, which included 4386 HBV patients, the summary Sp of TE for staging fibrosis $F \geq 2$, $F \geq 3$ and $F = 4$ were 0.806, 0.819 and 0.863, respectively, while the summary Sp were 0.824, 0.866 and 0.875, respectively. The corresponding AUROCs were 0.88, 0.91 and 0.93, respectively [68].Considering all these data TE has been accepted as a reliable method for staging fibrosis in chronic HBV hepatitis [2 - 5], in patients with normal ALT performing even better than serologic tests [2].

HBsAg inactive carriers (HBeAg-negative, with HBV-DNA < 2000 IU/ml and normal ALT levels) should be also mentioned since in this category of patients, fibrosis and cirrhosis can also be present, even if in a small percentage of cases. In a study from our group the mean LS values in inactive HBsAg carriers was 5.6±2.1kPa, significantly higher than in normal subjects (4.8 ± 1.2 kPa, $p = 0.0002$), while in patients with undetectable viral loads, the mean liver stiffness was 4.9 ± 1.2 kPa, significantly lower than in those with detectable DNA (< 2000 IU/ml) (6.7 ± 2.7 kPa, $p < 0.001$) [69]. International guidelines concluded that TE

can be used to exclude severe fibrosis and cirrhosis in these patients [2].

c. TE in Other Chronic Hepatopathies

Regarding the value of LS measurements by TE in evaluating chronic hepatopathies of other etiologies, several studies were performed, in order to identify significant fibrosis in patients with in HIV-HCV co-infection [70, 71], in chronic cholestatic hepatopathies: primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) [72] and in NASH [73]. In these studies, the AUROCs varied between 0.72 and 0.93, and the cut-off values for F≥2 ranged between 4 and 8.7 kPa (Table **3**).

Table 3. Performance of TE for evaluating significant fibrosis in patients with chronic hepatopathies other than HCV (PPV – Positive Predictive Value).

Authors	**De Ledinghen *et al.* [70]**	**Vergara *et al.* [71]**	**Corpechot *et al.* [72]**	**Yoneda *et al.* [73]**
Etiology	**HCV-HIV**	**HCV-HIV**	**PBC and PSC**	**NAFLD**
No. of patients F≥ 2	44	105	57	33
Proposed cut-off (kPa)	4.5	7.2	7.3	6.6
Sensitivity (%)	93.2	88	84	82.7
Specificity (%)	17.9	66	87	81.3
NPV (%)	61	75	79	59.1
PPV (%)	65	88	91	93.5
AUROC	0.72	0.83	0.92	0.87

Regarding ***HCV-HIV coinfection***, several studies demonstrated that TE is a useful method for fibrosis assessment in patients co-infected with HCV and HIV. In the study performed by de Ledinghen *et al.*, LS was significantly correlated to the fibrosis stage (Kendall tau-b=0.48; P<0.0001). The AUROC of LS measurement was 0.72 for F≥2 (cut-off 5.4 kPa) and 0.97 for F=4 [70]. In the Vegara study, the AUROCs were 0.87 for significant fibrosis (cut-off 7.2 kPa) and 0.95 for cirrhosis (cut-off 14.6 kPa). To diagnose significant liver fibrosis, a cut-off value of 7.2 kPa was associated with a positive predictive value of 88% and a negative predictive value of 75% [71]. In a more recent Spanish study, the AUROCs of LS were 0.80 for F>2, 0.93 (0.85-1.00) for F>3 and 0.99 for F4 (cut-

offs 7 kPa, 11 kPa and 14 kPa) [74].

The first study regarding LS by TE in ***cholestatic hepatitis*** (primary biliary cirrhosis – PBC and primary sclerosing cholangitis – PSC) was published in 2006 [72]. In this study, LS was correlated to both fibrosis (Spearman's rho=0.84, P<0.0001) and histological (0.79, P<0.0001) stages. These correlations were still found when PBC and PSC patients were analyzed separately. Areas under ROC curves were 0.92 for F≥2, 0.95 for F≥3 and 0.96 for F=4, for the following optimal cut-off values 7.3, 9.8, and 17.3 kPa respectively. In another study published in 2008 on 80 patients with PBC, LS by TE was significantly correlated to the histological fibrosis stage (Kendall coefficient: 0.56; P<0.005), the AUROCs being 0.89 for F>2 and 0.96 for F=4 [75]. A smaller study in 45 patients with PBC showed that the adjusted accuracy of LS by TE for the diagnosis of F≥2 was 80%, while for liver cirrhosis it was 95% [76]. A study published in 2012 by Corpechot found that the cut-offs to discriminate fibrosis stages in PBC were 7.1, 8.8, 10.7, and 16.9 kPa for F≥ 1, F≥ 2, F≥ 3, and =F4, respectively. TE performed significantly better than biochemical markers [77].

Regarding TE evaluation with nonalcoholic fatty liver disease (***NAFLD***) and nonalcoholic steato-hepatitis (***NASH***), a positive correlation was found between LS values and the histological stage of fibrosis, since even if steatosis may attenuate shear waves, it does not modify their speed [78]. LS measurements can be difficult in patients with NAFLD or NASH, since these conditions are often associated with obesity. A first step towards increasing the feasibility of TE in these patients was the introduction of the XL probe that increased the number of patients that could be evaluated by TE [13, 14, 79].

Yoneda *et al.* evaluated 97 NAFLD patients by TE and LB [73]. LS was well correlated with the stage of liver fibrosis (Kruskal-Wallis test p<0.0001). The AUROCs were: 0.927 for F≥1, 0.865 for F≥2, 0.904 for F≥3, and 0.991 for F4. Only fibrosis stage was correlated significantly with the LS measurement by multiple regression analysis. Lupşor *et al.* [80] evaluated 72 consecutive NASH patients LS was correlated with fibrosis (r=0.661; p<0.0001), steatosis (r=0.435, p<0.0001), ballooning (r=0.385; p=0.001) and lobular inflammation (r=0.364; p=0.002). In multivariate analysis, only fibrosis significantly correlated with LS

($p<0.0001$). Cut-off values were calculated for predicting each fibrosis stage: 5.3 kPa (AUROC=0.879) for F1, 6.8 kPa (AUROC=0.789) for F2 and 10.4 kPa (AUROC=0.978) for F3.

Wong *et al.* evaluated TE as a predictor of fibrosis and cirrhosis in NAFLD patients and the factors associated with discordance between TE and histology in 246 consecutive patients, who had successful LS measurement and satisfactory liver biopsy specimens [81]. The AUROCs of TE for F≥3 and F4 were 0.93 and 0.95, respectively. At a cut-off value of 7.9 kPa, the sensitivity, specificity, and positive and negative predictive values for F≥3 were 91%, 75%, 52%, and 97%, respectively. LS was not affected by hepatic steatosis, necroinflammation or body mass index. Discordance of at least two stages between TE and histology was observed in 33 (13.4%) patients. By multivariate analysis, liver biopsy length less than 20 mm and F0-2 disease were associated with discordance [81].

In an Indian study in NAFLD patients, LSM significantly correlated with fibrosis ($r = 0.68$, $p < 0.001$). The areas under receiver-operating characteristics (AUROC) curve of LSM for F ≥1, F≥2,F ≥3, and F4 were 0.82, 0.85, 0.94, and 0.96, respectively, while the best LSM cut-offs were 6.1, 7.0, 9.0, and 11.8 kPa, respectively. The negative predictive value of LSM for excluding advanced fibrosis was 95% [82].

In a ***meta-analysis*** published in 2014, including 856 NAFLD patients, evaluated with the M probe, TE proved to have good diagnostic accuracy to diagnose F≥3 (Se 85%; Sp 82%) and F4 (Se 92%; Sp, 92%) and only moderate accuracy for F≥2 (Se 79%; Sp 75%) [83].

A new technique, related to TE and performed with a FibroScan® device is the ***Controlled Attenuation Parameter*** (***CAP***) and it enables steatosis quantification in fatty liver (Fig. **6**). This parameter is an estimate of the total ultrasonic attenuation (go-and-return path) at the central frequency of the regular or M probe of the FibroScan® (3.5 MHz) and is expressed in decibel per meter (dB/m, range 100-400 dB/m) [84]. CAP was first validated as an estimate of ultrasonic attenuation at 3.5 MHz using Field II simulations and tissue-mimicking phantoms. Performance of the CAP was then evaluated on 115 patients, taking the

histological grade of steatosis as reference. CAP was significantly correlated to steatosis (Spearman $\rho=0.81$, $p<0.00001$). AUROCs for the detection of >10% and >33% steatosis were 0.91 and 0.95 respectively [84]. Recently, CAP was evaluated also on the XL probe in a cohort of 59 patients. The AUROCs to detect >2% and >16% liver fat were 0.83/0.84 and 0.92/0.91 for the M/XL probes, respectively [85].

Fig. (6). Transient Elastography and CAP measurement.

Several studies were published regarding CAP accuracy for diagnosing steatosis severity. In a study performed on 440 patients, in which CAP was compared with liver biopsy, the AUROCs of CAP for the diagnosis of steatosis >10% (S1), steatosis >33% (S2), and steatosis >66% (S3), were 0.79, 0.84, and 0.84, respectively. By multivariate analysis, factors significantly associated with elevated CAP were BMI 25-30 kg/m^2, BMI >30 kg/m^2, metabolic syndrome,

alcohol >14 drink/week and liver stiffness >6kPa [86]. In a smaller study on 261 patients, at a cut-off value of 310 dB/m, CAP had 79% the sensitivity, 71% specificity, 86% positive and 71% negative predictive values for ≥ S2 steatosis [87]. In a Romanian study on 201 patients, steatosis was the only histopathological factor independently influencing CAP. Maximal diagnostic accuracy was obtained for the prediction of ≥S2 and S3 (82.06% and 81.59%, respectively), for cut-off values of 285 and 294 dB/m, while for the prediction of S1, the accuracy reached only 76.11% (cut-off 260 dB7M) [88].

In a ***meta-analysis*** assessing the CAP accuracy for steatosis detection, the median optimal CAP cut-off values for ≥S1, ≥S2 and S3, were 232.5 dB/m, 255 dB/m and 290 dB/m respectively, and the summarized sensitivity and specificity values were 0.78 and 0.79 for ≥S1, 0.85 and 0.79 for ≥S2, and 0.83 and 0.79 for S3 [89].

Starting from these data and considering that diabetic patients have a high prevalence of NAFLD and advanced fibrosis, screening strategies for steatosis and fibrosis have been proposed in diabetics, with promising results [90]. International guidelines also recommend non-invasive assessment including serum biomarkers or TE as first line procedure for the identification of patients at low risk of severe fibrosis/ cirrhosis in NAFLD patients [2]. Also follow-up assessment by either serum biomarkers or TE for progression of liver fibrosis should be performed among NAFLD patients at a 3 year interval [2].

Regarding TE evaluation in patients with ***alcoholic liver disease(ALD)***, one must consider that in most of these patients, inflammation coexists with fibrosis and steatosis and it can influence the results of LS measurements, as showed above. Higher cut-off values for cirrhosis were reported in patients with ALD, than in those with viral hepatitis: 19.5 kPa in the study by Nguyen-Khac *et al.* [91] and 22.6 kPa in the Nahon study [92], but the patients included in those studies had high ALT levels that were not taken into consideration. In a study by Mueller *et al.* [93], LS was evaluated by TE in a learning cohort of 50 patients with ALD, admitted for alcohol detoxification, before and after normalization of serum aminotransferases. LS decreased in almost all patients, within a mean observation interval of 5.3 days. Of the serum aminotransferases, the decrease in LS correlated best with the decrease in glutamic oxaloacetic transaminase (GOT). No significant

changes in LS were observed below GOT levels of 100 U/L. In the study cohort of 101 patients with histological confirmed ASH, LS was measured only in patients with GOT >100 U/L at the time of LS assessment. In this group, the AUROC for cirrhosis detection by FS improved from 0.921 to 0.945 while specificity increased from 80% to 90%, at a sensitivity of 96%. A similar AUROC was obtained for lower F3 fibrosis stage, if LS measurements were restricted to patients with GOT <50 U/L. The conclusion of this study was that postponing cirrhosis assessment by TE, during alcohol withdrawal, until GOT decreases to <100 U/mL, significantly improves the diagnostic accuracy [93].

In a recently published ***meta-analysis*** the optimal cut-off values for the prediction of each fibrosis stage in alcoholic liver disease could not be established, due to the large variability of published cut-offs, which is probably due to the presence of inflammation as assessed by elevated aminotransferases [94]. However, this meta-analysis suggests that TE may be used to rule out severe fibrosis or cirrhosis using cut-offs of 9.5 and 12.5 kPa, respectively, but with caution to the risk of overestimation in patients that are continuing alcohol consumption.

5. TE FOR THE DIAGNOSIS OF LIVER CIRRHOSIS

If the performances of TE for the differentiation of mild from significant fibrosis are only moderate, its real value is for the diagnosis of cirrhosis. Data from 9 studies were evaluated by Talwalkar *et al.* [42] showing that TE has an 87% pooled sensitivity [95% confidence interval (CI): 84–90%)] and 91% pooled specificity (95% CI: 89–92%) for the diagnosis of cirrhosis. In a ***meta-analysis*** on 50 studies, the mean AUROCs for the diagnosis of significant fibrosis, severe fibrosis, and cirrhosis were 0.84, 0.89, and 0.94, respectively [43]. Another meta-analysis from 2010 [95] evaluated 22 published papers. For a cut-off value of 15.08 kPa, it showed a pooled sensitivity of 84.45% (95% CI: 84.2-84.7%) with a pooled specificity of 94.69% (95% CI: 94.3%-95%). Finally, in a recently published meta-analysis which included 40 studies, the summary sensitivity and specificity of TE for diagnosing cirrhosis were 0.83 (95% CI: 0.79-0.86) and 0.89 (95% CI: 0.87-0.91), respectively [50]. The mean optimal cut-off was 15±4.1 kPa.

Different ***cut-off values*** for the diagnosis of cirrhosis were proposed for different

etiologies: 12.5 kPa in HCV infection [44], 13.4 kPa in HBV infection [60], 10.3 kPa in NAFLD [81], 22.4 kPa in ASH [93], 17.3 kPa in cholestatic chronic diseases (primary biliary cirrhosis and primary sclerosing cholangitis) [72].

All elastography guidelines state the high diagnostic accuracy of TE for the diagnostic of liver cirrhosis [2 - 5]. According to the EASL Guidelines on Elastography, TE is a reliable method for the diagnosis of cirrhosis in patients which performs better at ruling out than ruling in cirrhosis (NPV> 90%) [2]. Also, the same guideline states that even if TE and biologic tests have similar performances to diagnose F≥2, TE is significantly better for diagnosing cirrhosis.

6. TE FOR THE DIAGNOSIS OF CIRRHOSIS COMPLICATIONS

The advantage of FibroScan® evaluation of liver fibrosis on other non-invasive methods, is that transient elastography can also assess the severity of cirrhosis (values up to 75 kPa), as shown in some studies, which proposed cut-off values of LS that predict the presence of cirrhosis complications (esophageal varices, variceal bleeding, vascular decompensation or hepatocellular carcinoma).

Esophageal varices and upper digestive hemorrhage are feared complications of cirrhosis. The hemorrhage risk depends on the varices' size so that primary prevention of variceal bleeding should be applied to patients with large EV (grade 2 or 3) diagnosis established by periodical upper digestive endoscopy (Baveno V and AASLD Consensuses) [96, 97]. Such a screening program of periodical gastroscopy in all cirrhotics would be very expensive, and repeated endoscopies are poorly accepted by the patients. Published studies demonstrated that LS values <19 kPa are highly predictive for the absence of significant EV (≥ grade 2) [98]. Cut-off values for at least grade 2 EV range from 27.5 [98] to 47.2 kPa [99], while for esophageal bleeding, one study reported a cut-off value of 62.7kPa [100]. In a study from 2009, performed on 298 HCV patients (70 with cirrhosis; 25 with EV), Castera concluded that TE cannot replace upper endoscopy for EV diagnosis, even if it predicted their presence with 76% sensitivity and 78% specificity [101].

Nguyen-Khac *et al.* demonstrated that there are different cut-off LS values for predicting at least grade 2 EV, according to the etiology of cirrhosis [100]. The cut-offs for predicting significant EV were: 47.2 kPa in alcoholic cirrhosis (84.6%

sensitivity, 63.6% specificity, 44% positive predictive value and 92.5% negative predictive value, AUROC=0.77) and 19.8 kPa in cirrhotic patients with viral etiology (88.9% sensitivity, 55.1% specificity, 26.7% positive predictive value, and 96.4% negative predictive value, AUROC=0.73). Similar results were obtained by Vizzutti *et al*. [102].

Portal hypertension is best assessed by measuring the hepatic venous pressure gradient (HVPG), an invasive procedure. In an Italian study on 61 patients, LS cut-off values of 13.6 kPa and 17.6 kPa predicted significant HVPG of ≥10 and ≥12 mm Hg, with 97% and 94% sensitivity (AUROCs 0.99 and 0.92, respectively). For predicting the presence of EV, the cut-off was 17.6 kPa, with 90% sensitivity (AUROC 0.76) [103].

The correlation between LS by TE and HVPG was also assessed in a French study on 150 patients [105]. For a cut-off of 21 kPa, TE accurately predicted significant portal hypertension (HVPG > 10 mmHg AUROC 0.945).

Robic *et al.* compared LS measurement by TE to HVPG, as predictors of cirrhosis complications. One hundred patients with chronic liver disease were evaluated in the same session by TE and HVPG measurements and followed-up for 2 years. HVPG and LS measurements showed similar performances for predicting portal hypertension: AUROCs 0.830 *vs.* 0.845. All patients with LS lower than the 21.1 kPa cut-off value remained free of portal hypertension complications during the 2 years follow-up, as compared to 47.5% of those with higher values. The performances of LS and HVPG were similar also in the cirrhotic subgroup of patients [104].

Reiberger *et al.* performed a study on 122 cirrhotics with EV who were evaluated by means of TE and HVPG. There was a better correlation of LS values assessed by TE and HVPG in patients with HVPG ≤12 mmHg than in those with HVPG >12 mmHg (r=0.951*vs.* r=0.538). Also, the authors observed an improvement in the correlation of LS with HVPG under beta-blockers, mainly in hemodynamic responders (r=0.864), but not in non-responders (r=0.535), while changes of blood pressure, heart rate and LS were similar in responders *vs.* non-responders. For discriminating cirrhotic patients with at least grade 2 EV, from those with grade 1

EV, for a cut-off value of 47.5 kPa, LS had 80.6% sensitivity and 47.7% specificity [105].

In a review published in 2011, Castera concluded that "diagnostic performances of TE are acceptable for the prediction of clinically significant portal hypertension, but far from satisfactory to confidently predict the presence of OV in clinical practice and to screen cirrhotic patients without endoscopy" [106]. But all the studies included in this review evaluated only small numbers of patients (ranging from 47 to 211), with contradicting results (cut-off values for significant EV ranging from 19.8 to 48 kPa, and AUROCs ranging from 0.73 to 0.87).

In a study published by our group [107], not available for the Castera review, including 1000 consecutive cirrhotic patients, we found out that negative and positive predictive values (NPV and PPV) for at least grade 2 EV were 76.2% and 71.3%, respectively, for a cut-off value of 31 kPa, chosen to maximize the sum of sensitivity and specificity. For >40 kPa criterion, chosen to have a PPV of more than 85%, the sensitivity was 77.8%, the specificity 68.3%, with 86% PPV and 55% NPV (95%CI: 49.60–60.23). We also searched for a cut-off value as close as possible to a NPV of 90%, and we found out that for 17.1 kPa, the NPV was 89.3%, with 43.2% PPV, 92.6% sensitivity and 33.5% specificity (AUROC 0.7807). So, according to our data, at least 8 out of 10 patients with TE values >40 kPa will have significant portal hypertension, therefore it seems reasonable to recommend prophylactic beta-blocker therapy in these patients, without endoscopy. Similarly, 5 out of 10 patients with TE values <40 kPa will have significant EV (NPV 54.9%), and in these cases we recommend endoscopic evaluation. In patients with LS <17.1 kPa, we cannot recommend endoscopic evaluation, since they have only 1 in 10 risk to present significant EV (NPV 89.3%).

In our study group, we also observed that the mean LS value in patients with a history of variceal bleeding was significantly higher than in those with no bleeding history: 51.92±1.56 *vs.* 35.20±0.91kPa, $p<0.0001$. For a cut-off value of 50.7 kPa, LS had 53.33% sensitivity and 82.67% specificity, with 82.71% PPV and 53.66% NPV (AUROC 0.7300, $p<0.0001$) for predicting esophageal bleeding [107].

A ***meta-analysis*** regarding TE and portal hypertension (PH), that included more than 3,500 patients was published in 2013 [108]. It showed that TE had a 0.90 summary Se and a 0.79 summary Sp (AUROC=0.93) for predicting HVPG≥10 mmHg, a 0.87 summary Se and a 0.53 summary Sp (AUROC=0.84) for predicting the occurrence of any EV, and a 0.86 summary Se and a 0.59 summary Sp (AUROC=0.78) for predicting significant (grade 2 and 3) EV. The conclusion was that, due to the low specificity of this method, TE cannot replace upper gastrointestinal endoscopy for EV screening [108]. The same recommendation is maintained in international guidelines regarding elastography, but mentioning the fact that TE can be used to stratify patients at risk for PH [2 - 5].

Hepatocellular carcinoma (HCC) is another feared complication of cirrhosis, being one of the most common causes of death in these patients. Several studies assessed the predictive value of LS by TE for the presence of HCC. In a study by Foucher *et al.*, the cut-off values for the presence of grade 2/3 EV, cirrhosis Child-Pugh B or C, past history of ascites, HCC, and esophageal bleeding were 27.5, 37.5, 49.1, 53.7, and 62.7 kPa, respectively [99]. In a Japanese study LS values in patients with HCC were significantly higher than in those without HCC (24.9±19.5 kPa *vs.* 10.9±8.4 kPa; P<0.0001). Multivariate analysis identified LS ≥12.5 kPa, age ≥60 years, and serum total bilirubin ≥1.0 mg/dL, as significantly correlated with development of HCC [109]. These data were similar to the ones from another Japanese study, that proved a significant increase in the risk of developing HCC that paralleled the increase of LS values, from 16.7 folds when LS was 10.1-15 kPa, to 20.9 folds when LS was 15.1-20 kPa, to 25.6 folds when LS was 20.1-25 kPa, and to 45.5 folds when LS was >25 kPa, as compared to patients with LS values<10 kPa [110].

In a study performed in HBV patients, Jung demonstrated a stepwise increased risk of developing HCC in patients with higher LS values: LS 8.1–13 kPa, HR, 3.07; LS 13.1–18 kPa, HR, 4.68; LS 18.1–23 kPa, HR, 5.55 and LS >23 kPa, HR, 6.60 [111].

Several authors tried to develop risk scores for HCC in cirrhotics, including LSM with promising results [112 - 114], so that the EASL Guidelines on Elastography state that: “although TE could be useful to identify patients at risk of developing

HCC, more data are needed before it can be integrated into an HCC surveillance program" [2]

7. TE IN TRANSPLANTED PATIENTS

It is a known fact that recurrence of HCV infection is a rule in transplanted patients, with cirrhosis developing in a few years. Several studies proved that TE could be a valuable tool for assessing the severity of recurrent HCV hepatitis, following liver transplantation, reducing the need for follow-up liver biopsies [115 - 121]. Carrion *et al.* evaluated 124 transplanted HCV patients, who underwent 169 liver biopsies and LS measurements by TE. For a cut-off value of 8.5 kPa, TE had 90% sensitivity, 81% specificity, 79% negative predictive value, and 92% positive predictive value for diagnosis of fibrosis F≥2, with AUROC 0.90, while for F4 the AUROC was 0.98 [115]. Another study that evaluated 95 transplanted HCV patients by means of paired liver biopsies and TE, showed that LS changed in parallel with grading (r=0.63) and staging (r=0.71), with good sensitivity (86%) and specificity (92%) in predicting staging increases [116].

In a systematic review published in 2010, Cholongitas *et al.* showed that TE had a good discrimination power for significant fibrosis in transplanted patients (median AUROC: 0.88, median sensitivity 0.86, median NPV 0.90 and median PPV 0.8) [121]. In a recent meta-analysis, the pooled data of 5 studies that estimated at least F2 in transplant HCV patients were 83% for sensitivity and specificity, 4.95 for the positive likelihood ratio, 0.17 for the negative likelihood ratio, and 30.5 for the diagnostic odds ratio. Five studies assessed cirrhosis, and their pooled estimates were 98% for sensitivity, 84% for specificity, 7 for the positive likelihood ratio, 0.06 for the negative likelihood ratio, and 130 for the diagnostic odds ratio [122].

As demonstrated above, TE reliably predicts severity of recurrent HCV hepatitis following liver transplantation, but its accuracy in non-viral liver graft damage is unknown. Rigamontti *et al.* evaluated 69 transplant recipients (37 hepatitis B/D recurrence-free, 20 autoimmune/cholestatic liver disease, 6 alcoholic liver disease and 6 mixed) by means of both protocol or on demand liver biopsy and concomitant TE. 94% of patients had reliable TE examinations during post-transplant follow-up (median 18 months, range 7-251). Liver biopsy showed graft

damage in 43% (28) patients. LS values were significantly higher in patients with graft damage as compared to the ones without (median 7.8 kPa *vs.* 5.3 kPa, $p<0.0001$). By ROC curve analysis, TE cut-off for the diagnosis of graft damage with 100% sensitivity was 5.3 kPa, while 100% specificity was obtained by a 7.4 kPa cut-off. Thus, only patients with LS values ranging from 5.3 to 7.4 kPa should undergo liver biopsy to assess graft damage [123].

More recently published studies confirmed the value of TE to: predict fibrosis severity in transplanted patients, with or without HCV infection recurrence [124], to assess fibrosis severity in those with HCV infection recurrence in order to stratify them for antiviral treatment [125], but also to evaluate the severity of acute rejection [126].

8. TE IN CHILDREN

One of the first studies regarding TE in children was the one published by de Ledinghen *et al.* [127], which evaluated the feasibility and performance of TE as compared to FibroTest, APRI and LB for fibrosis assessment in pediatric patients. One hundred and sixteen consecutive children with various liver diseases were evaluated, and only in one TE was not feasible. TE showed the best correlation to clinical and biological severity parameters. Also, TE was significantly correlated with the Metavir fibrosis score. The AUROCs of TE, FibroTest and APRI for predicting cirrhosis were 0.88, 0.73 and 0.73, respectively.

Nobili *et al.* evaluated 52 consecutive NASH pediatric patients by means of LB and TE [128]. Even if an adult probe was used and most patients were overweight and obese, TE proved to be a highly feasible (96% of patients with reliable measurements) and highly reproducible (intraclass correlation coefficient 0.961) method in children. The AUROCs for prediction of "any" (>1), significant (>2), or advanced fibrosis (>3) were 0.977, 0.992, and 1, for cut-offs <5, <7, and <9 kPa, respectively.

TE was also evaluated as a predictor of hepatic fibrosis in children with cystic fibrosis. A good correlation of liver stiffness measurements with histological fibrosis was found to exist in those patients [129, 130].

Also TE was evaluated as a predictor of portal hypertension in children, but only in small studies, and no threshold could be established, even if stiffness values were significantly higher in children with esophageal varices [129, 131, 132].

9. TE AS COMPARED TO OTHER NON-INVASIVE MARKERS OF FIBROSIS

Several studies compared TE to fibrosis biomarkers for fibrosis assessment in chronic viral hepatitis. In a French multicenter prospective study (Fibrostic) [133], performed in 23 French university hospitals, 1307 subjects were evaluated by means of TE, biomarkers and liver biopsy, and the authors found out that the accuracy of FibroScan® in predicting cirrhosis was high (0.90), higher than that of biomarkers (0.77-0.86). On the other hand, this study showed that the performance of all the non-invasive methods for predicting significant fibrosis (F≥2) was moderate to poor (AUROC 0.72-0.78). This study evaluated a heterogeneous group of patients with HCV chronic hepatitis and also with HCV and HIV co-infection, with better results in HCV chronic hepatitis. Also, this study showed that in 21.3% of the cases evaluated by means of TE, valid results could not be obtained [measurements with Success Rate (SR) <60% and/or Interquartile Range (IQR) ≥30%] [133].

Combining FibroScan® with serum fibrosis markers [44] or with Acoustic Radiation Force Impulse Elastography (ARFI) [134], can further improve the diagnostic accuracy of non-invasive liver fibrosis measurement.

But, we must underline that TE (FibroScan®) has some disadvantages for the assessment of liver fibrosis: measurement failure in patients with ascites, valid measurement can be obtained only in approximately 80% of cases [6], impossibility to discriminate between contiguous narrow stages of fibrosis, false overestimated results during ALT flares, multiple factors influencing LS besides fibrosis [135], and last but not least, the high cost of the FibroScan® machine. Despite these limitations, TE is used in daily practice in many centers in different countries (from Europe, Asia, Canada) and the results are taken into consideration for prognosis assessment and therapeutic decisions. The EASL Guidelines on Elastography state that even if TE and biologic tests have similar performance to

diagnose F≥2, TE is significantly better to diagnose cirrhosis [2].

In conclusion, TE is the most used elastographic method in daily practice. This method is validated by multiple guidelines. The body of evidence regarding this method is huge. Using all types of probes, this method can be used in adults (including severely obese patients) and the pediatric population. This method's good reproducibility, its usefulness in almost all types of hepatic diseases ('apart from patients with ascites, obstructive jaundice, severe heart failure) have made it the most used worldwide.

Main advantages and weaknesses of liver fibrosis evaluation by means of TE

Advantages	Weaknesses
- reproducible method - good results for non-invasive liver fibrosis evaluation in patients with chronic hepatitis B and C, especially for detecting patients with severe fibrosis and liver cirrhosis - promising results for non-invasive liver fibrosis evaluation in patients with chronic liver diseases other than viral - promising results for predicting liver cirrhosis complications - technical parameters IQR and SR available in real time, automatically calculated by the device's software	- expensive equipment - not feasible in patients with ascites - influenced by an elevated aminotransferases level - increased number of unreliable measurements in patients with high BMI, partially corrected by the introduction of XL probe - not very accurate for differentiating patients without fibrosis and those with mild fibrosis and patients with moderate *vs.* mild fibrosis

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare for this publication.

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CHAPTER 2

Point Shear Wave Elastography

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Abstract: VTQ **(**ARFI) elastography is a new method developed in the last 5-6 years for the non-invasive evaluation of liver fibrosis, integrated into a Siemens Acuson ultrasound system. Ten valid measurements are performed in the right liver lobe, a median value is calculated and the result is expressed in meters/second. The AUROC's range between 0.75-0.85 for predicting significant fibrosis and for predicting cirrhosis between 0.85-0.95. To increase the accuracy of liver cirrhosis diagnosis, the spleen stiffness (SS) assessed by VTQ (ARFI) can be used. VTQ (ARFI) it is a reproducible method (intraclass correlation coefficient ranging from 0.81-0.87), especially in patients with severe fibrosis and cirrhosis. Similar with Transient Elastography (TE), elevated levels of aminotransferases are associated with the increase of liver stiffness (LS) values assessed by VTQ (ARFI). Even if the manufacturer did not recommend the use of technical parameters IQR (interquartile range interval) and SR (success rate) well-known from TE, published data proved that the accuracy of the method significantly increased with the use of these quality parameters. Regarding the prediction of liver cirrhosis complications, especially portal-hypertension, data regarding the usefulness of LS and/or SS are not so solid, but VTQ (ARFI) accuracy can be increased by combining different parameters.

ElastPQ is a newly developed point Shear Waves elastographic method. Only few data, but with promising results, were published until now regarding this technique.

Keywords: ARFI elastography, Chronic hepatitis, ElastPQ, Liver cirrhosis, Liver stiffness, Portal hypertension, VTQ.

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II.A. ACOUSTIC RADIATION FORCE IMPULSE (ARFI) ELASTOGRAPHY

1. VTQ (ARFI) Elastography Technique

Virtual Touch™ Quantification (VTQ) uses Acoustic Radiation Force Impulse (ARFI) technology in a Siemens Acuson S2000TM ultrasound system (Siemens AG, Erlangen, Germany) with 4C1 and 4V1 transducers to evaluate the elastic properties of a targeted anatomical region with the use of a region of interest (ROI) cursor, while performing real-time B-mode imaging.

The principle of VTQ (ARFI) elastography is that compression of the examined tissue induces a strain into the tissues. The ultrasound probe automatically produces an acoustic "push" pulse that generates shear-waves which propagate into the tissue, perpendicular to the "push" axis. The speed of the shear-waves, measured in meters/second (m/s), is displayed on the screen. The highest theoretically reachable velocity in the hardest medium corresponds to approximately 6 m/s. The propagation speed increases with tissue stiffness, thus with fibrosis severity. Shear wave speed may be quantified, in a precise anatomical region, focused on a region of interest, with a predefined size, provided by the system. Speed measurement value and depth are reported and the results of the elasticity are given in meters/second (m/s) [1, 2].

The operator can select the depth at which liver elasticity is evaluated, by placing a "measuring box" (10/5 mm) in the desired place (Fig. **1**). Scanning is performed between the ribs in the right liver lobe (*e.g.* segment 8 or 5) (in order to avoid cardiac motion), approximately in the place where a liver biopsy is usually performed, 1-2 cm under the capsule, with minimal scanning pressure applied by the operator, while the patient is asked to stop breathing for a moment, in order to minimize breathing motion. Usually, 10 valid measurements are performed and a median value is calculated (expressed in m/s). If the measurement is not reliable "X-X-X" is displayed on the screen.

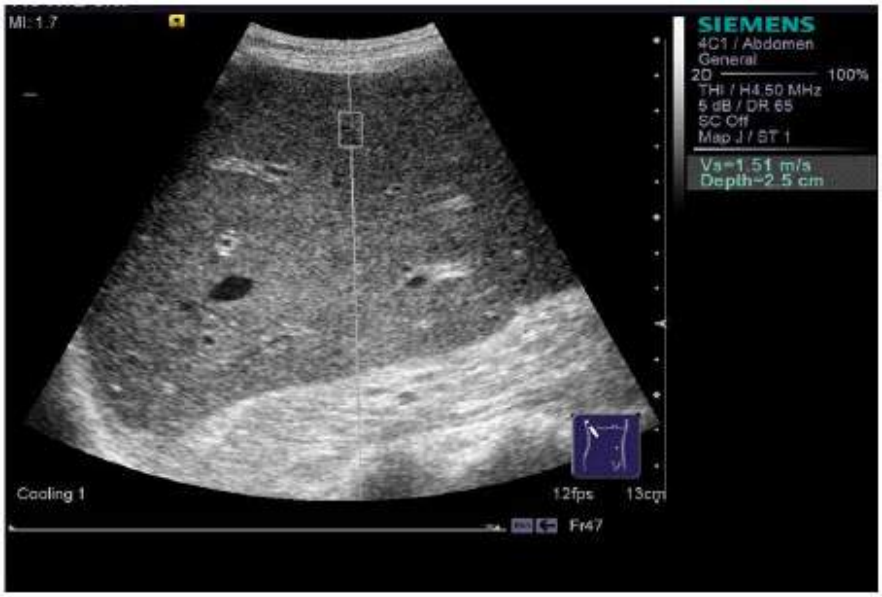

Fig. (1). VTQ (ARFI measurement).

Our study published in 2011 [3] showed that the best correlation with histological fibrosis was observed for measurements made 1-2 cm and 2-3 cm under the liver capsule (0.675 and 0.714, respectively), but in up to 15% of cases, valid measurements could not be obtained for profound measurements (2-3 cm). Another study [4] showed that VTQ (ARFI) assessments with the lowest rate of invalid measurements are obtained by an intercostal approach to segments VII/VIII of the liver, while our study [5] demonstrated that similar VTQ (ARFI) values are obtained in segments VIII and V of the liver.

The device's manufacturer did not made specific recommendations regarding the technique that should be used for liver fibrosis evaluation in children.

2. Reproducibility of VTQ (ARFI) and Factors which Influence the Correlation of Liver Stiffness with Fibrosis

Non-invasive methods for liver fibrosis evaluation should have a good diagnostic accuracy and must be reproducible in order to be used in clinical practice. Also, it is imperative to know which factors influence the correlation of liver stiffness (LS) assessed by VTQ (ARFI) with fibrosis.

a. VTQ (ARFI) Reproducibility

The VTQ (ARFI) inter-operator agreement was evaluated by Friedrich-Rust *et al.* [6] in a cohort of 61 patients with chronic hepatopathies. The authors used the following cut-off values for liver fibrosis staging: F≥2 - 1.37 m/s; F≥3 - 1.45 m/s; F4 - 1.75 m/s. There was an 87% agreement between repeated VTQ (ARFI) measurements, for different stages of fibrosis. For differentiation between patients with at least significant fibrosis (F≥2) from those with F<2, the inter-operator agreement was 90%.

Another study showed a very good inter-operator agreement (intraclass correlation coefficient - ICC=0.84 in a cohort of 50 patients with different etiologies of chronic hepatopathies [7].

In a study by Piscaglia *et al.* the correlation of VTQ (ARFI) values obtained by 2 operators was also very good (Spearman r correlation coefficient=0.874, p<0.0001) [8]. Other two studies showed similar results [9, 10].

Guzman-Aroca *et al.* assessed ARFI reproducibility in 50 healthy volunteers. The inter-operator agreement was very good (ICC=0.86) [11]. A nonsignificant negative correlation was observed between VTQ (ARFI) measurements and age, sex and body mass index (BMI). Similar results (ICC=0.87) were obtained by D'Onofrio *et al.* [12].

Our group showed an excellent intra- and inter-operator agreement for VTQ (ARFI) measurements: ICC - 0.90 *vs.* ICC - 0.81 [13]. For both intra and inter-operator reproducibility, the ICC's were smaller in women *vs.* men (0.88 *vs.* 0.91 and 0.67 *vs.* 0.86 respectively), in patients with high BMI (≥ 25 kg/m^2) *vs.* BMI <25 kg/m^2 (0.88 *vs.* 0.91 and 0.79 *vs.* 0.82, respectively), in patients with ascites *vs.* no ascites (0.80 *vs.* 0.93 and 0.78 *vs.* 0.84, respectively) and in non-cirrhotic *vs.* cirrhotic patients (0.77 *vs.* 0.82 and 0.70 *vs.* 0.83, respectively).

b. Factors which Influence the Correlation of LS Values Assessed by VTQ (ARFI) with Fibrosis

In a study published by our group [5], which included 471 subjects, the factors which can influence the VTQ (ARFI) accuracy for assessment of liver fibrosis

were studied: the technical parameters, namely IQR (interquartile range interval, defined as the difference between the 75th and the 25th percentile, essentially the range of middle 50% of the data) and SR (success rate, defined as the ratio of successful acquisitions over the total number of acquisitions); the location of VTQ (ARFI) measurements; the quality of the liver biopsy specimen; and the presence of liver steatosis. A direct, strong correlation (r=0.694) was observed between VTQ (ARFI) measurements and fibrosis severity (p<0.0001). In patients in whom the quality parameters for VTQ (ARFI) measurements were fulfilled (IQR<30% and SR≥60% - 415 patients), there was a very strong correlation with fibrosis (r=0.722, p<0.0001), while in patients with unsatisfactory technical parameters (SR<60% and/or IQR≥30%) there was no correlation between LS measurements by means of VTQ (ARFI) and histological fibrosis (r=0.268, p=0.07) (p=0.0001). High BMI was associated with the measurements with unsatisfactory technical parameters. The correlations between VTQ (ARFI) measurements and fibrosis were similar in segments V *vs.* VIII (r=0.836, p<0.0001 *vs.* r=0.784, p<0.0001) (p=0.33). LS values assessed by VTQ (ARFI) were correlated with histological fibrosis in patients with no or mild steatosis (Hepburn I, II and III) (r=0.535 p<0.0001), while in patients with moderate and severe steatosis (Hepburn IV and V) there was no significant correlation (r=0.223, p=0.48). We found no significant differences between the correlations of LS values with fibrosis stage according to the length of the liver specimen: >3 cm, *vs.* those 2-3 cm long (r=0.456, p=0.01 *vs.* r=0.480, p=0.002; p=0.89). It should be mentioned that in our study all specimens were longer than 2 cm.

A recently published study also obtained similar LS values in segment V/VIII *vs.* VIII when measured in supine position, but the values obtained in segment VIII in semidecubitus were significantly higher than those obtained in supine position [14].

D'Onofrio *et al.* compared the LS values obtained deep in the right lobe *vs.* the ones obtained immediately underneath the surface of the right lobe (1.56 *vs.* 1.90 m/s) and between the mean values obtained deep in the right lobe *vs.* those obtained deep in the left lobe (1.56 *vs.* 1.84 m/s). In both cases the differences were significant [12]. Piscaglia *et al.* [8], Karlas *et al.* [15] and Toshima *et al.* [16] obtained also significantly higher LS values assessed by VTQ (ARFI) in the left

as compared with the right liver lobe.

Another factor which can influence VTQ (ARFI) accuracy are elevated aminotransferases, which has also been demonstrated to influence LS values assessed by Transient Elastography [17 - 19].

Karlas *et al.* [20] evaluated by means of VTQ (ARFI) 3 patients with acute liver failure, 21 patients with liver fibrosis and 30 healthy controls. ARFI values in patients with acute liver failure were similar with those observed in patients with liver fibrosis, so the authors concluded that high aminotransferases values increase ARFI values.

Kuroda *et al.* [21] published a case report regarding the influence of liver functional tests on LS assessed by VTQ (ARFI) in a patient with acute liver failure. At admission, VTQ (ARFI) values were very high (3.6 m/s), similar to those observed in cirrhotic patients and they decreased in parallel with the improvement of liver function (down to 1.6 m/s 39 days after admission).

Another study evaluated 250 patients with chronic liver disease by liver biopsy and VTQ (ARFI) [22]. The optimum cut-off values for VTQ (ARFI) were 1.13 m/s for F≥2 and 1.98 m/s for F4. The optimum cut-off values decreased to 1.09 m/s for F≥2 and to 1.81 m/s for F4 when only patients with normal alanine aminotransferase (131) were selected.

We conducted an international multicenter study which evaluated the influence of aminotransferases level on VTQ (ARFI) measurements in a cohort of 1242 patients [23]. The mean LS values assessed by VTQ (ARFI) for the same stage of histological fibrosis increased with the ALT level. The optimum VTQ (ARFI) cut-off value to predict liver cirrhosis (F4) was 1.57 m/s (AUROC=0.856). The cut-offs for predicting significant fibrosis (F≥2) and cirrhosis (F=4) in the groups with normal ALT, ALT=1.1-5 x upper limit of normal (ULN) and >5 x ULN were: 1.29 m/s, 1.36 m/s, 1.44 m/s for F≥2 and respectively 1.59 m/s, 1.57 m/s, 1.75 m/s for F=4. In a subgroup of 512 patients in whom TE was also available, VTQ (ARFI) was the least influenced by increased ALT values between 1.1-5 x ULN.

Our group also demonstrated that LS values by VTQ (ARFI) significantly increase after food intake (similar with TE) and for this reason it is recommended to perform measurements in fasting condition or at least 3-4 hours after the last meal [24].

Other factors which influence the accuracy of VTQ (ARFI) for liver fibrosis evaluation are: right heart failure [25] and the presence of extrahepatic cholestasis [26].

In conclusion, VTQ (ARFI) is a reproducible non-invasive method for liver fibrosis assessment. In order to have a better accuracy, similar with TE, technical parameters IQR and SR should be used. Also, measurements should be performed in the right liver lobe. Non-fasting condition, elevated aminotransferases levels, right heart failure, extrahepatic cholestasis and moderate/severe steatosis are associated with a lower accuracy of VTQ (ARFI) for fibrosis evaluation.

3. Liver Stiffness Assessed by VTQ (ARFI) in Healthy Volunteers

In order to use non-invasive methods for liver fibrosis evaluation in patients with chronic hepatopathies, we should know their normal range. The first data regarding normal LS values as assessed by VTQ (ARFI) were published by Gallotti *et al.* in 35 young healthy volunteers [27]. In this study, the mean LS value assessed by VTQ (ARFI) was 1.59 m/s, significantly higher than that obtained by Goertz *et al.* in a healthy control group (1.16±0.11 m/s) [28]. In the study of Goertz *et al.* VTQ (ARFI) values were not significantly correlated with gender, age, height, weight or body mass index (BMI) [28].

Our study evaluated 82 healthy subjects [29]. Reliable LS measurements (10 valid measurements with an IQR ≥ 30% and/or SR <60%) were obtained in 76 (92.6%) subjects, which were included in the final analysis. The mean LS value determined by VTQ (ARFI) was 1.15±0.21 m/s (range: 0.69-1.6 m/s). We did not find significant differences between the mean ARFI values in men *vs.* women, also among different age groups.

Another study evaluated 68 healthy in which the mean LS value was 1.19 (range: 0.77-1.63 m/s) [30]. Age, gender, Valsalva maneuver, the type of ultrasound

probe (4C1 *vs.* 4V1), the intercostal or abdominal approach to liver segment 8 did not influence the LS values obtained by VTQ (ARFI). Only the skin-liver distance significantly influenced the LS values ($p<0.05$). The success rate was highest using the intercostal approach (97.2%).

Son *et al.* evaluated 108 healthy liver and kidney donors [31]. The mean VTQ (ARFI) velocity was 1.07±0.11 m/s (range: 0.79-1.27 m/s). The mean LS values were similar in patients with BMI<23.5 kg/m^2 and in those with BMI≥23.5 kg/m^2. Also, similar with previously presented studies, VTQ (ARFI) measurements were not influenced by age and gender.

In the study of Kim *et al.* 133 healthy subjects were analyzed and the mean VTQ (ARFI) was 1.08±0.15m/s, significantly lower than in patients with chronic liver diseases (1.66±0.60) ($p<0.001$) [32]. The mean LS values assessed by VTQ (ARFI) obtained in normal subjects was similar with the one observed in patients with fatty liver (1.02±0.16 m/s).

Guzman-Aroca *et al.* evaluated by VTQ (ARFI) elastography a cohort of 50 normal subjects using 2 operators [11]. The mean LS values were similar for the 2 operators: 1.03±0.17 m/s *vs.* 1.01±0.17 m/s, p=n.s.

Another study showed that the mean LS values assessed by VTQ (ARFI) in the left liver lobe were significantly higher than those obtained in the right liver lobe: 1.28±0.19 m/s *vs.* 1.15±0.17 m/s, $p<0.001$ [15]. In resting respiratory position, the SR rate was >95% in the right intercostal approach and in the left liver lobe, while in the subcostal approach to the right liver lobe the SR was <75%. After deep inspiration the SR by using subcostal approach increased to 92%, but VTQ (ARFI) values also increased. Higher LS values in the left as compared with the right liver lobe were also observed in the study published by Toshima *et al.* [15].

Rifai *et al.* evaluated by means of VTQ (ARFI) 23 healthy controls, 70 non-cirrhotic patients with chronic liver diseases and 29 patients with liver cirrhosis [33]. The mean LS values assessed by VTQ (ARFI) were significantly lower in normal subjects as compared with those with chronic liver diseases and cirrhotic patients: 1.10±0.17 m/s *vs.* 1.33±0.39 m/s *vs.* 2.92±1.11 m/s ($p<0.001$). Madhok *et al.* evaluated 137 healthy volunteers and obtained a mean value VTQ (ARFI) of

1.19±0.25 m/s [34].

In conclusion, VTQ (ARFI) elastography is feasible in most healthy volunteers, with a very good reproducibility, the mean LS values ranging from 1.05 to 1.19 m/s.

4. Usefulness of VTQ (ARFI) for Liver Fibrosis Assessment in Chronic Hepatopathies

Published data suggested that VTQ (ARFI) and TE have similar predictive value for different stages of histological fibrosis. The first data concerning the value of VTQ (ARFI) was published by Friedrich-Rust *et al.* [6]. A total of 86 patients with chronic hepatitis B and C were evaluated by means of liver biopsy (LB), serological tests (FibroTest and APRI), VTQ (ARFI) and TE. AUROCs for detection of significant fibrosis (F≥2) for ARFI, TE, FibroTest and APRI were 0.86, 0.86, 0.84 and 0.79, while for detection of liver cirrhosis (F=4) they were 0.91, 0.91, 0.82 and 0.76. The LS cut-offs assessed by VTQ (ARFI) for predicting F≥2, F≥3 and cirrhosis (F=4) were: 1.37 m/s, 1.45 m/s and 1.75 m/s respectively.

A study from our group evaluated 114 subjects with and without chronic liver diseases by means of TE and VTQ (ARFI) [3]. A direct, strong correlation was found between VTQ (ARFI) measurements (1-2 cm bellow the liver capsule) and fibrosis ($r=0.675$, $p<0.0001$). LS values obtained by subcapsular measurements showed a poor correlation with fibrosis ($r=0.469$). The best test for predicting F≥2 was TE with AUROC=0.908, significantly higher than the AUROCs for VTQ (ARFI). If only VTQ (ARFI) was considered, measurements made 1 - 2 and 2 - 3 cm below the capsule had the best predictive value, with AUROCs not significantly different from each other (0.767 and 0.731, respectively). According to the measurement depth, the VTQ (ARFI) cut-offs for F≥2, were: 1.4 m/s, AUROC=0.747 (1 - 2 cm), and 1.26 m/s AUROC=0.721 (2 - 3 cm). For predicting cirrhosis, the optimized VTQ (ARFI) cut-offs were: 1.8 m/s, AUROC=0.970 (1-2cm) and 1.78 m/s, AUROC=0.951 (2 - 3 cm underneath the capsule). The conclusion of this study was that the best place to perform LS measurements assessed by VTQ (ARFI) is 1-2 cm underneath the liver capsule.

In another study, our group evaluated 71 patients with chronic hepatitis B and C

by means of LB, VTQ (ARFI) and TE [35]. The correlation of LS values assessed by VTQ (ARFI) and fibrosis was lower than that between TE values and histological fibrosis: r=0.469 *vs.* r=0.707, p<0.0001. By comparing the AUROC curves, TE and VTQ (ARFI) had similar predictive values for the presence of F≥2 (AUROC-ARFI=0.649 *vs.* AUROC-TE=0.731, p=0.47) and also for F=4 (AUROC-ARFI=0.868 *vs.* AUROC-TE= 0.936, p=0.29).

We conducted a bicentric Romanian study, including 223 subjects (38 healthy volunteers, 162 patients with chronic hepatopathies in which LB was performed and 23 with liver cirrhosis – without biopsy) [36]. LS measurements were performed by VTQ (ARFI) and also by TE. The correlation of LS with fibrosis was better for TE as compared with VTQ (ARFI): r=0.870, *vs.* r=0.646, p<0.0001. The optimum LS cut-off assessed by VTQ (ARFI) for predicting significant fibrosis was 1.27 m/s: AUROC=0.890, with 88.7% sensitivity (Se), 67.5% specificity (Sp), 64.5% positive predictive value (PPV) and 90% negative predictive value (NPV). For predicting cirrhosis, the best VTQ (ARFI) cut-off value was 1.7 m/s (AUROC=0.931) with 93% Se, 86.7% Sp, 73.6% PPV and 96.9% NPV.

Takahashi *et al.* evaluated 55 patients with chronic liver diseases by means of VTQ (ARFI) and LB and 25 healthy volunteers by means of VTQ (ARFI) [10]. LS determined by VTQ (ARFI) was correlated with histological liver fibrosis (r=0.800, p<0.0001). The AUROCs for predicting the presence of significant fibrosis, severe fibrosis and cirrhosis were: 0.94, 0.94 and 0.96 respectively. The optimum LS cut-off values assessed by VTQ (ARFI) for predicting different stages of fibrosis were: >1.34 m/s for significant fibrosis (91.4% Se and 80% Sp); >1.44 m/s for severe fibrosis (96.2% Se and 79.3% Sp); and >1.80 m/s for liver cirrhosis (94.1% Se and 86.8% Sp). VTQ (ARFI) values were strongly negatively correlated with albumin (r= - 0.719, p<0.0001), platelet count (r= - 0.657, p<0.0001), prothrombin time (r= - 0.630, p<0.0001), total cholesterol (r= - 0.554, p<0.0001) and positively correlated with aspartate aminotransferase - AST (r=0.649, p<0.0001), type IV collagen (r=0.609, p<0.0001), hyaluronic acid (r=0.575, p<0.0001), glutamyl transpeptidase (r=0.379, p=0.0005), total bilirubin (r=0.294, p=0.009) and alanine aminotransferase - ALT (r=0.292, p=0.008). There was no correlation between VTQ (ARFI) values and BMI.

Goertz *et al.* obtained a significant correlation between LS values by VTQ (ARFI) and histological liver fibrosis ($r=0.64$, $p<0.001$) in a cohort of 57 patients with chronic hepatitis B and C [4]. The AUROCs for predicting $F\geq2$, $F\geq3$ and $F=4$ were 0.85, 0.92 and 0.87 respectively.

Another study included 22 patients with chronic liver diseases evaluated by LB and VTQ (ARFI). An AUROC of 0.85 was calculated for predicting the presence of cirrhosis [37].

Kim *et al.* evaluated whether VTQ (ARFI) provides a better diagnostic performance for the diagnosis of chronic liver disease and if it correlates better with Child-Pugh scores and liver function tests, as compared with an ultrasound scoring system based on visual assessment of conventional B-mode US images by experienced radiologists [32]. The study included 521 patients. The mean VTQ (ARFI) values were compared with US-based scores (assessing liver surface nodularity, parenchyma echo texture and hepatic vein contour) evaluated by two radiologists, Child-Pugh scores and liver function tests. The mean LS values assessed by VTQ (ARFI) elastography were: for the normal liver group 1.08±0.15 m/s; for the fatty liver group 1.02±0.16 m/s; and for the chronic liver disease group 1.66±0.60 m/s ($p<0.001$). The AUROC of VTQ (ARFI) for predicting the presence of chronic liver disease was significantly higher than that of the conventional B-mode US-based scores (0.89 *vs.* 0.74 and 0.77, $p<0.05$), with 75.4% Se and 89.5% Sp for a cut-off value of 1.22 m/s. The mean LS values assessed by VTQ (ARFI) elastography showed higher correlation with Child-Pugh scores than either reviewer's US-based score (0.459 *vs.* 0.342 and 0.333).

An Italian study evaluated 133 patients with chronic liver disease [8]. Ninety of these patients underwent both VTQ (ARFI) and TE and 70 patients assessed with VTQ (ARFI) were submitted also to LB. The best LS cut-off value assessed by VTQ (ARFI) for cirrhosis was then tested in the 70 patients with biopsy. Mean VTQ (ARFI) values in controls, in patients with chronic hepatitis and cirrhosis were 1.13, 1.47 and 2.55 m/s, respectively. The AUROC of LS assessed by VTQ (ARFI) for the diagnosis of cirrhosis (reference TE) was 0.941 with 1.75 m/s as the best cut-off (93.0% Se and 85.1% Sp). VTQ (ARFI) elastography also showed a good performance in patients with bioptic diagnosis of cirrhosis (AUROC

0.908, 81.5% Se and 88.4% Sp).

Another study obtained significantly higher LS values assessed by VTQ (ARFI) in cirrhotic patients as compared with non-cirrhotic chronic liver disease patients and healthy controls: 2.91±1.11 m/s *vs.* 1.33±0.39 *vs.* 1.10±0.17 m/s ($p<0.001$) [33]. The rate of invalid measurements was lower in VTQ (ARFI) than in TE ($p<0.04$). Furthermore, VTQ (ARFI) correlated to histological staging of liver fibrosis ($r=0.54$, $p<0.001$) and to inflammatory activity ($r=0.37$, $p<0.05$). Liver steatosis had no influence on LS values assessed by VTQ (ARFI), but significantly influenced the LS values assessed by TE.

Ebinuma *et al.* evaluated LS in 131 patients with chronic liver disease by VTQ (ARFI), TE and LB [38]. The mean values of LS measurements assessed by VTQ (ARFI) were: F0=1.29±0.51 m/s, F1=1.35±0.39 m/s, F2=1.68±0.52 m/s, F3=2.24±0.57 m/s and F4=2.31±0.78 m/s. VTQ (ARFI) values were significantly correlated with TE values ($r=0.722$, $p<0.0001$). The AUROC curves of VTQ (ARFI) and TE for predicting different stages of fibrosis were similar: fibrosis- 0.690 *vs.* 0.724 ($p=0.74$), significant fibrosis - 0.871 *vs.* 0.891 ($p=0.51$), severe fibrosis - 0.890 *vs.* 0.908 ($p=0.34$) and liver cirrhosis - 0.817 *vs.* 0.888 ($p=0.90$). The optimum LS cut-offs assessed by VTQ (ARFI) for predicting F≥1, F≥2, F≥3 and F+4 were: 1.02 m/s, 1.3 m/s, 1.65 m/s and 1.88 m/s, respectively.

In another study the authors evaluated 45 patients with various etiologies of chronic liver disease (with liver biopsy) and 23 healthy subjects by means of VTQ (ARFI), TE and Hi-RTE (RealTime-Elastography) [39]. Failure or unreliable measurements occurred in 12.5% of the attempts at TE, but in none of the attempts at Hi-RTE and VTQ (ARFI). The three methods showed high correlation with fibrosis: $r=0.646$, $p<0.0001$ for TE; $r=0.535$, $p<0.0001$ for VTQ (ARFI) and $r=0.363$, $p<0.002$ for Hi-RTE. VTQ (ARFI) (AUROC=0.934) and TE (AUROC=0.922) exhibited high diagnostic accuracy in diagnosing cirrhosis, while for Hi-RTE the AUROC was significantly lower (0.852). For diagnosing F≥1, the AUROCs of TE, ARFI and Hi-RTE were 0.878, 0.807 and 0.834 respectively, while for predicting F≥2 the AUROCs were: 0.897, 0.815 and respectively 0.751.

Cassinotto *et al.* evaluated 349 consecutive patients with chronic liver diseases who underwent liver biopsy and LS measurement by VTQ (ARFI), TE and 2D-SWE (Aixplorer® system) [40]. 2D-SWE, TE and VTQ (ARFI) correlated significantly with histological fibrosis score ($r=0.79$, $p<0.00001$; $r=0.70$, $p<0.00001$ and $r=0.64$, $p<0.00001$, respectively). AUROCs of 2D-SWE, TE and VTQ (ARFI) were 0.89, 0.86, and 0.84 for the diagnosis of F≥1; 0.88, 0.84, and 0.81 for the diagnosis of F≥2; 0.93, 0.87, and 0.89, for the diagnosis of F≥3; 0.93, 0.90, and 0.90 for the diagnosis of compensated cirrhosis (F=4), respectively. 2D-SWE had a higher accuracy than TE for the diagnosis of severe fibrosis ($p=0.0016$) and a higher accuracy than VTQ (ARFI) elastography for the diagnosis of significant fibrosis ($p=0.0003$). No significant difference was observed between the three elastographic methods for the diagnosis of mild fibrosis and cirrhosis.

In a ***published meta-analysis*** regarding VTQ (ARFI) 8 studies with 518 patients were included [41]. The AUROC for predicting F≥2, F≥3 and F4 were: 0.87, 0.91 and 0.93, respectively. A subgroup of 312 patients were evaluated by both VTQ (ARFI) and TE. The AUROCs for predicting F≥2 and F=4 were significantly higher for TE as compared with VTQ (ARFI), while for predicting F≥3 they were similar.

Another ***meta-analysis*** compared the diagnostic performance of VTQ (ARFI) and TE for liver fibrosis assessment, using LB as the "gold-standard" [42]. This meta-analysis included 1163 patients from 13 studies. Inability to obtain a reliable measurement was more than twice higher for TE than for VTQ (ARFI) (6.6% *vs.* 2.1%, $p<0.001$). For detection of significant fibrosis the summary Se for VTQ (ARFI) was 0.74 and the summary Sp was 0.83, while for TE the summary Se was 0.78 and the summary Sp was 0.84. For the diagnosis of cirrhosis, for VTQ (ARFI), the summary Se was 0.87 and the summary Sp was 0.87, while for TE the summary Se was 0.89 and the summary Sp was 0.87. The diagnostic odds ratios were similar for VTQ (ARFI) and TE for detection of F≥2 and F=4. The mean optimal cut-off value of LS assessed by VTQ (ARFI) for detection of significant fibrosis was 1.30±0.07 m/s (median 1.31 m/s) and for detection of cirrhosis it was 1.80±0.16 m/s (median 1.8 m/s).

The last ***meta-analysis*** published that included 36 studies and 3951 patients (with liver biopsy as "gold-standard" method), showed the following AUROCs for predicting the presence of significant fibrosis, severe fibrosis and liver cirrhosis: 0.84, 0.89 and 0.91 respectively [43].

a. VTQ (ARFI) in Patients with Chronic Hepatitis C

The first data was published by Friedrich-Rust *et al.*, who evaluated 64 patients by LB, VTQ (ARFI), TE and serological tests (FibroTest and APRI) [6]. The AUROCs of VTQ (ARFI), TE, FibroTest and APRI for predicting the presence of F≥2 were: 0.86, 0.87, 0.86, and 0.81 respectively; for predicting the presence of F≥3: 0.93, 0.90, 0.93 and 0.80 respectively while for predicting F=4 they were: 0.95, 0.91, 0.84 and 0.73 respectively. The best cut-off LS values assessed by VTQ (ARFI) for predicting F≥2, F≥3 and F=4 were: 1.35 m/s, 1.55 m/s and 1.75 m/s respectively.

Another study evaluated 112 patients by LB, VTQ (ARFI) and TE [44]. The following LS cut-off values assessed by VTQ (ARFI) for predicting F≥1, F≥2, F≥3 and F=4 were proposed: 1.19 m/s, 1.34 m/s, 1.61 m/s and 2 m/s, respectively. The AUROCs of VTQ (ARFI) and TE for predicting different stages of fibrosis were similar for F≥3 (0.869 *vs.* 0.926, p=0.15) and F4 (0.911 *vs.* 0.945, p=0.33), while for detection of F≥1 and F≥2, TE performed significantly better than VTQ (ARFI): 0.902 *vs.* 0.709 (p=0.006) and 0.941 *vs.* 0.851 (p=0.02), respectively.

The study of Fierbințeanu-Braticevici *et al.* evaluated 79 patients by LB and VTQ (ARFI) [45]. The optimum LS cut-offs values assessed by VTQ (ARFI) for predicting the presence of fibrosis, significant fibrosis, severe fibrosis and cirrhosis were: 1.18 m/s, 1.21 m/s, 1.54 m/s and 1.94 m/s, respectively.

We conducted a multicenter Romanian study, which compared the value of VTQ (ARFI) to liver biopsy in 274 patients with chronic hepatitis C [46]. A direct, strong correlation was found between LS values assessed by VTQ (ARFI) and histological fibrosis ($r=0.707$, $p<0.0001$). For predicting the presence of F≥1, F≥2, F≥3 and F=4, the VTQ (ARFI) cut-off values were 1.19, 1.21, 1.58 and 1.82 m/s respectively. LS measurements assessed by VTQ (ARFI) had 73%, 84%, 84% and 91% sensitivity respectively; 93%, 91%, 94%, 90% specificity respectively; with

AUROCs 0.880, 0.893, 0.908 and 0.937 respectively for predicting F≥1, F≥2, F≥3 and F=4 (thus the accuracy of VTQ (ARFI) increasing with the severity of fibrosis).

We also conducted an international multicenter study, including 914 patients from 5 countries evaluated by means of LB and VTQ (ARFI), and in a subgroup of patients also by means of TE [47]. In this study, valid VTQ (ARFI) measurements were obtained in 911 (99.6%) of 914 cases. A highly significant correlation was found between LS values assessed by VTQ (ARFI) and histological fibrosis ($r=0.654$, $p<0.0001$). The predictive values of VTQ (ARFI) for various stages of fibrosis were: F≥1 – cut-off >1.19 m/s, AUROC=0.779; F≥2 – cut-off >1.33 m/s, AUROC=0.792; F≥3 – cut-off >1.43 m/s, AUROC=0.829; F=4 – cut-off >1.55 m/s, AUROC=0.842.

In the cohort of 400 patients with LB and valid VTQ (ARFI) and TE measurements, the correlation with histological fibrosis was similar for TE in comparison with VTQ (ARFI): $r=0.728$ *vs.* 0.689, $p=0.28$. TE was better than VTQ (ARFI) for predicting the presence of liver cirrhosis (AUROC 0.932 *vs.* 0.885, $p=0.01$) and any fibrosis (F≥1) (AUROC 0.857 *vs.* 0.772, $p=0.01$), while for predicting F≥2 and F≥3 the AUROCs were similar. This study showed that VTQ (ARFI) is non-inferior to TE for non-invasive assessment of liver fibrosis [47, 48].

When TE is compared to VTQ (ARFI), we must consider that valid measurements ("intend-to-diagnose") can be obtained with TE only in approximately 80-85% of cases (less in obese and impossible in patients with ascites) [49], while for VTQ (ARFI) the success rate is higher than 97% [6, 33, 36, 38, 39].

One solution proposed by our group is to combine these two elastographic methods in order to increase the accuracy of the non-invasive evaluation of liver fibrosis [50]. When both elastographic methods were taken into consideration, for predicting F≥ (cut-off for TE ≥ 6.7 kPa and for VTQ ≥ 1.2 m/s) we obtained 60.5% Se, 93.3% Sp, 96.8% PPV, 41.4% NPV and 68% accuracy, while for predicting F=3 (cut-off for TE ≥ 12.2 kPa and for VTQ ≥ 1.8 m/s) we obtained 84.9% Se, 94.4% Sp, 84.9% PPV, 94.4% NPV and 91.8% accuracy. The study

concluded that TE used in combination with VTQ (ARFI) is highly specific for predicting significant fibrosis and therefore when the two methods are concordant, liver biopsy can be avoided.

Kuroda *et al.* studied 30 non-cirrhotic patients with chronic hepatitis C, 30 patients with HCV liver cirrhosis and 10 healthy subjects (controls) [51]. The mean VTQ (ARFI) values in cirrhotic, non-cirrhotic and control groups were 2.67±1.18 m/s, 1.33±0.54 m/s and 0.99±0.21 m/s, respectively. LS values assessed by VTQ (ARFI) were significantly higher in the liver cirrhosis group as compared with the other two groups. The diagnostic accuracy of VTQ (ARFI) for predicting the presence of liver cirrhosis was superior to other non-invasive methods (AUROC=0.930 for VTQ-ARFI; 0.846 for aspartate aminotransferase to platelet ratio index; 0.829 for Forns' index and 0.785 for platelet count).

Goertz *et al.* evaluated 36 patients by means of VTQ (ARFI) and LB [4]. A significant correlation was observed between LS values assessed by VTQ (ARFI) and liver fibrosis (r=0.55, p=0.001).

Rizzo *et al.* evaluated 139 patients with chronic hepatitis C by means of LB, VTQ (ARFI) and TE [9]. LS assessed by TE was unreliable in nine patients (6.5%), while by VTQ (ARFI) valid measurements were obtained in all cases (p=0.02). The best LS cut-off values assessed by VTQ (ARFI) for predicting significant fibrosis, severe fibrosis and cirrhosis were: ≥1.3 m/s, ≥1.7 m/s and ≥2.0 m/s respectively, while the AUROCs were: 0.86, 0.94 and 0.89, respectively. By pair wise comparison of AUROCs, VTQ (ARFI) was significantly more accurate than TE for the diagnosis of F≥2 and F≥3 (0.86 *vs.* 0.78, p=0.024 and 0.94 *vs.* 0.83, p=0.002, respectively), while for predicting F=4 they were similar (0.89 *vs.* 0.80, p=0.09). The average concordance rates of TE and VTQ (ARFI) *vs.* liver biopsy were 45.4 and 54.7%, respectively.

Chen *et al.* evaluated 127 patients by liver biopsy, VTQ (ARFI) and Fibrotest (complex serological test for liver fibrosis evaluation) [52]. The AUROCs of VTQ (ARFI) and TE for predicting the presence of F≥2, F≥3 and F=4 were: 0.847 *vs.* 0.823, 0.902 *vs.* 0.812 and 0.831 *vs.* 0.757 respectively.

Li *et al.* evaluated 128 patients by liver biopsy, VTQ (ARFI) and APRI (simple

serological score) [53]. The mean values of LS assessed by VTQ (ARFI) for F1, F2, F3 and F4 were 1.23 ± 0.34 m/s, 1.48 ± 0.43 m/s, 2.06 ± 0.45 m/s, and 2.30 ± 0.87 m/s respectively. VTQ (ARFI) showed a better correlation with liver fibrosis stages than APRI ($r=0.649$ *vs.* $r = 0.478$, $p<0.05$). The areas under the ROC curves for VTQ (ARFI) and APRI were 0.775 and 0.721 for F≥2, 0.901 and 0.787 for stages F≥3, and 0.792 and 0.780 for F = 4, respectively.

b. VTQ (ARFI) in Patients with Chronic Hepatitis B

Our group assessed the value of VTQ (ARFI) in comparison with LB in 160 patients with chronic hepatitis B and C [54]. In most patients (156/160) TE measurements were also performed. Reliable LS measurements (10 valid measurements with IQR<30% and SR≥60%) by both elastographic methods were obtained in 146/160 (91.2%) patients, which were included in the final analysis. The correlation of LS measurements by VTQ (ARFI) with histological liver fibrosis was better in patients with chronic hepatitis C *vs.* those with chronic hepatitis B, but not statistically significant so: $r=0.490$, $p<0.0001$ *vs.* $r=0.356$, $p=0.01$ ($p=0.36$). In patients with chronic hepatitis B, the correlations of LS values by VTQ (ARFI) and TE with histological fibrosis were similar: $r=0.356$, $p=0.01$ *vs.* $r=0.403$, $p=0.004$ ($p=0.78$).

We also conducted an international multicenter study comprising 1095 patients (181 with chronic hepatitis B and 914 with chronic hepatitis C) [55]. In each patient LB and VTQ (ARFI) measurements were performed. The correlation of LS assessment by VTQ (ARFI) with histological fibrosis was significantly better in patients with chronic hepatitis C as compared with those with chronic hepatitis B: $r=0.653$, $p<0.0001$ *vs.* $r=0.511$, $p<0.0001$ ($p=0.007$). In this study, the mean LS values as determined by VTQ (ARFI), depending on the stage of fibrosis in patients with chronic hepatitis B and C were similar (Table **1**).

In a German multicenter study VTQ (ARFI) was evaluated as a predictor of fibrosis severity in a cohort of 133 chronic hepatitis B patients [56]. In most patients (104/133) TE was also performed. VTQ (ARFI) and TE were significantly correlated to the histological fibrosis stage. the AUROCs for VTQ (ARFI) for predicting F≥2, F≥3 and F=4 were: 0.69, 0.83 and 0.96, respectively.

No differences were found between VTQ (ARFI) and TE for the diagnosis of significant fibrosis, severe fibrosis and cirrhosis.

Table 1. Mean LS values assessed by VTQ (ARFI), depending on the stage of fibrosis in patients with chronic hepatitis B and C

Fibrosis (F)	Number of HBV patients	Mean VTQ (ARFI) HBV (m/s)	Number of HCV patients	Mean VTQ (ARFI) HCV (m/s)	P
0	11	1.24±0.17	61	1.09±0.42	0.24
1	59	1.20±0.21	241	1.22±0.41	0.71
2	46	1.38±0.30	202	1.37±0.48	0.89
3	34	1.52±0.48	187	1.70±0.59	0.09
4	31	2.04±0.60	223	2.23±0.71	0.15

In the study of Goertz *et al.*, 21 patients with chronic hepatitis B and 36 with hepatitis C were evaluated by means of VTQ (ARFI) and LB [4]. The correlation of VTQ (ARFI) values with histological fibrosis was higher in patients with chronic hepatitis B as compared with those with chronic hepatitis C: $r=0.71$, $p<0.001$ *vs.* $r=0.55$, $p=0.001$.

Zhang *et al.* evaluated 180 chronic hepatitis B patients by liver biopsy, VTQ (ARFI) and TE [57]. VTQ (ARFI) and TE were significantly correlated with histological fibrosis ($r = 0.599$, $p < 0.001$, for VTQ (ARFI) ; $r = 0.628$, $p < 0.001$, for TE) and necro-inflammatory activity ($r = 0.591$, $p<0.001$, for VTQ (ARFI) ; $r = 0.616$, $p<0.001$, for TE). AUROCs for VTQ (ARFI) and TE were 0.764 and 0.813 ($p = 0.302$) for $F\geq2$, 0.852 and 0.852 ($p = 1.000$) for $F\geq3$ and 0.825 and 0.799 ($p = 0.655$) for F4, respectively. The optimum cut-off values for VTQ (ARFI) were 1.63 m/s for $F \geq2$, 1.74 m/s for $F \geq3$ and 2.00 m/s for F4 in all cohort of patients. The cut-off values decreased to 1.24 m/s for $F\geq 2$, 1.32 m/s for $F\geq3$ and 1.41 m/s for cirrhosis in patients with normal alanine aminotransferase levels.

Dong *et al.* evaluated 81 patients by liver biopsy, VTQ (ARFI), TE and Forns index (another simple serological test) [58]. The AUROCs of VTQ (ARFI), TE and Forns index were similar for predicting the presence of significant fibrosis (0.762 *vs.* 0.753 *vs.* 0.735) and severe fibrosis (0.882 *vs.* 0.888 *vs.* 0.832). Regarding the prediction of cirrhosis, VTQ (ARFI) was inferior to TE and Forns

(0.732 *vs.* 0.873 *vs.* 0.876). The optimal LS values assessed by VTQ (ARFI) for prediction of F≥2, F≥3 and F=4 were: 1.29 m/s, 1.54 m/s and 1.83 m/s, respectively.

c. VTQ (ARFI) in Patients with Chronic Viral Hepatopathies after Antiviral Treatment

Goertz *et al.* [59] evaluated 38 patients with chronic hepatitis B and C. The authors divided the patients in 2 groups: one group of 25 patients with responded at the antiviral treatment and one group of 13 patients which did not received antiviral treatment or did not respond at treated or the relapse. The LS measurements by VTQ (ARFI) were performed at baseline and approximately 2 years later. In the group of patients with responded at the treatment, the mean VTQ (ARFI) values decreased significantly (from 1.55 ± 0.60 m/s to 1.34 ± 0.47 m/s, $p<0.05$), while in the group with no sustained antiviral response (or no treatment) the mean VTQ (ARFI) values were not significantly different (1.57 ± 0.70 m/s *vs.* 1.93 ± 0.77 m/s, $p=0.08$).

d. VTQ (ARFI) in Patients with Non-alcoholic Fatty Liver Disease (NAFLD)

Osaki *et al.* evaluated 23 patients with non-alcoholic steatohepatitis (NASH) by means of VTQ (ARFI) and LB [59]. The mean VTQ (ARFI) values for different stages of fibrosis were: F0-1=1.34±0.26 m/s, F2=1.79±0.78 m/s, F3=2.20±0.74 m/s and F4=2.90±1.01 m/s. For a cut-off value >1.47 m/s, LS assessed by VTQ (ARFI) had 100% Se and 75% Sp (AUROC=0.942) for predicting severe fibrosis (F≥3). In addition, the correlation between ARFI values and hyaluronic acid was significant ($p<0.0001$), while a tendency toward negative correlation was observed with serum albumin ($p=0.053$).

Another study evaluated 54 patients with NAFLD by means of LB, VTQ (ARFI) and TE [60]. The following median VTQ (ARFI) values for patients without fibrosis (F0), mild fibrosis (F1), significant fibrosis (F2), severe fibrosis (F3) and cirrhosis were obtained: 1.04 m/s, 1.12 m/s, 1.13 m/s, 1.78 m/s and 2.18 m/s, respectively. The optimum LS cut-off assessed by VTQ (ARFI) for predicting the presence of F≥3 was 1.77 m/s (AUROC=0.973), with 100% Se, 91% Sp, 71% PPV and 100% NPV. For diagnosing F=4, the best VTQ (ARFI) cut-off value was

1.9 m/s (AUROC=0.976), with 100% Se, 96% Sp, 75% PPV and 100% NPV. The AUROCs of TE for diagnosing F≥3 and F=4 were high: 0.990 and 0.998 respectively.

Palmeri *et al.* evaluated 172 patients with NAFLD by means of LB, VTQ (ARFI) and a simple serological score (APRI) [61]. In each patient, VTQ (ARFI) measurements were performed in 3 locations: superior intercostal (9-10^{th} rib intercostal space, coinciding with the place of liver biopsy), inferior intercostal (10-11^{th} rib intercostal space, typically 1-2 rib spaces inferior to the superior location) and lateral subcostal. Three replicate acquisitions were performed at each imaging location for a total of nine data acquisitions per patient. The RANSAC algorithm was used for all the shear wave speed estimates. Quantitative criteria were used to eliminate spurious estimates corrupted by an excessive motion artifact, poor signal-to-noise ratio, and an inadequate imaging window. Patients who had an IQR/mean >0.3 after outlier rejection were considered too variable and not successfully reconstructed. The shear waves' speed was transformed from m/s in kPa. Liver stiffness values were not associated with ballooned hepatocytes (p=0.11), inflammation (p=0.69), nor imaging location (p=0.11). Using a predictive shear stiffness threshold of 4.24 kPa, shear stiffness distinguished low (F≤2) from high (F≥3) fibrosis stages, with a sensitivity of 90% and a specificity of 90% (AUROC=0.90). Shear stiffness had a mild correlation with APRI (r=0.22). BMI > 40 kg/m^2 was not a limiting factor for VTQ (ARFI) imaging and no correlation was noted between BMI and shear stiffness.

In another published study, 61 patients with NAFLD/NASH were evaluated by means of LB, VTQ (ARFI) and TE (using normal M probe and XL probe) [62]. Liver stiffness measurements failure by TE was observed in 8 patients with the M probe and in 3 patients with the XL probe. Valid LS measurements by VTQ (ARFI) were obtained in all patients. The diagnostic accuracy for TE measurements with the M and XL probe and for VTQ (ARFI) in the right and left liver lobe were 0.73, 0.84, 0.71 and 0.60 for predicting F≥3, and 0.93, 0.93, 0.74 and 0.90 for predicting F=4, respectively. No significant differences were observed between TE and ARFI performance in the subgroup of patients with reliable TE measurement when taking into account the best results of both methods. A significant correlation was obtained between LS assessed by TE (M

probe and XL probe) and histological liver fibrosis (r=0.36, p=0.04 and r=0.53, p=0.008, respectively). The correlation of LS assessed by VTQ (ARFI) in the right and left liver lobe with the histological fibrosis was not significant (r=0.20, p=0.10 and r=0.22, p=0.10, respectively).

Guzman-Aroca *et al.* evaluated 32 patients with morbid obesity by VTQ (ARFI) before bariatric surgery, using LB performed during the surgery as "gold-standard" method [63]. They performed in each patients 3 valid VTQ (ARFI) measurements and a mean value was calculated. The patients were divided into 3 groups: simple steatosis, inflammation and fibrosis. Significant differences between the mean VTQ (ARFI) values in these 3 groups of patients were observed: 1.34 ± 0.90 m/s, 1.55 ± 0.79 m/s and 1.86 ± 0.75 m/s ($p < 0.001$), respectively. For a LS cut-off value of 1.3 m/s, they obtained 85% Se and 83.3% Sp (AUROC=0.899) for differentiating NAFLD from NASH or fibrosis.

Fierbinteanu-Braticevic *et al.* evaluated 64 NAFLD patients by VTQ (ARFI) and liver biopsy [64]. VTQ (ARFI) was able to distinguishing between patients with NASH from those with simple steatosis, with an AUROC of 0.867. There was a highly significant correlation between LS measurements by VTQ (ARFI) and fibrosis severity (r=0.843, $p < 0.001$). In patients with NASH, the AUROCs of VTQ (ARFI) elastography for predicting significant fibrosis and cirrhosis were 0.944 and 0.984, respectively.

e. VTQ (ARFI) in Post Transplant Patients

Crespo *et al.* evaluated 168 patients (87 liver transplant recipients, 59 non-transplant patients) by means of LB, VTQ (ARFI), TE and ELF score [65]. The best LS cut-off values assessed by VTQ (ARFI) for predicting F≥2 and F=4 were relatively similar for transplant and non-transplant patients: 1.43 *vs.* 1.39 m/s and respectively 2.05 *vs.* 1.92 m/s. The AUROC's of VTQ (ARFI), TE and ELF score for predicting F≥2 in transplant and non-transplant patients were: 0.90, 0.86, 0.81 and respectively 0.89, 0.89, 0.80; while for diagnosing F=4 the AUROC's were: 0.94, 0.93, 0.83 and respectively 0.97, 0.96, 0.89. The AUROC's of the VTQ (ARFI), TE and ELF score were similar for predicting F≥2 in transplant and non-transplant patients, while for diagnosing F=4, for both categories of patients, VTQ

(ARFI) performed significantly better than ELF score.

Wildner *et al.* evaluated 58 post transplant patients by VTQ (ARFI) and APRI and in a subgroup of 22 patients also by liver biopsy [66]. LS values assessed by VTQ (ARFI) were significantly correlated with APRI ($r=0.44$, $p<0.001$). The histological fibrosis was also significantly correlated with the VTQ (ARFI) values ($r=0.55$, $p=0.008$). The mean VTQ (ARFI) values were significantly increased in advanced fibrosis ($F\leq2$: 1.57±0.57 m/s *vs*.$F\geq3$ 2.85±0.66 m/s, $p<0.001$), obstructive cholestasis and active viral hepatitis. The AUROCs of VTQ (ARFI) elastography for predicting the presence of $F\geq2$, $F\geq3$ and $F=4$ were: 0.74, 0.93 and 0.80, respectively.

Liao *et al.* included in their study 57 post transplant patients, evaluated by liver biopsy and VTQ (ARFI) [67]. The LS values increased with severity of liver fibrosis and had a significant linear correlation with the results of histological fibrosis staging. The optimal cut-offs, Se and Sp for predicting different stages of liver fibrosis were: $F\geq1$: 1.06 m/s (Se=95.5%, Sp= 25.7%), $F\geq2$: 1.81 m/s (Se=50%, Sp=83.6%) and $F\geq3$: 2.33 m/s (Se=100%, Sp=92.9%).

f. VTQ (ARFI) for Liver Fibrosis Evaluation in Children

The manufacturer did not make specific recommendation regarding the evaluation of liver fibrosis with VTQ (ARFI) in children.

Noruegas *et al.* published the first data regarding the use of VTQ (ARFI) elastography for non-invasive assessment of liver fibrosis in children (10 children with chronic hepatopathies, 22 children from the liver transplantation waiting list and 20 healthy controls) [68]. The reference method for liver fibrosis assessment was LB and a 4 MHz convex probe was used for VTQ (ARFI) assessment. The scans was performed by intercostal approach and/or sub-xiphoid approach. Three LS measurements by VTQ (ARFI) were performed at different positions (4-6 locations), during slow breathing. The mean LS values assessed by VTQ (ARFI) for different stages of fibrosis were: F0=1.19±0.17 m/s, F1=1.48±0.32 m/s, F2=1.66±0.43 m/s and F4=2.93±0.97 m/s. The AUROCs of VTQ (ARFI) for diagnosing $F\geq1$, $F\geq2$ and $F=4$ were: 0.834, 0.818 and 0.983 respectively.

Marginean *et al.* evaluated by means of VTQ (ARFI) elastography 103 children divided into four categories: healthy control group (32 children), children with chronic liver disease with LB (19 subjects), overweight or obese children with NAFLD (13 children) and patients with malignancy (39 children) [69]. Ten valid LS measurements by VTQ (ARFI) were performed in the segment 8 and respectively in the liver segment 1 and median values were calculated. Global VTQ (ARFI) values were also calculated taking into consideration all the values obtained in the left and right lobe. In the healthy controls and NAFLD children the mean VTQ (ARFI) values obtained in segment 1 were significantly lower than those obtained in the liver segment 8. In the group of children with NAFLD, the mean global VTQ (ARFI) values were significantly higher than those obtained in the control group: 1.65 ± 0.49 m/s *vs.* 1.18 ± 0.27 m/s, p=0.002.

g. Usefulness of Spleen Stiffness Assessed by VTQ (ARFI) for Predicting Liver Cirrhosis

In order to increase the diagnostic accuracy in liver cirrhosis, the spleen stiffness (SS) assessment by VTQ (ARFI) was evaluated. Similar with LS assessment, 10 valid measurements are performed by intercostal approach and a median value is calculated, expressed in m/s (Fig. **2**).

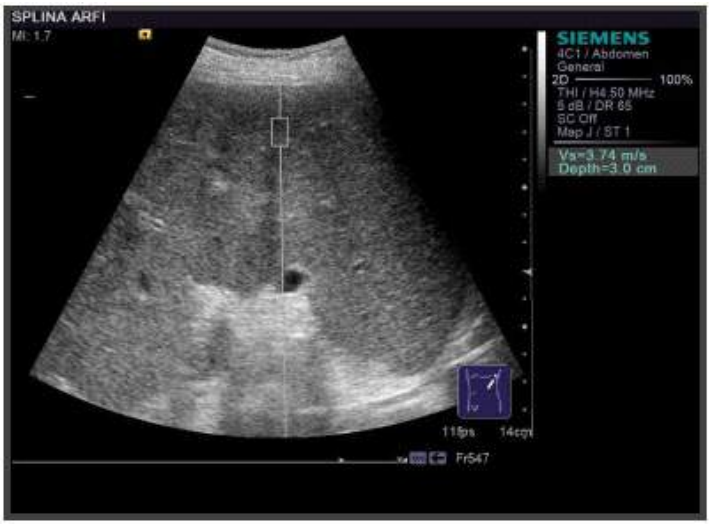

Fig. (2). VTQ (ARFI) measurement in the spleen (in a patient with splenomegaly).

Our group performed a study which evaluated 82 subjects (15 healthy volunteers, 57 cirrhotic patients, 10 with chronic liver disease with various stages of liver fibrosis) [70]. The mean SS (spleen stiffness) values (m/s) were: 2.04±0.28 in healthy subjects and 3.10±0.55 in cirrhotic patients ($p<0.001$). For a cut-off value of >2.51 m/s, SS had 85.2% Se, 91.7% Sp, 95.8% PPV, 73.3% NPV and 87.1% accuracy (AUROC=0.91) for predicting liver cirrhosis. The optimum LS cut-off value assessed by VTQ (ARFI) for predicting the presence of cirrhosis was 1.8 m/s, with 96.4% Se, 92% Sp, 96.4% PVP, 92% NPV and 95% accuracy. If LS and SS combined values are used, when one of the parameters is higher than the proposed cut-offs, 98.1% Se, 95.8% Sp, 98.1% PPV, 95.8% NPV and 95.8% accuracy are obtained to predict cirrhosis, and when both parameters are higher than the proposed cut-offs, 94.7% Se, 96% Sp, 98.1% PPV, 88% NPV and 95.1% accuracy are obtained.

An Italian study obtained the following mean SS values in healthy subjects, non-cirrhotic patients with chronic liver disease and cirrhotic patients: 2.23 m/s, 2.62 m/s and 3.36 m/s, respectively [8]. They also calculated the ***spleno-hepatic index*** (LS assessed by VTQ-ARFI in the right liver lobe multiplied by SS). The mean values of spleno-hepatic index in healthy subjects, patients with chronic hepatopathies and cirrhotic patients were: 2.61 m/s, 3.77 m/s and 8.13 m/s. For a cut-off value of 4.9 m/s, the spleno-hepatic index had 95.2% Se, 80.9% Sp, 81.6% PPV and 95% NPV (AUROC=0.945) for predicting the presence of liver cirrhosis.

Grgurevic *et al.* assessed LS and SS by VTQ (ARFI) in 58 subjects (20 healthy volunteers, 18 non-cirrhotic patients with chronic hepatopathies with LB and 20 patients with known liver cirrhosis) [71]. The mean SS values increased with liver fibrosis severity: 2.27±0.35 m/s in healthy subjects, 2.58±0.47 m/s in patients with chronic hepatopathies and 3.29±0.65 m/s in cirrhotic patients ($p<0.001$). For a cut-off value of 2.73 m/s, SS assessed by VTQ (ARFI) had 90% Se and 77.8% Sp (AUROC=0.822) for predicting F=4. The best LS cut-off value assessed by VTQ (ARFI) for predicting cirrhosis was 1.86 m/s (95% Se and 94.4% Sp, AUROC=0.989).

Cabassa *et al.* evaluated LS and SS by VTQ (ARFI) in 84 subjects (33 healthy

volunteers and 51 patients with chronic hepatopathies) [72]. Spleen stiffness assessed by VTQ (ARFI) was able to discriminate early (F1) from severe (≥F3) liver fibrosis with an optimal cut-off of 3.05 m/s (AUROC = 0.807). Liver stiffness assessed by VTQ (ARFI) was superior to SS, using a cut-off of 2.11 m/s (AUROC=0.879). Neither spleen nor liver VTQ (ARFI) was able to differentiate healthy volunteers from F1 patients.

Chen *et al.* evaluated the value of SS for predicting different stages of liver fibrosis in a cohort of 163 patients with chronic hepatitis B and C [73]. The AUROCs of SS for predicting the presence of F≥2, F≥3 and F=4 were: 0.839, 0.936 and 0.932 respectively. The optimal cut-offs of SS assessed by VTQ (ARFI) for predicting the presence of significant fibrosis, severe fibrosis and liver cirrhosis were: 2.74 m/s, 3.14 m/s and 3.32 m/s, respectively.

In conclusion, VTQ (ARFI) seems to be a useful method for liver fibrosis assessment in patients with chronic hepatopathies (especially chronic hepatitis C), non inferior as compared to TE. VTQ (ARFI) performed better for predicting severe fibrosis and cirrhosis. For patients with non-alcoholic fatty liver disease and other non-viral etiologies of chronic liver disease, post transplant patients and in children, further studies for the evaluation of VTQ (ARFI) as a predictor of liver fibrosis are required.

5. Usefulness of VTQ (ARFI) for Predicting Liver Cirrhosis Complications

Liver cirrhosis is the final stage of chronic hepatopathies of diverse etiologies. It has several complications such as portal hypertension, decompensation, hepatocellular carcinoma, hepatorenal syndrome, hepatopulmonary syndrome. Invasive evaluation of hepatic vein pressure gradient (HVPG) remains the most precise method for portal hypertension assessment. A HVPG value higher than 10 mm Hg predicts the presence of clinically significant portal hypertension, while a value higher than 12mm Hg is predictive for variceal bleeding [74].

Splenomegaly is a common finding in cirrhotic patients and the spleen density is modified in these patients, due to tissue hyperplasia and fibrosis and/or due to portal and spleen congestion due to the splanchnic hyper dynamic state [75, 76].

Several non-invasive methods have been evaluated as predictors of portal hypertension and esophageal varices (EV): aspartate aminotransferase to platelet ratio index - APRI or platelet count to spleen diameter ratio [77, 78]. In the last years, another 3 methods which evaluate liver stiffness (LS) and/or spleen stiffness (SS), have demonstrated some results in this field: Magnetic Resonance Elastography (MRE) [79, 80], TE [81 - 83] and VTQ (ARFI) [70, 84, 85].

a. Usefulness of VTQ (ARFI) for Predicting Portal Hypertension

Salzl *et al.* [86] presented data regarding the correlation of LS assessed by VTQ (ARFI) with HVPG in 48 patients (36 cirrhotic and 12 non-cirrhotic patients). A good correlation of LS measurements assessed by VTQ (ARFI) with HVPG measurements (r=0.709) was obtained. The authors calculated an AUROC of 0.874 for predicting the presence of clinically significant portal hypertension.

We evaluated in one study 157 patients [87]. The mean value of VTQ (ARFI) measurements in patients with large EV (at least grade 2) was not significantly different from the one in patients with no or small EV: 2.73±0.71 *vs.* 2.80±0.71m/s (p=0.49), nor in patients with or without a history of variceal bleeding: 2.78±0.81 *vs.* 2.77±0.7m/s (p=0.99).

In another study published by our group, 82 subjects were evaluated (15 healthy volunteers, 57 cirrhotic patients, 10 non-cirrhotic patients with various stages of liver fibrosis on LB) [70]. The mean SS values (m/s) were: 2.04±0.28 in healthy subjects and 3.10±0.55 in cirrhotic patients ($p<0.001$). For a cut-off value of >2.51 m/s, SS had 85.2% Se, 91.7% Sp, 95.8% PPV, 73.3% NPV, and 87.1% accuracy (AUROC=0.91) for predicting liver cirrhosis. No significant differences regarding SS were observed between patients with and without EV, also between those with and without a history of variceal bleeding.

In a subsequent study we tried to combine several parameters in order to increase the accuracy of VTQ (ARFI) for predicting significant EV [88]. We evaluated LS and SS by means of VTQ (ARFI) in 145 newly diagnosed cirrhotic patients who did not receive beta-blockers before LS measurements, 62 (42.7%) of them having significant EV (grade 2 and 3). The following parameters were significantly higher in patients with significant EV as compared to those without

EV or grade 1 EV: the mean SS assessed by VTQ (ARFI) (m/s) (3.28±0.50 *vs.* 3.08±0.61, p=0.04), the mean LS assessed by VTQ (ARFI) (m/s) (3.06±0.67 *vs.* 2.81±0.80, p=0.03), and the percentage of patients with ascites (70.9% *vs.* 34.9%, p=0.0001). The spleen size was similar in the 2 groups of patients. By multiple regression analysis we obtained the following formula for predicting significant EV:

Prediction of Significant EV (Pred EV_{2-3}): -0.572 + 0.041 x LS (m/s) + 0.122 x SS (m/s) + 0.325 x Ascites (1-absent, 2-present).

For cut-off values > 0.395, Pred EV_{2-3} had 75% Se, 61.8% Sp, 61.4% PPV, 78.2% NPV and 69.6% accuracy (AUROC=0.721) for predicting significant EV. In a cohort of 24 patients, the value of PredEV_{2-3} score for predicting significant EV was prospectively analyzed. Using the same cut-off value, the PredEV_{2-3} score had 66.7% Se, 75% Sp, 72.7% PPV, 69.2% NPV and 70.8% accuracy to predict significant EV in this cohort of patients.

Rifai *et al.* evaluated SS and LS in 125 subjects (25 healthy control subjects, 70 patients with chronic hepatopathies without portal hypertension and 30 cirrhotic patients with portal hypertension) [85]. The mean SS values were higher in patients with portal hypertension *vs.* those without portal hypertension: 3.25±0.56 m/s *vs.* 2.86±0.60 m/s (p<0.008). In this study, the authors obtained a significantly better performance of LS as compared with SS for predicting significant portal hypertension (AUROC 0.90 *vs.* 0.68), but the LS cut-off value proposed for predicting significant portal hypertension (1.67 m/s), is lower than the VTQ (ARFI) cut-off values proposed by the most published studies for diagnosing liver cirrhosis [6, 9, 37, 43]. The best SS cut-off value for predicting portal hypertension was 3.29 m/s, with 47% Se, 73% Sp, 36% PPV and 81% NPV.

Vermehren *et al.* evaluated 166 cirrhotic patients by means of VTQ (ARFI) (LS and SS), TE (LS) and FibroTest [84]. In an intention-to-diagnose analysis, the AUROCs for predicting significant EV were 0.58, 0.58, 0.53 and 0.50 for VTQ (ARFI) liver, VTQ (ARFI) spleen, TE and FibroTest, respectively (p>0.20). Logistic regression analysis showed that SS assessed by VTQ (ARFI) predicted better the presence of significant EV as compared with LS assessed by VTQ (ARFI). The best SS cut-off value for predicting significant EV was 4.13 m/s

(35% Se, 83% Sp, 54% PPV and 69% NPV). The authors also calculated the SS by VTQ (ARFI) cut-off for which the Se for predicting significant EV was >90. This cut-off was 3.04 m/s (90% Se and 25% Sp).

The performance of VTQ (ARFI) for non-invasive prediction of esophageal varices reported by the Asian studies was significantly better in comparison with the one reported in studies including European population.

Ye *et al.* evaluated SS and LS by VTQ (ARFI) in a cohort of 60 healthy volunteers (classified as stage 0), 66 patients with chronic hepatitis B who had undergone liver biopsy and 138 patients with hepatitis B-related cirrhosis [89]. LS was not correlated with the varices grade, whereas a significant linear correlation (Spearman $\rho = 0.65$; $P < .001$) between SS and the varices' grade was found. The optimal SS cutoff value for predicting the presence of esophageal varices was 3.16 m/s (AUROC = 0.83).

Morishita *et al.* studied the value of SS assessed by VTQ (ARFI) for prediction of high risk esophageal varices in a cohort of 135 HCV cirrhotic patients (92 included in the training cohort und 43 in the validation group) [90]. In the training set, the SS values assessed by VTQ (ARFI) increased with the EV grade ($p<0.001$). The mean VTQ (ARFI) values in the group with high-risk EV was significantly higher than in the group with low-risk EVs ($p < 0.001$). AUROC values for diagnosis of EV presence and high-risk EVs by VTQ (ARFI) were 0.890 and 0.868. The optimal cutoff value of VTQ (ARFI) for EV presence was 2.05 m/s with good sensitivity (83%), specificity (76%), PPV (78%), and NPV (81%), and that for high-risk EV was 2.39 m/s with good sensitivity (81%), specificity (82%), PPV (69%), and NPV (89%). These cut-off values obtained in the training cohort also showed excellent performance in the validation set.

Takuma *et al.* evaluated LS and SS assessed by VTQ (ARFI) for prediction of varices in a large cohort of 340 cirrhotic patients [91]. Spleen stiffness had the greatest diagnostic accuracy for the identification of patients with EV or high-risk EV as compared with LS, independent of the etiology of cirrhosis. An SS cut-off value of 3.18 m/s identified patients with EV with 98.4% NPV, 98.5% Se and 75% accuracy. A SS cut-off value of 3.30 m/s identified patients with high-risk

EV with 99.4% NPV, 98.9% Se and 72.1% accuracy. SS values less than 3.3 m/s ruled out the presence of high-risk varices in patients with compensated or decompensated cirrhosis.

b. Usefulness of VTQ (ARFI) for Predicting Decompensation of Liver Cirrhosis

A study published by our group assessed the mean LS value assessed by VTQ (ARFI) in cirrhotic patients [87]. The mean LS values were significantly lower in patients with compensated liver cirrhosis as compared with patients with decompensated liver cirrhosis (Child B and C): 2.67±0.73 *vs.* 3.05±0.8m/s (p=0.021).

In another study from our group, 211 cirrhotic patients were evaluated [92]. We found a direct, weak correlation between LS values and the Child-Pugh score (r=0.264, p<0.001) and also with MELD score (r=0.194, p=0.005). We also found a direct, weak correlation between LS measurements and total bilirubin (r=0.271, p<0.001) and an inverse, weak correlation with albumin (r= - 0.270, p<0.001), prothrombin time (r= - 0.196, p=0.006) and cholinesterase (r= - 0.241, p=0.003). The mean values of VTQ (ARFI) measurements were significantly higher in patients with Child-Pugh B *vs.* A (2.93±0.72 m/s *vs.* 2.59±0.68 m/s, p=0.002) and in Child-Pugh C *vs.* A (3.18±0.63 m/s *vs.* 2.59±0.68 m/s, p<0.001), but the values were not significantly different in patients with Child-Pugh B *vs.* C (2.93±0.72 m/s *vs.* 3.18±0.63 m/s, p=0.06). VTQ (ARFI) had 50% Se, 75% Sp, 70% PPV, 56.2% NPV, with 61.5% accuracy (AUROC - 0.65) for predicting the presence of at least B class Child-Pugh cirrhosis for a cut-off value of 3.11 m/s.

In a German study the mean SS values assessed by VTQ (ARFI) were significantly higher in patients with ascites *vs.* those without ascites: 3.33±0.59 m/s *vs.* 2.91±0.59 m/s (p<0.04) [85].

c. Usefulness of VTQ (ARFI) for Predicting Hepatocellular Carcinoma Occurrence

We observed in a study performed by our group that LS values assessed by VTQ (ARFI) were similar in patients with and without hepatocellular carcinoma: 2.70±0.64 *vs.* 2.88±0.81m/s (p=0.19) [87].

In a German study the AUROCs for predicting the occurrence of hepatocellular carcinoma for LS assessed by VTQ (ARFI), SS assessed by VTQ (ARFI), LS assessed by TE and FibroTest were: 0.54, 0.58, 0.56 and 0.72, respectively ($p>0.20$) [84]. Logistic regression analysis showed that SS assessed by VTQ (ARFI) predicted better the presence of hepatocellular carcinoma as compared with LS assessed by VTQ (ARFI). The best SS cut-off value for predicting hepatocellular carcinoma was 3.4 m/s (87% Se, 31% Sp, 11% PPV and 96% NPV). For a cut-off of 2.87 m/s the SS assessed by VTQ (ARFI) had 93% Se and 15% Sp for predicting the presence of hepatocellular carcinoma.

In conclusion, VTQ (ARFI) elastography seems to be a useful non-invasive method for predicting liver cirrhosis complications (especially portal hypertension and especially in Asian patients) and the accuracy can be increased by combining different elastographic parameters.

The main advantages and weaknesses of liver fibrosis evaluation by means of VTQ (ARFI)

Advantages	Weaknesses
-integrated into a standard ultrasound system -real-time elastographic method -can be performed in patients with ascites -higher rate of valid measurements as compared with Transient Elastography -it is a reproducible method -good results for non-invasive liver fibrosis evaluation in patients with chronic hepatitis B and C, especially for detecting patients with severe fibrosis and liver cirrhosis -promising results for non-invasive liver fibrosis evaluation in patients with NASH, post-transplant patients and children	-influenced by elevated aminotransferases level -the technical parameters IQR (interquartile range interval) and SR (success rate) need to be used (similar with Transient Elastography) in order to increase the accuracy of liver fibrosis evaluation -high BMI increases the number of measurements with improper IQR and/or SR -it is not very accurate to differentiate between patients without fibrosis and those with mild fibrosis; and between patients with moderate and mild fibrosis -severe steatosis can influence the accuracy of VTQ (ARFI) for liver fibrosis evaluation -weaker performance than Transient Elastography for the assessment of liver cirrhosis complications (especially in European population)

II.B. ELASTPQ ELASTOGRAPHY

1. ElastPQ Technique

ElastPQ technique is integrated into a Philips ultrasound system (iU22, Philips Medical Systems, Bothell,WA, USA, newly in Affinity 70 or in EPIC). Currently, little information is available regarding the physical principles of ElastPQ technique. According to the data provided by the manufacturer in the application for approval, submitted to the US Food and Drug Administration (FDA), ElastPQ system is relatively similar to 2D-SWE, even if this method is classified as a point SWE method [93]. The ElastPQ system generates an electronic voltage pulse, which is transmitted to the transducer. In the transducer, a piezo electric array converts the electronic pulse into an ultrasonic pressure wave. When coupled to the body, the pressure wave transmits through body tissues. The Doppler functions of the system process the Doppler shift frequencies from the echoes of moving targets, such as blood, to detect and graphically display the Doppler shift of these tissues as flow. The Doppler mode creates waves in soft tissues and estimates tissue stiffness by determining the speed at which these shear waves travel through the ROI [93]. Similar with VTQ Elastography, ROI has a predefined size, provided by the system (15 mm long and 5 mm wide). The shear wave speed is displayed on the screen (Fig. **3**). The operator can choose to display the results in m/s or in kPa.

2. Examination Technique

The measurements are performed on patients in supine position, with minimal scanning pressure applied by the operator, usually the operator asking the patient to suspend their breathing in a neutral, relaxed state, in order to minimize breathing motion. According to the data provided by the manufacturer, an intercostal scanning is needed for accurate stiffness measurements and the recommended liver stiffness (LS) measurements should be taken in segment 7 or 8 of the right lobe of the liver using a sagittal view, keeping in mind to hold the transducer very still during measurements, maintaining steady pressure. On the other hand according to published data LS measurements by ElastPQ should be performed in the liver segment V, because the lowest variation of LS

measurements was observed in this location [94]. The operator places the ROI in a region without large vessels and far from the heart, the diaphragm, liver/kidney interface and liver capsule. Ten valid measurements are performed and average and median value are calculated (expressed in m/s or kPa). A "0.00" value means the system did not detect liver tissue. An accurate stiffness average needs at least 10 non-zero measurements. There is no information regarding if the measurements must be made under fasting conditions. Also, there are no published studies, nor information from the manufacturer regarding the use of quality criteria parameters for LS measurements.

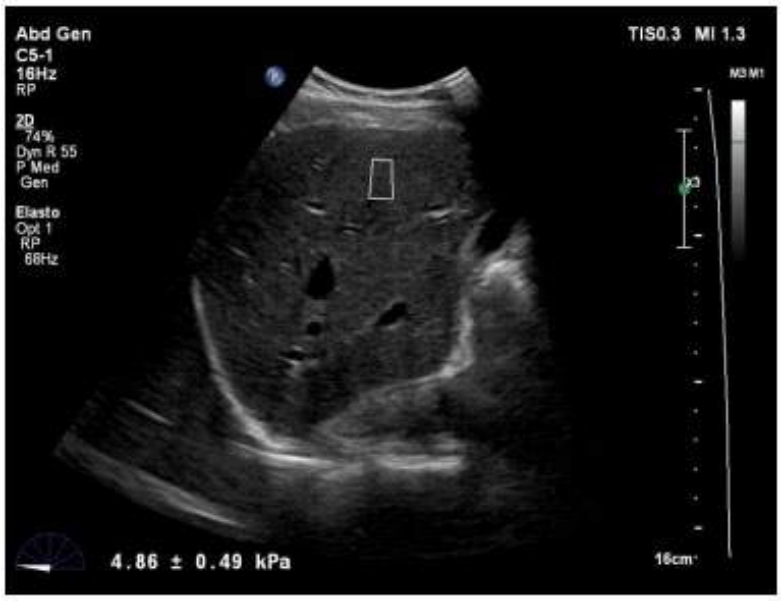

Fig. (3). ElastPQ measurement.

3. Feasibility of ElastPQ

Ten valid LS measurements can be obtained in more than 95% of patients [94 - 96] or without chronic hepatopathies [97]. In our cohort reliable LS measurements were obtained in a significantly higher proportion of patients by means of ElastPQ as compared with TE, 2D-SWE and VTQ: 99.3% *vs.* 87.4% ($p<0.0001$), 99.3% *vs.* 87.4% ($p<0.0001$) and 99.3% *vs.* 92.7% ($p=0.86$). In another study conducted by our group we compared the performance of ElastPQ in 121 consecutive subjects with chronic hepatopathies, using TE as the reference method, since it is

a validated method for liver fibrosis assessment [98]. Reliable LS measurements were obtained in 74.4% of patients by means of TE and in 99.3% with ElastPQ.

There is no information about the learning curve of this method, but similar with VTQ elastography, considering the excellent feasibility in the few published studies, probably a special training is not needed for physicians with basic knowledge in liver ultrasonography.

4. Reproducibility of ElastPQ Elastography

Ma JJ *et al.* evaluated the inter-operator reproducibility of ElastPQ elastography in 291 successive patients with hepatitis B who underwent liver partial hepatectomy or biopsy due to liver neoplasm [95]. The ICC of 10 measurements of liver stiffness with ElastPQ technique was 0.798, which indicated a good reproducibility. Ferraioli *et al.* assessed the reproducibility of ElastPQ in 116 subjects, including 47 consecutive patients scheduled for liver biopsy (Group 1) and 69 consecutive healthy volunteers (Group 2) [99]. The intraobserver agreement ranged from 0.83 (95%CI: 0.79-0.88) to 0.96 (95%CI: 0.95-0.97) for rater 1 and from 0.84 (95%CI: 0.79-0.88) to 0.96 (95%CI: 0.95-0.97) for rater 2. The interobserver agreement yielded values from 0.83 (95%CI: 0.78-0.88) to 0.93 (95%CI: 0.91-0.95).

In a study conducted by our group we also evaluated ElastPQ reproducibility [100]. The intra and inter-operator reproducibility were studied in 33 and respectively in 50 patients. The overall intraobserver agreement was better than the interobserver one: ICC 0.92 *vs.* ICC 0.85. A strong correlation was obtained between measurements assessed by both operators ($r=0.86$, $p<0.0001$) and also between measurements assed by a single operator 0.76 ($p<0.0001$). For both intra- and interobserver reproducibility, the ICCs were similar in patients with high body mass index (BMI) ≥ 25 kg/m^2 *vs.* <25 kg/m^2 (0.90 *vs.* 0.93 and 0.89 *vs.* 0.82, respectively).

5. Factors which Influence the Correlation of Liver Stiffness Values Assessed by ElastPQ with Fibrosis

Ling *et al.* evaluated in 21 healthy individuals the impact of liver location

(segments I-VIII), breathing phase (end-inspiration and end-expiration), probe position (sub-costal and inter-costal position) and examiner, in what LS measurements by ElastPQ were concerned [94]. More studies were also performed in 175 healthy individuals in order to determine the influence of gender and age on LS. This study found significant impact of liver location on LSM, while the liver segment V displayed the lowest coefficient of variation (CV 21%). The liver stiffness at the end-expiration was significantly higher than that at the end-inspiration. The liver stiffness was 8% higher in men than in women (3.8 ± 0.7 kPa *vs*. 3.5 ± 0.4 kPa, p=0.0168). In contrast, the liver stiffness was comparable in the different probe positions, examiners and age groups (p>0.05).

The influence of necroinflammation on liver stiffness is controversial; some studies have found an influence [95] and others have not [99]. In a study conducted by Ma JJ *et al.* the stage of liver fibrosis and the grade of necroinflammatory activity were associated with higher values of LS by ElastPQ ($p< 0.05$) [95].

6. Liver Stiffness Values by ElastPQ in Healthy Volunteers

According to available data, LS values by ElastPQ in Romanian healthy volunteers are 1.08 ± 0.12 m/s, value equivalent with 3.5 ± 0.04 kPa [96]. An Asian study showed that LS values by ElastPQ technique are significantly higher in men as compared with women and that they are not influenced by age [95].

7. ElastPQ Technique for Liver Fibrosis Evaluation in Chronic Hepatitis C Patients

No information available until now.

8. ElastPQ Technique for Liver Fibrosis Evaluation in Chronic Hepatitis B Patients

Published data showed a good value of ElastPQ technique for predicting the presence of significant fibrosis (F≥2) and cirrhosis (F=4), the best LS cut-off values being: 6.99 kPa (AUROC=0.94) and 9 kPa (AUROC=0.89), respectively [95].

9. ElastPQ Technique for Liver Fibrosis Evaluation in a Cohort of Patients with both Chronic Hepatitis B and C

In a cohort of patients with both viral etiologies of chronic liver disease, the median LS values for different stages of liver fibrosis were: F0-1 = 4.6 kPa, F2 = 5.9 kPa, F3 = 7 kPa and F4 = 12 kPa, respectively [101]. Also, ElastPQ technique had similar value with TE for predicting different stages of liver fibrosis.

10. The Usefulness of ElastPQ Technique for Predicting the Complications of Liver Cirrhosis

No information available until now.

11. The Usefulness of ElastPQ Technique in HCC

Ling *et al.* evaluated the usefulness of ElastPQ in a cohort of 99 patients with pathology-proven HCC [102]. They found that *in vivo* stiffness was significantly higher than *in vitro* stiffness (20 of the 99 surgical HCC specimens). Significantly higher stiffness was observed in hyper-vascular and poorly differentiated lesions than in hypo-vascular ones (p = 0.0352) and moderately to well-differentiated lesions (p = 0.0139).

The main advantages and weaknesses of liver fibrosis evaluation by means of ElastPQ

Advantages	*Weaknesses*
- integrated into a standard ultrasound system - real-time elastographic method - can be performed in patients with ascites - higher rate of valid measurements as compared with Transient Elastography - it is a reproducible method - promising results for non-invasive liver fibrosis evaluation	- influenced by liver location, breathing phase - insufficient data for the evaluation of accuracy in chronic liver diseases

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare for this publication.

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CHAPTER 3

2D-ShearWaves Elastography (2D-SWE)

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Abstract: Shear waves elastography is a technique designed to overcome some of the disadvantages of other elastographic techniques. It is based on supersonic share imaging, an ultrasound-based technique used for real-time visualization of soft tissue viscoelastic properties. This technique is based on the combination of a radiation force induced into the tissues by focused ultrasonic beams and a very high frame rate ultrasound imaging sequence able to capture in real time the transient propagation of the resulting shear waves. Shear waves' propagation induces small tissue displacements which are recorded by the imaging system, and measured using tissue Doppler techniques. 2D-SWE offers as major innovations the ability to measure area and distance ratios, a high spatial resolution and real-time capabilities. The technique produces an image where true local tissue elasticity is displayed in a color map in "real time". Elasticity is displayed using a color coded image superimposed on a B-mode image. The true elasticity is assessed based on Shear Waves propagation speed into the tissue. Thus the technique permits a quantitative mapping of liver tissue viscoelasticity. The technique was first available on the Aixplorer® system (SuperSonic Imagine, France) and initially was used for the evaluation of breast nodules, of prostate elasticity, for the evaluation of muscle and tendon stiffness and for thyroid disease diagnosis. Published data showed a real value of this method for liver stiffness estimation in patients with chronic hepatitis. It has the advantage that it can be also used in patients with ascites. A similar technique is now available on the Logiq E9 system (General Electric) with promising results.

Keywords: Liver stiffness, Shear waves elastography, Viscoelasticity.

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1. 2D-SHEARWAVES ELASTOGRAPHY TECHNIQUE

2D-Shear Waves Elastography (2D-SWE) is a new technique designed to overcome some of the disadvantages of other elastographic techniques. It is based on supersonic share imaging, an ultrasound-based technique, used for real-time visualization of soft tissue viscoelastic properties. The technique is based on the combination of a radiation force induced into the tissues by focused ultrasonic beams and a very high frame rate ultrasound imaging sequence, able to capture in real time the transient propagation of resulting shear waves [1].

Thus, 2D-SWE uses transient pulses to generate shear waves into the body [2 - 4], the only approach able to provide measureable and local elastic information in "real time" [5] - a major advantage. Fully automatic generated acoustic radiation force impulses induced by ultrasound beams perturb the underlying tissues, generating mechanical waves and shear waves, which propagate transversely into the tissue. Using SonicTouch™ technology, ultrasound beams are successively focused at different depths into tissues, all resulting shear waves interfering constructively along a "Mach cone", creating two quasi-plane shear Waves fronts propagating in opposite directions through the tissue. The shear waves generated using the SonicTouch™ excitation are captured by the ultrasound system. In order to capture shear waves in sufficient detail, frame rates of a few thousand of images per second are needed, 100 times faster than the frame rates offered by current state-of-the-art ultrasound technology. This ultrafast imaging mode acquires raw radiofrequency data at a very high frame rate, up to 5000 frames/s.

Shear waves' propagation induces small tissue displacements, which are recorded by the Ultrafast™ imaging system and measured using tissue Doppler techniques. 2D-SWE offers as major innovations, the ability to measure area and distance ratios, a high spatial resolution and real-time capabilities. Fully automated shear waves generation from the ultrasound transducer also allows user-skill independent and reproducible imaging.

2D-ShearWaves™ Elastography (2D-SWE) produces an image where true local tissue elasticity is displayed in a color map in "real time". Elasticity is displayed using a color coded image superimposed on a B-mode image. Stiffer tissues are

coded in red and softer tissues in blue, with an image resolution of approximately 1 mm. The true elasticity is assessed based on shear Waves propagation speed into the tissue. Thus the technique permits a quantitative mapping of liver tissue viscoelasticity [1].

The 2D-SWE method was used for the evaluation of breast nodules, of prostate elasticity, for the evaluation of muscle and tendon stiffness and for thyroid disease diagnosis. Preliminary results have shown the value of this method for liver stiffness (LS) estimation in patients with chronic hepatitis.

The technique has several advantages. The elasticity estimation is performed over a large area (10 cm^2) and probably reduces sampling errors; it also allows a mapping of local stiffness heterogeneities, thus allowing a precise location of hepatic lesions. Another interesting aspect of the supersonic share imaging technique relies on its ultrafast imaging characteristics, the high frame rates up to 5000 frames/s removing the influence of low-frequency displacement artifacts, such as respiratory motion or cardiac vibrations, which are error factors for the other elastographic techniques [1]. Thus the method is proven to be rapid, easy to perform, repeatable and reproducible [1].

On the other hand, the frequency bandwidth of the generated shear Waves is large, typically ranging from 60 to 600 Hz, different from transient elastography (FibroScan®) for example. By averaging shear Waves speed over a large bandwidth, supersonic share imaging seems to provide a more discriminator parameter for fibrosis evaluation [6] increasing the diagnosis accuracy.

The technique was first available on the ***Aixplorer® system*** (SuperSonic Imagine, France), integrated in an ultrasound system. The evaluation protocol requires placing the patient in supine position with the right arm in maximum abduction. The patient has to be fasted and the evaluation is recommended to be performed in normal breathing. The convex probe is placed in an intercostal space, using the best acoustic window available for liver evaluation. It is recommended to perform the acquisition on the right liver lobe and slow or no movement of the probe is preferable in order to avoid motion artifacts and to allow map stabilization. The 2D-SWE box has to be placed in vessel free parenchyma, in a uniform zone, not

close to the liver capsule. The best acquisition is performed 3 to 7 cm deep, 1-2 cm below the liver capsule [7]. The patient has to hold breath in the expiration phase for 4 sec. to acquire a stable image. The quantification box (ROI) is next placed in a homogeneous area and the elasticity value is displayed on the image (expressed as mean value and standard deviation measured either in kPa or in m/s) (Figs. **1**, **2**). The ROI size can be adjusted and allows the interrogation of a larger area of parenchyma. There is a large range of values that can be obtained (2-150 kPa), another advantage together with the possibility to use this technique also in patients with ascites (Fig. **3**). Some ultrasound experience is needed in order to obtain a high feasibility rate [8], especially in obese patients, and difficulties can also appear in patients with narrow intercostal spaces [9].

There is still no consensus regarding the number of measurements that should be performed, some authors using three [10], four [9] or five [11 - 13] valid 2D-SWE measurements. Other studies tried to see which would be the optimal number of measurements [14, 15] but the small number of patients did not allow the authors to reach a conclusion. Also, some studies used the mean value of LS measurements [9, 10], while in others the median was used [13]. In a study by Sporea *et al.* the mean LS values of three or five measurements, or the median value of five valid 2D-SWE measurements were compared. All correlated with TE showing similar results [16].

There are no quality criteria well defined yet, but some authors used the standard deviation/median liver stiffness ≤ 0.10 and measurement depth < 5.6 cm as quality technical parameters [17].

Similar to other ultrasound based elastographic methods, 2D-SWE should be performed in fasting conditions of at least 2 hours in order to avoid falsely elevated LS values [18]. There are no available data regarding the influence of elevated aminotransferases level, congestive heart failure, excessive alcohol intake or obstructive jaundice on the LS values, but in all these cases measurements can be unreliable.

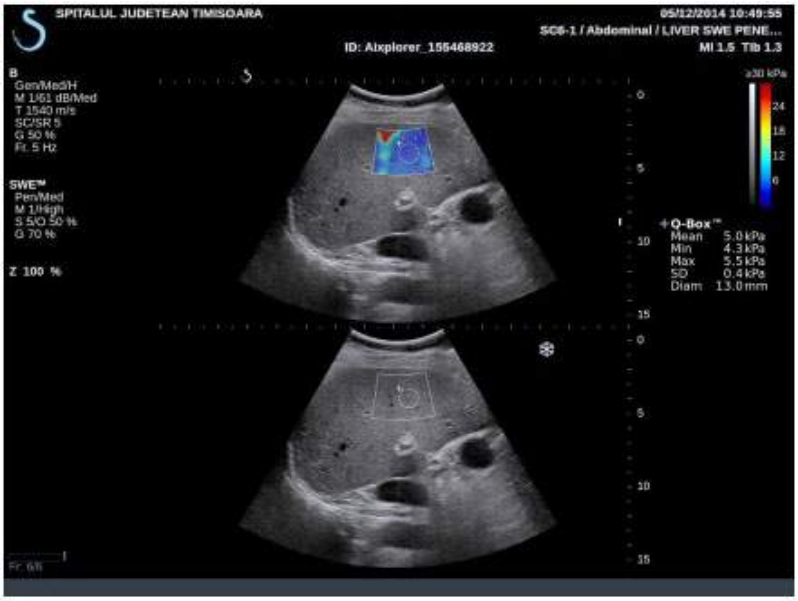

Fig. (1). Liver stiffness measurement by 2D-SWE using the Aixplorer® system in a patient with mild hepatitis.

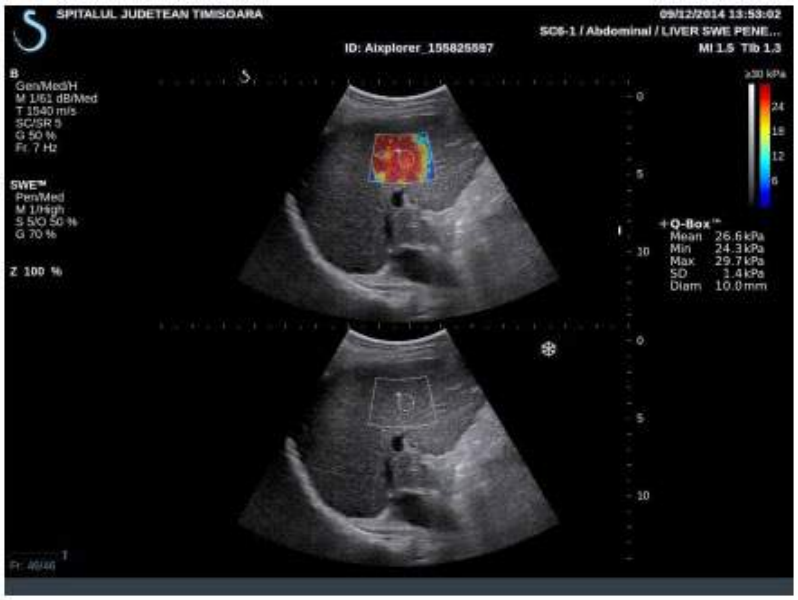

Fig. (2). Liver stiffness measurement by 2D-SWE using the Aixplorer® system in a patient with cirrhosis.

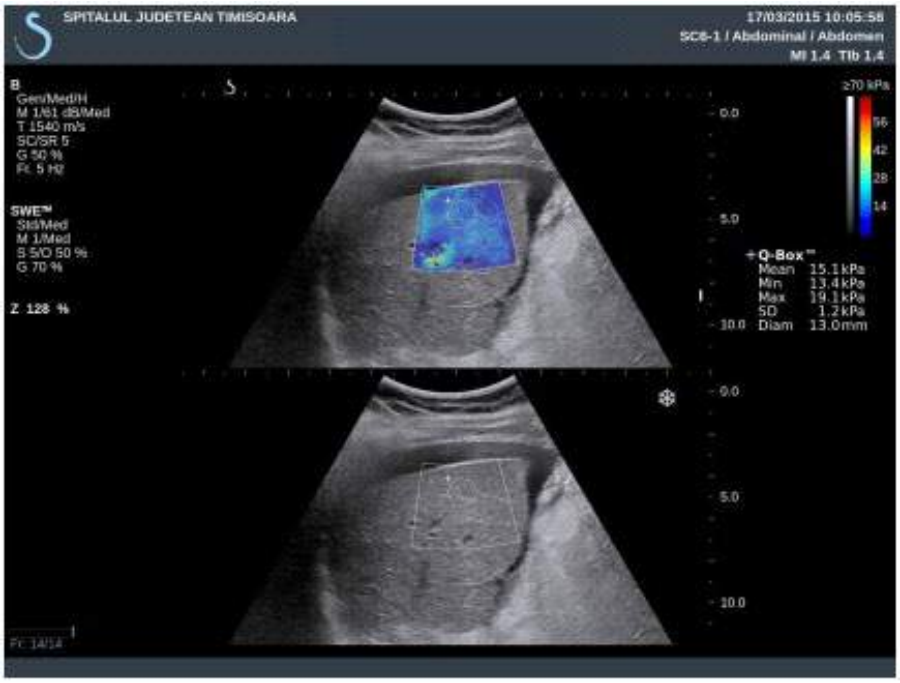

Fig. (3). Liver stiffness measurement by 2D-SWE using the Aixplorer® system in a patient with liver cirrhosis and ascites.

2D-SWE is proved to be a *feasible and reproducible technique*. Most published studies showed that reliable LS measurements by means of 2D-SWE can be obtained in 90-98.9% of cases [9, 10, 19, 20], but if the TE quality criteria are applied (IQR<30% and SR≥30%), the rate of reliable measurements can decrease to 71.3% [13]. The technique showed a good intra- and inter-observer reproducibility in assessing LS [19, 21, 22].

2. CLINICAL RESULTS

a. Healthy Volunteers

The mean LS in healthy volunteers varied from 4.1 kPa [23], to 6 ± 1.4 kPa (median 5.7 kPa), higher values being obtained in men than in women (6.6 ± 1.5 kPa *vs.* 5.7 ± 1.3 kPa, p=0.01.) [24], to values ranging from 2.6 to 6.2 kPa in another study [25].

b. Chronic Liver Diseases

HCV Hepatitis

In one of the first studies, Bavu *et al.* included 133 patients with HCV chronic infection in whom 2D-SWE was compared with TE and liver biopsy (LB) [11]. Analysis of variance (ANOVA) showed a good correlation between fibrosis and elasticity assessment using 2D-SWE and TE ($p<0.0001$). AUROCs for elasticity values assessed by 2D-SWE were: 0.948 for F≥2, 0.962 for F≥3 and 0.968 for F=4. In this study, the AUROCs for 2D-SWE were better than those from TE performed in the same session (AUROCs for TE for F≥2, F≥3 F4 were 0.846, 0.857 and 0.940 respectively).

Another small study compared LS values in subjects with chronic C hepatitis obtained by means of ultrasound elastographic methods [26]. In a cohort of 33 patients with proven HCV hepatitis, the LS was evaluated with FibroScan® (Echosens®, XL probe, 10 valid measurements), ARFI (VTQ-Siemens, 10 valid measurements) and Aixplorer® (Supersonic, 4 valid measurements). Successful measurements were obtained with FibroScan® and ARFI in 100% and with Aixplorer® in 97% of cases. Mean values were as follows: ARFI: 1.30 m/s; FibroScan®: 8.57 kPa; Aixplorer®: 10.05 kPa. In this study, there was a significant correlation between ARFI and both FibroScan® and Aixplorer® ($r2=0.6720$; $r2=0.5408$, $p<0.0001$).

More recently published data proved 2D-SWE as a good, reliable method for assessing LS in chronic hepatitis C patients [9], the best cut-off values for different stages of liver fibrosis in this study being: F≥2: 7.1 kPa (AUROC=0.92), F≥3: 8.7 kPa (AUROC=0.98) and F4: 10.4 kPa (AUROC=0.98). Another study on 55 patients with HCV chronic hepatitis evaluated by 2D-SWE, FIB-4 index, aspartate aminotransferase-to-platelet ratio index (APRI) and Forns' index and liver biopsy [27] showed an AUROC for 2D-SWE for diagnosing significant liver fibrosis of 0.94, with an accuracy of 90.9%, both higher than the results for the other techniques.

HBV Hepatitis

The data available regarding LS assessment by means of 2D-SWE in patients with chronic hepatitis B show almost similar values as for HCV patients: F≥1: 6.5 kPa (AUROC=0.86), F≥2: 7.1 kPa (AUROC=0.88), F≥3: 7.9 kPa (AUROC=0.93) and F4: 10.1 kPa (AUROC=0.98) [10].

In another study on 303 patients with chronic hepatitis B, in which the first 202 patients were the index cohort who were validated on the next 101 patients (validation cohort), the AUROC curves for significant fibrosis, severe fibrosis and cirrhosis were all greater than 0.90 [28]. Using the cut-off values generated from the index cohort, the validation cohort 2D-SWE had NPV of 82.6% (95% confidence interval [CI]: 68.4% - 92.3%) for significant fibrosis; 95.1% (95% CI: 86.3% - 99.0%) for severe fibrosis; and 97.4% (95% CI: 90.8% - 99.7%) for cirrhosis. The PPV were 83.6% (95% CI: 71.2% - 92.2%); 65.0% (95% CI: 48.1 - 79.5%); and 60.0% (95% CI: 38.7% - 78.9%), respectively, proving that the method is better to rule out the diagnosis of cirrhosis.

NAFLD

Even if data are still limited, 2D-SWE seems to be also accurate for the evaluation of LS in NAFLD patients. Thus, 291 NAFLD patients were evaluated by 2D-SWE, TE (M probe), and ARFI (VTQ) using liver biopsy as reference method. The AUROCs for 2D-SWE, TE, and ARFI (VTQ) 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 [29]. Also the cut-off values for 2D-SWE and TE for staging fibrosis with a sensitivity ≥90% were very close: 6.3/6.2 kPa for ≥F2, 8.3/8.2 kPa for ≥F3, and 10.5/9.5 kPa for F4.

Various Etiologies of Liver Disease

In a preliminary study performed by Bavu and al, evaluation of hepatic elasticity with 2D-SWE was performed in 104 patients with chronic hepatitis, in comparison with liver biopsy, serum tests and TE [6]. Sensitivity and specificity of 2D-SWE for F≥2 were 0.72 and 0.86; for F≥3 were 0.69 and 0.82 and for F=4 were 0.90 and 0.91.

Ulterior studies seemed to confirm the good value of 2D-SWE for liver fibrosis assessment. Thus, in a cohort of 79 patients with chronic hepatitis, Ferraioli *et al.* compared 2D-SWE with TE and liver biopsy [30]. The cut-off value found for F≥2 was 7.4 kPa (Se=80%, Sp=83%), for F≥3 it was 8.7 kPa (Se=100%, Sp=95%) and for F=4 it was 9.2 kPa (Se=100%, Sp=87%). The AUROCs for prediction of F≥2, F≥3 and F4 were 0.91, 0.99 and 0.97 respectively. In cases in which 2D-SWE was compared to TE, the two methods showed similar diagnostic performance.

In a study on Romanian patients with various etiologies of chronic liver disease, the cut-off values for predicting each stage of fibrosis were the following: F≥1: 7.1 kPa (AUROC=0.825), F≥2: 7.8 kPa (AUROC=0.859), F≥3: 8 kPa (AUROC=0.897) and for F=4: 11.5 kPa (AUROC=0.914) [31]. In another study on a cohort of 127 consecutive patients with chronic liver diseases, the optimal 2D-SWE cut-off values for predicting significant fibrosis and cirrhosis were slightly higher: 8.03 kPa for F≥2 (AUROC=0.832) and 13.1 kPa for F=4 (AUROC=0.915), respectively [32].

Good results were also obtained in a study that compared 2D-SWE, TE and ARFI (VTQ) in 349 consecutive patients with chronic liver diseases who underwent liver biopsy [33]. 2D-SWE performed better then TE and ARFI (VTQ): AUROCs of 2D-SWE, TE, and ARFI (VTQ) 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. Another similar study on 132 patients with chronic hepatopathies, in whom LS was evaluated using TE, ARFI (VTQ) and 2-D SWE, with liver biopsy as the reference method for liver fibrosis assessment (n = 101) and magnetic resonance imaging/computed tomography for the diagnosis of liver cirrhosis (n = 31), showed no significant differences between the three elastographic methods for diagnosing significant fibrosis, advanced fibrosis and cirrhosis [34].

The good accuracy of 2D-SWE for liver fibrosis assessment was also proved in overweight and obese patients with chronic liver disease, including HCV patients [35].

In a more recent individual patient data based meta-analysis that included 1340 patients and which compared 2D-SWE with liver biopsy, the overall performance of 2D-SWE was good to excellent in patients with HCV, HBV and NAFLD with AUROCs of 86.3%, 91.6%, 85.9% for diagnosing significant fibrosis and 96.1%, 97.1% and 95.5% for diagnosing cirrhosis, respectively [36]. The optimal cut-off for diagnosing significant fibrosis in all patients was 7.1 kPa, while for diagnosing liver cirrhosis the cut-offs were 13.5 kPa in HCV and NAFLD patients, and 11.5 kPa in HBV patients.

c. Liver Cirrhosis Complications Estimation

This technique is rather new and there are limited data regarding its place in predicting liver cirrhosis complications. Kim *et al.* showed that for a cut-off value of 15.2 kPa, the sensitivity and specificity of 2D-SWE for predicting clinically significant portal hypertension were 85.7% and 80% respectively (AUROC 0.819) (HVPG > 10 mmHg) [37]. Procopet *et al.* [17] used as quality technical parameters the standard deviation/median liver stiffness ≤ 0.10 and measurement depth < 5.6 cm, and when applying them, for the optimal cut-off value of 15.4 kPa, 2D-SWE showed a very good accuracy in predicting clinically significant portal hypertension (AUROC =0.948, with both sensitivity and specificity higher than 90%).

In another study on 79 patients with liver cirrhosis in whom LS and spleen stiffness (SS) were measured by 2D-SWE, TE and in whom HVPG measurements were also performed, the technical success rate of 2D-SWE was significantly better than that of TE for both LS and SS (97% and 97% *vs.* 44% and 42%, respectively; P < .001) [38]. 2D-SWE LS of more than 24.6 kPa had 81% sensitivity, 88% specificity, and 82% accuracy for clinically significant portal hypertension, the diagnostic performance of LS being better than of SS (AUROC of 0.87 *vs.* 0.64, P = .003).

In a larger study, on 401 consecutive cirrhotic patients, the liver stiffness cut-offs values for a NPV≥90% to exclude high-risk esophageal 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 [39]. The AUROCs for SS and LS (2D-

SWE), and LS (TE) were 0.80, 0.77 and 0.73 respectively for detection of esophageal varices.

All these papers concerning 2D-SWE in patients with chronic hepatopathies showed very good accuracies for LS evaluation (similar or slightly superior diagnostic performance as compared to TE, but with the advantage that with 2D-SWE, valid measurements are obtained in a high proportion of cases), placing it as a reliable alternative of LS evaluation, with further advantages that that require more research.

2D-SWE.GE

Another system that implemented the 2D-ShearWaves Elastography (2D-SWE) technique is the **LOGIQ E9** ultrasound system from General Electric. The examination protocol requires the subjects to lie supine with their right arm raised over their head. Through the inter-costal space, the right liver lobe is scanned and the Shear Waves elastography region of interest (ROI) is placed below the liver capsule, in a region free of vessels (if possible). Once a suitable image window is found, the subject is asked to suspend breathing and afterwards the Shear Waves acquisition is initiated. After approximately five seconds, during which usually 2 or 3 Shear Waves frames are acquired, the subject is asked to resume breathing. The acquisition process needs to be repeated until at least 10 Shear Waves frames are acquired. The measurements are than performed by placing a circular measurement region of interest over each saved Shear Waves elastographic image (Figs. **4**, **5**). The measurement regions are chosen to exclude obvious artifacts.

The average stiffness expressed in terms of Young's Modulus within each measurement region is automatically recorded by the system in a worksheet. The ten measurement regions are typically placed on different Shear Waves image frames or at non-overlapping locations on the same frame, so that ten independent measurements of liver stiffness are obtained for each subject. The system automatically calculates the median value and the interquartile range of the valid measurements [40].

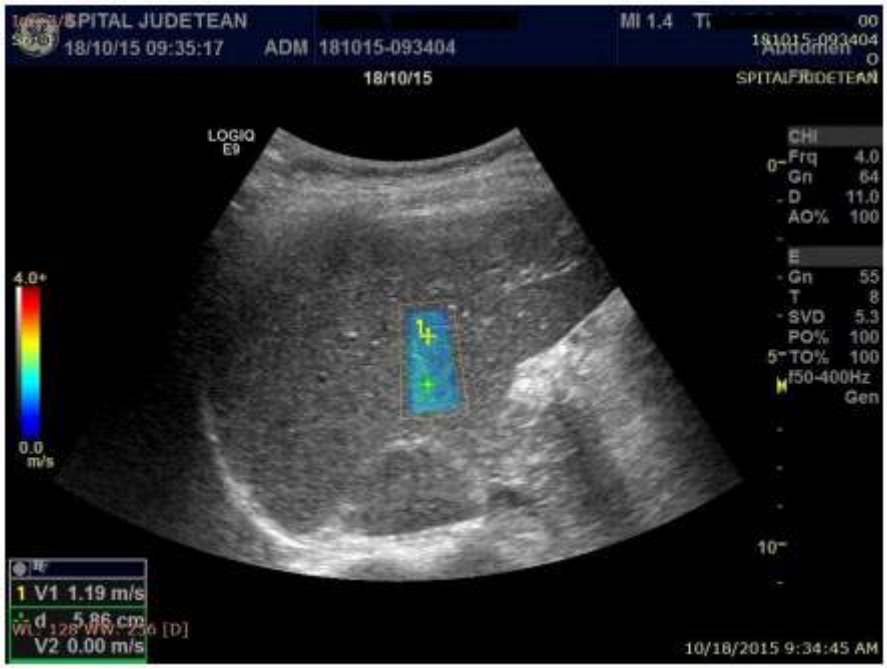

Fig. (4). Liver stiffness measurement by 2D SWE using Logiq E9 system in a mild hepatitis.

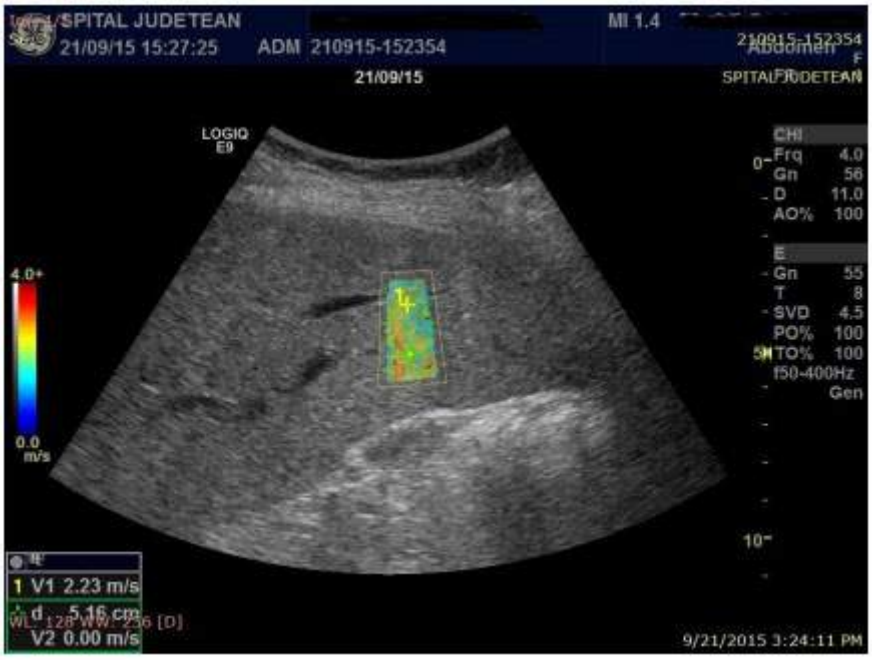

Fig. (5). Liver stiffness measurement by 2D SWE using the Logiq E9 system in a patient with cirrhosis.

Main advantages and weaknesses of liver fibrosis evaluation by means of 2D-SWE.

Advantages	Weaknesses
- integrated into an standard ultrasound system - real-time elastographic method - feasible in patients with ascites - standardized technique - large and adjustable size of the ROI - promising results for non-invasive liver fibrosis evaluation, comparable to transient elastography and other techniques	- insufficient data for the evaluation of accuracy in all chronic liver diseases - some ultrasound experience is needed - quality criteria not yet established

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare for this publication.

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CHAPTER 4

Real-Time Strain Elastography (HI-RTE)

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Abstract: Real-Time Strain Elastography performed by the Hitachi System (HI-RTE) uses a conventional ultrasound probe to compare and analyze echo signals before and under slight compression. Initially, HI-RTE offers only qualitative results. To overcome this limitation several quantitative methods in RTE have been developed, such as Elastic Ratio, Elastic Index, Elasticity Score and Liver Fibrosis Index (LFI). Despite being the first ultrasound-based elastography technique, HI-RTE has not yet yielded the desired results in the evaluation of liver fibrosis. This lack of performance is a consequence of inconsistency between the ultrasound-systems, methods and data analysis among different research teams. In the past few years, it seems that the technique has become more standardized and the elastographic assessment parameters are already established. The overall results of a meta-analysis suggested that LFI was excellent in diagnosing F≥3 and has moderate accuracy for F≥2 and F=4. However, LFI could not be applied to accurately differentiate F2 *versus* F0-1 and F=4 *versus* F0-3. HI-RTE is readily available with the ultrasound machine, is feasible in patients with ascites and inflammation and has promising results for non-invasive liver fibrosis evaluation in patients with chronic viral hepatitis and fatty liver diseases. In the future, a large, prospective, international multicenter study is essential to obtain a further evaluation of the potential diagnostic value of HI-RTE.

Keywords: Elasticity index, Liver stiffness, Real Time Elastography.

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As mentioned in the previous chapters, there is a current trend towards replacing liver biopsy with ultrasound-based elastography in the evaluation of liver fibrosis in chronic diffuse liver diseases. In all elastographic methods mechanical stress acted upon the liver induces a tissue displacement. Measuring tissue displacement offers an estimation of the elastic properties of the liver, which in turn allows a reliable assessment of liver fibrosis severity.

1. REAL-TIME STRAIN ELASTOGRAPHY TECHNIQUE

Real-Time Strain Elastography (RTE) is an add-on module that can be incorporated, similar to acoustic radiation force impulse (ARFI) technology, in standard ultrasound devices; this represents an advantage when compared with transient elastography (TE), for example, where a new unit must be purchased. On the other hand, both Real-Time Elastography and ARFI use conventional ultrasound transducers for the examination, allowing a direct visualization of liver parenchyma while performing a liver stiffness (LS) evaluation. Thus, the examiner is able to avoid the liver capsule, to adjust the transducer's position, and thus to obtain the best acoustic window, even in difficult patients, such as overweight ones. The method is also reliable and reproducible in patients with ascites [1].

Real-Time Strain Elastography was performed for the first time with Hitachi systems (EUB-8500 and EUB-900) [2]. It uses a conventional ultrasound probe to compare and analyze echo signals before and under slight compression [3]. To perform free-hand HI-RTE, usually with the patient in supine position, the transducer is placed in the intercostal space and the examiner must apply stress by moving the transducer [4]. The examination is usually performed in the right liver lobe. The Hitachi Real-Time Elastography (HI-RTE) module uses an extended combined autocorrelation method to produce a real-time elasticity image, by using a freehand approach and compressing the tissues with the ultrasound transducer. The relative tissue elasticity is calculated and displayed as a color overlay on the conventional B-mode image. Stiffer structures are displayed in blue, while the more easily deformed tissues are displayed in red.

Initially, HI-RTE offered only qualitative results. To overcome this limitation

several quantitative methods in RTE have been developed, such as **Elastic Ratio, Elastic Index, Elasticity Score and Liver Fibrosis Index (LFI).**

Despite being the first ultrasound-based elastography technique, HI-RTE has not yet yielded the desired results in the evaluation of liver fibrosis. This lack of performance is a consequence of inconsistency between the ultrasound-systems, methods and data analysis among different research teams. In the past few years, though, along with the development of the new HI-RTE systems (HI VISION Avius, Preirus, Ascendus systems - Hitachi Medical Systems Europe Holding AG), it seems that the technique has become more standardized and the elastographic assessment parameters are already established. The examination is performed using a linear probe (3.5-7 MHz), positioned in the intercostal space, without compressing, seeing that the device already uses the internal pressure generated by the heart beats on the liver parenchyma. In this fashion, the sampling errors produced by the examiner compression are avoided. A well trained examiner with sufficient experience is needed in order to keep clear of any artifacts related to obesity, ROI setting, avoidance of large vessels and costal shades, as well as adjustment of the probe position in order to obtain a reliable image of the liver parenchyma, where compression/relaxation is homogeneous and axial to the probe.

1. Feasibility and Reproducibility

Ultrasound examinations are operator-dependent techniques and different levels of training and experience could influence the results of the HI-RTE as well. A prospective study in which patients were examined by two doctors with different levels of experience in ultrasound obtained good intra- and inter-observer variability values [5]. The authors did not find significant differences between the two physicians, regardless of the patients' real status (cirrhosis, chronic hepatitis, steatosis, or healthy subjects). In a study published by Koizumi *et al.*, elastography was performed at four liver locations by two independent observers. The authors found no difference in reproducibility for the four measurement positions, while the interobserver agreement was very good (k=95%) [6].

2. Clinical Results

a. Liver Stiffness Values in Healthy Volunteers

Very little information is available to date. Hu *et al.* conducted a study in adults without liver disease, in order to investigate normal liver stiffness evaluated by RTE. The mean LFI in healthy participants with a normal BMI was 1.31 ± 0.25. The optimal LFI threshold value for discriminating normal liver from noncirrhotic chronic liver disease was 2.12 in participants with a normal BMI. The authors found no significant differences in the mean LFI between sexes or among different age groups, but only a positive correlation between BMI and the liver fibrosis index [7].

b. RTE for Liver Fibrosis Evaluation in Chronic Viral Hepatitis

The first report [2] regarding chronic viral hepatitis evaluated by HI-RTE (Hitachi EUB-8500 and EUB-900) included 79 patients with chronic HCV or HBV hepatitis (all of them had had a liver biopsy), 20 patients with proven cirrhosis and a control group of 20 healthy volunteers. In those participants, the amount of displacement of the reflected ultrasound echoes before and under compression were measured (stress field). A strain field was then reconstructed from the measured displacements (strain image).

The calculation of tissue elasticity distribution was performed in real-time and the examination results were represented as color-coded images with a conventional B-mode image in the background. 10 valid measurements were performed. The investigators attempted to find a new elasticity score using a specially developed Matlab computer program.

This ***German Elasticity Score*** was generated as follows: numerical values were determined from 0 – 10, according to color mapping from blue (1) to red (0), followed by the calculation of mean, median, minimum, maximum, frequency of pixel values above 0.75 of a single measurement, and descriptive statistics of all measurements. The elasticity score was then calculated by the following formula, which was developed by stepwise multivariate logistic regression analysis:

Elasticity score = 177 + 50 × Log10 (Median [Freq(pixel 3 0.75)] –

13000 ×Min [Min (pixel with values above 0)]).

This elasticity score ranged from 65 to 122. The comparison of histological liver fibrosis with HI-RTE showed a good correlation; the increasing elasticity scores corresponded to increasing stages of fibrosis. Spearman's correlation coefficient between elasticity scores and histological fibrosis stages was highly significant, with a value of 0.48 ($p<0.001$). The accuracy was 0.75 for significant fibrosis ($F\geq2$), 0.73 for severe fibrosis ($F\geq3$) and 0.69 for cirrhosis ($F=4$). In this study, 80% of patients with significant fibrosis ($F\geq2$) could be correctly identified with HI-RTE and the elasticity score was not influenced by the severity of liver steatosis.

Tatsumi *et al.* [8] performed HI-RTE in 119 patients with chronic liver disease, who had had a liver biopsy and compared the results with TE and serum fibrotic markers. Tissue elasticity was calculated using real-time tissue elastography, in which numerical values from 0–255 (256 stepwise grading) were determined according to color mapping from blue (0) to red (255). The percentage of blue areas in the ROI was then calculated. The authors elaborated the ***Japanese Elasticity Score***: numerical values were determined from 0 – 255 according to color mapping from blue (0) to red (255), followed by the calculation of means ± SD in the "region of interest" (ROI), the percentage of blue area in the ROI, the complexity (length squared divided by blue area), skewness, as well as image features using a co-occurrence matrix: inverse difference moment, angular second moment (ASM), and entropy. In this study, HI-RTE showed a negative correlation with fibrotic stages and FibroScan® findings, suggesting that real-time tissue elastography is a better test than FibroScan®.

The group of Friedrich-Rust who published the first study using HI-RTE for the evaluation of liver fibrosis [2], performed a validation study of their own elasticity score and the elasticity score developed in Japan and compared the results of HI-RTE with TE [9]. They evaluated a cohort of 134 patients with histological evaluated chronic hepatitis (n=112) or proven liver cirrhosis (n=20) and showed that HI-RTE, in its present form, cannot replace TE for non-invasive assessment

of liver fibrosis.

In the study performed by Koizumi *et al.*, 70 patients with chronic HCV hepatitis were evaluated through HI-RTE, biological tests, liver biopsy and the Elastic Ratio [6]. The Elastic ratio was calculated (ratio of the value in the intrahepatic venous small vessels, divided by the value in the hepatic parenchyma) and compared with the histological stage of fibrosis on liver biopsy. HI-RTE cut-off values were: 2.73 for F ≥ 2; 3.25 for F ≥ 3 and 3.93 for F4. The AUROCs for elastic ratio (HI-RTE), hyaluronic acid, type IV collagen, aspartate aminotransferase-to-platelet ratio index, FibroIndex, Forns score and Hepascore were: 0.95, 0.32, 0.73, 0.76, 0.76, 0.87, and 0.70, respectively. In this study, the Elastic Ratio performed better than the serum fibrosis markers and the AUROCs for this method are promising (0.95).

Another study conducted by Hu *et al.* evaluated the utility of the Elastic Ratio for assessing liver fibrosis in ninety-six patients with chronic hepatitis B, compared with histological fibrosis stage on liver biopsy [10]. Using the optimal cut-off value of 2.62 for liver fibrosis S > 2; 3.20 for S >3; and 3.86 for S≥ 4, the corresponding area under the ROC curves were 0.91, 0.93, and 0.94, respectively. These findings are consistent with the study of Koizumi *et al.* in patients with chronic hepatitis C.

In the study of Tatsumi *et al.* that evaluated HI-RTE + Strain Histogram in 44 patients with chronic HCV infection as compared to liver biopsy and TE, HI-RTE was better at detecting the differences between milder stages of fibrosis: F1/F2, and F2/F3 as well [11]. On the other hand, Hi-RTE was more successful than FibroScan® in diagnosing the severity of fibrosis.

Gheonea *et al.* [5] performed a study on 97 patients diagnosed with chronic viral B and C hepatitis, liver cirrhosis, fatty alcoholic liver disease, as well as healthy volunteers. A Hitachi EUB 8500 ultrasound system was used, with an embedded elastography module (Hitachi Medical Systems Europe Holding AG, Zug, Switzerland) using a 6.5-MHz linear probe. Three 10 seconds long movie clips were captured in each patient by each of the 2 operators. Each recorded elastography movie was then evaluated by computer-enhanced dynamic analysis

using a public domain Java-based image processing tool (Image J) with a special plug-in, developed by the authors. The study showed a good inter-observer variability and there were no significant differences between the two physicians, regardless of the patients' real status.

The group of Fujimoto evaluated the effectiveness of HI-RTE for liver fibrosis assessment in a cohort of 310 patients with chronic C hepatopathies [12]. Nine image features were extracted from each RTE image and multiple regression analysis was performed to obtain an equation for the ***Liver Fibrosis Index*** (LF Index), which had 78.4% accuracy to discriminate between F0-1/F3-4 and 80.3% accuracy to discriminate between F0-3/F4.

Further improvements were developed in analyzing the elastographic images. Thus, in another Japanese study, the authors used for image evaluation a novel software developed by Hitachi Medical, Elasto ver. 1.5.1 [13]. They demonstrated the utility of Mean, SD, Area and Complexity as RTE parameters, speculating that Mean and Area may directly represent liver elasticity, while SD and Complexity may imply the collapse of the uniform architecture of liver parenchyma, concomitant with progressing hepatic fibrosis.

Another improvement in this technique was the development of the new HI VISION Preirus (Hitachi Medical Systems Europe Holding AG) system with embedded an elastography module. The probe (3.5-7MHz linear probe) is applied in an intercostal space without compression, with the patient lying supine. The strain graph displayed is used as a quality control of the procedure. The device automatically captures the internal compression transmitted to the liver parenchyma by the heart beats. Practically, this examination technique accredited in the last years includes several steps [14 - 16]:

- In order to accommodate the patient and to get a good section of liver, initially a standard ultrasound exam with the convex transducer is performed.

- Dual imaging is set in such a manner as to display both 2D image as well as an elastographic image juxtaposed over the 2D image.

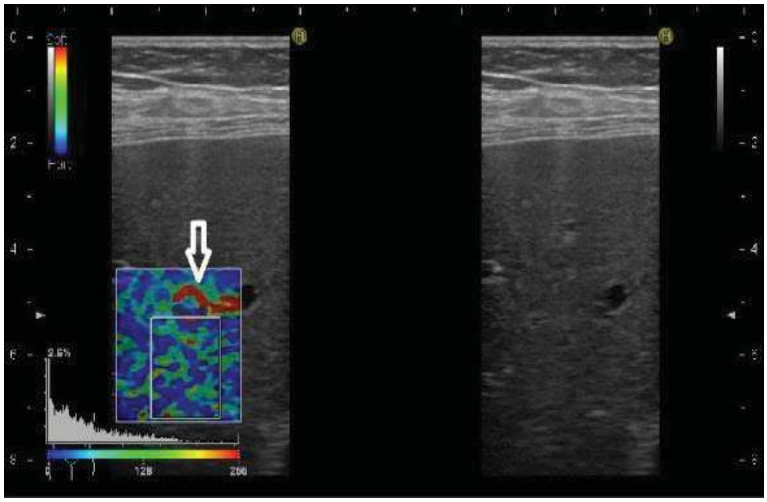
- The examination is performed on the right liver lobe, in the V-VIII intercostal

spaces, between the anterior and middle axillary line, with the transducer towards the direction of heart, while the patient is holding his breath, with constant compression on the probe. The strain induced into the liver tissues is dependent on the heart beats.

- The region of interest (ROI) is set inside the liver parenchyma, 1 cm under the liver capsule, in a selected box of 1/2.5 cm. The ROI is chosen so that the 2D image is as clear as possible, avoiding the large vessels and the RTE artifacts given by ribs and lung (Fig. **1a**, **1b**, **1c**).

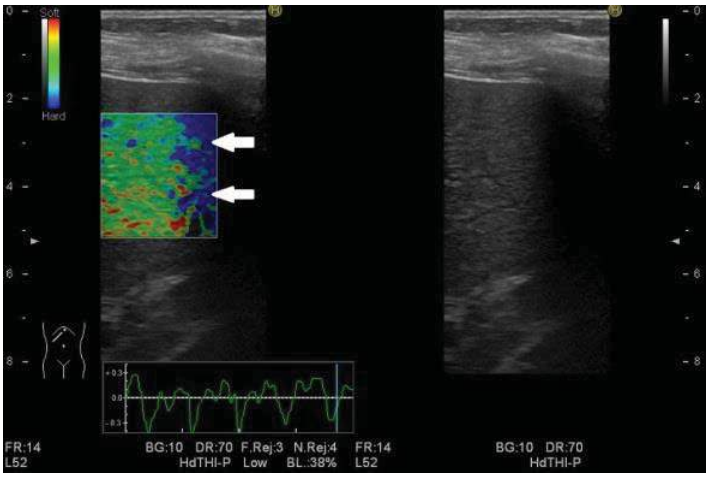
- For each patient, recording of 3 different clips that include a stable elastography image of at least 5 heart beats is required. A sequence on the negative pick is selected, and a value for each film is calculated (Fig. **2a**). **(a)**

The quantitative software elaborated by Hitachi provides many parameters from which two are the most important: Liver Fibrosis Index and mean strain histogram (mean of relative strain value within the ROI) (Fig. **2b**).

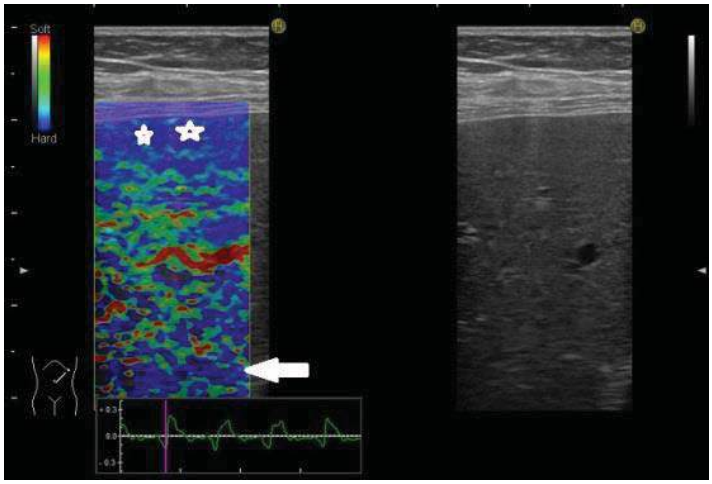


(a)

Fig. 1 contd.....



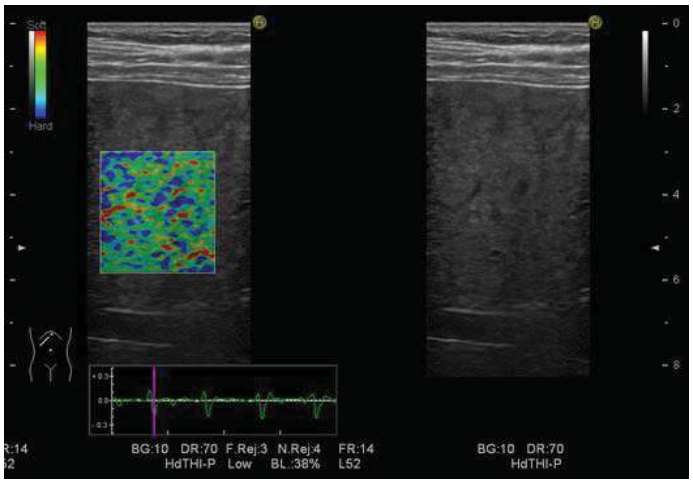
(b)



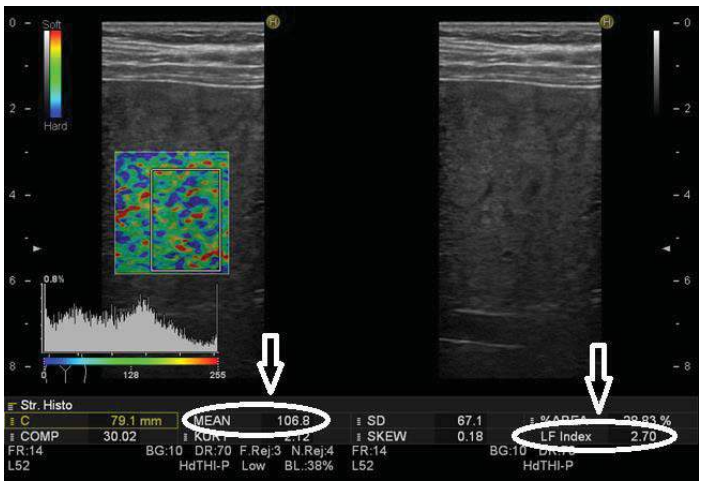
(c)

Fig. (1). Examination technique of HI-RTE. ROI is placed 1 cm under the liver capsule in order to avoid artifacts generated by vessels (**a**), rib shadows (**b**), capsular proximity or remoteness (**c**).

The ***new Hitachi Preirus system*** was used by Colombo *et al.* in a study including 45 patients with chronic liver diseases and 27 normal subjects, in whom they compared transient elastography (TE), HI-RTE and ARFI for liver fibrosis diagnosis [17].



(a)

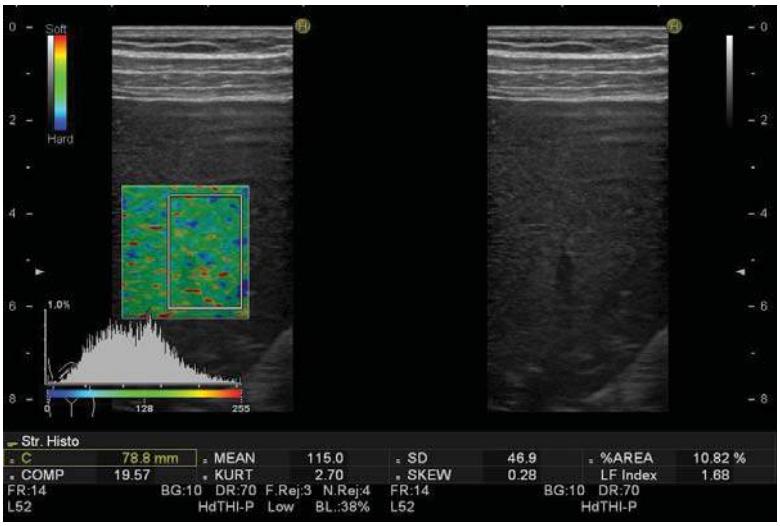


(b)

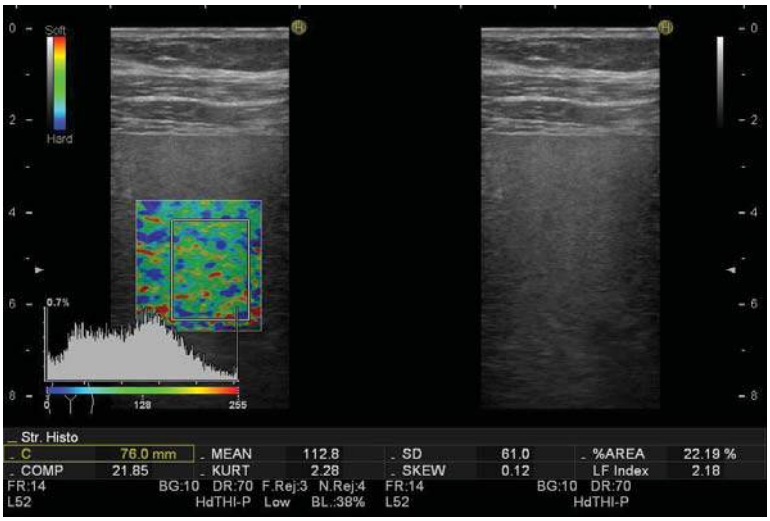
Fig. (2). Examination technique of HI-RTE. The displayed image is induced by internal compression of the heart beats (**a**). Parameters recorded in HI-RTE were Liver Fibrosis Index and mean strain histogram (**b**).

Ten static images were analyzed using the Elasto vers. 1.5.1 software, provided by Hitachi. The pixel distribution was represented by a histogram, from which eleven parameters were derived and analyzed by the software. Four main functions (Z1–Z4) were calculated and included in an integrative function, from which the common elastic index of RT-E was calculated, according to the formula: I =

(5.174Z1 + 2.154Z2 + 1.366Z3 + 0.985Z4). Results were expressed as the mean elastic index of all measurements.

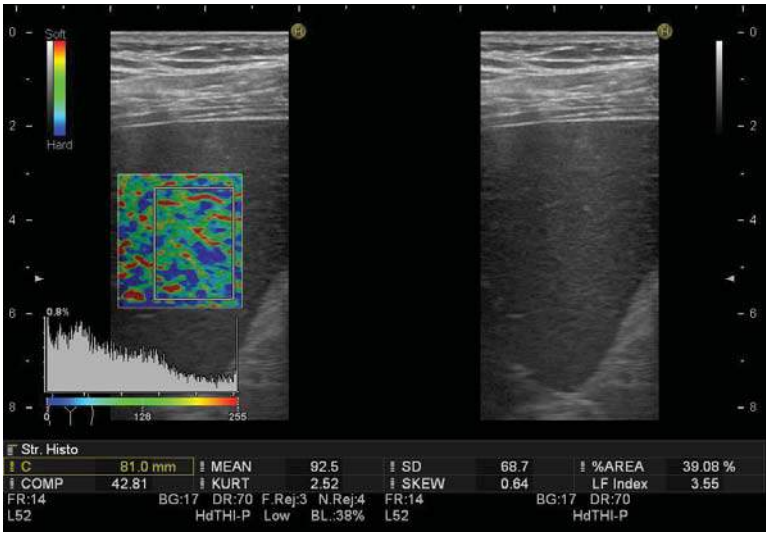


(a)

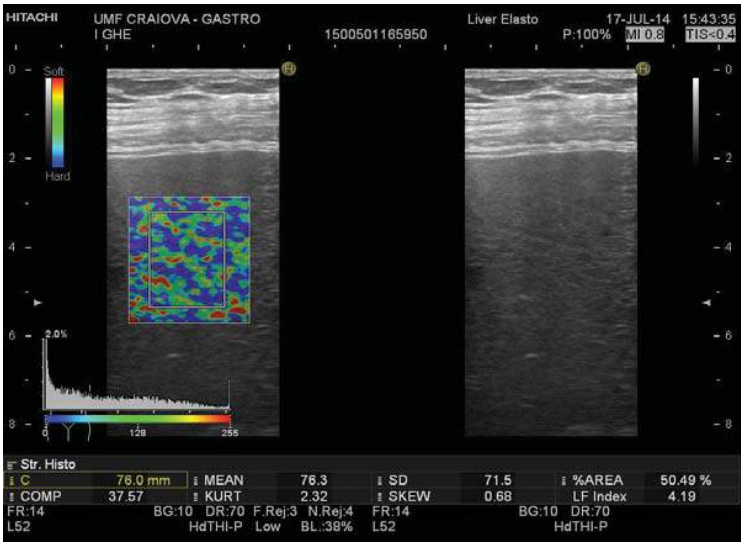


(b)

Fig. 3 contd.....



(c)



(d)

Fig. (3). HI-RTE images reflecting different stages of liver fibrosis in chronic viral hepatitis patients. From F1 to F4 fibrosis stage, color variation of the strain elastogram increases, from relatively low strain regions to a patched image pattern. F1 stage (**a**), F2 stage (**b**), F3 stage (**c**), F4 stage (**d**).

In this study, the performances of TE, HI-RTE and ARFI, expressed as AUROCs,

for predicting various stages of fibrosis were as follows: for predicting any fibrosis: TE 0.878, RTE 0.834 and ARFI 0.807 (no significant difference between the three curves); for predicting significant fibrosis: TE 0.897, RTE 0.751 and ARFI 0.815 (TE better than RTE with $p<0.01$, no significant difference between TE and ARFI, nor between ARFI and RTE); for predicting cirrhosis: TE 0.922, RTE 0.852, ARFI 0.934 (no significant difference between the three curves). The authors also specified that RTE and ARFI were feasible in all patients, while TE was unsuccessful (no valid measurements) in 15% of patients. Figs. (**3a**, **b**, **c** and **d**) represent strain elastograms for F1, F2, F3 and F4 respectively.

In another study performed on the same system by Wang *et al.* in patients with HBV chronic hepatitis, the Spearman's correlation coefficient between the elasticity index and the histological fibrosis stage was 0.81, which is highly significant ($p<0.001$) [18]. The AUROCs indicating diagnostic accuracy were 0.93 for F≥F1 ($p<0.001$), 0.92 for F≥F2 ($p<0.001$), 0.84 for F≥F3 ($p<0.05$) and 0.66 for F=F4 ($p>0.05$), respectively. These data are surprising since the accuracy seems to decrease with fibrosis severity (contrary to other elastographic methods).

Seventy-four patients with chronic liver diseases who had undergone a liver biopsy were analyzed in a study performed with Hitachi HI-Visions Preirus system by Chung *et al.* [19]. Ten valid measurements were performed in each patient and the elasticity score was determined on a scale of 0 to 5. TE, ARFI and RTE showed good correlations with histological fibrotic stages. The optimal cut-off values for significant fibrosis (≥F2) were 7.5 kPa for TE (AUROC=0.727), 1.19 m/s for ARFI (AUROC=0.715) and 2.54 for RTE (AUROC=0.507) ($p=0.0069$, $P=0.0277$). The optimal cut-off values for cirrhosis were 8.6 kPa for TE (AUROC=0.786), 1.39 m/s for ARFI (AUROC=0.807) and 2.79 for RTE (AUROC=0.767). TE, ARFI and RTE for predicting cirrhosis did not show significant differences from each other, while TE and ARFI had a better predictive value than RTE for predicting significant fibrosis (≥F2).

Liver Fibrosis Index (LFI), one of the most important parameters provided by Hitachi HI VISION Preirus software, was analyzed in many studies based in Asia, where the real-time elastography system is widely spread and LB is still a reference method for liver fibrosis assessment. Thus, Kim *et al.* found good AUCs

of LFI obtained by RTE: 0.683 and 0.744 for predicting advanced fibrosis (stage ≥ F3) and cirrhosis (stage F4), respectively, in a cohort of eighty-three patients with chronic hepatitis B or C [20]. The cut-off LFI value of >3.51 had 82.4% sensitivity and a 68.2% specificity for predicting cirrhosis (stage F4). In this study, RTE could discriminate between advanced fibrosis (F3) and cirrhosis (F4) more effectively than other serologic markers.

Fujimoto *et al.* studied 310 subjects with chronic hepatitis C and liver biopsy used as the gold standard [21]. In 15% of cases no valid measurements were obtained. LFI was calculated from image features of HI-RTE images, using multiple regression analysis performed on clinical data of 310 cases as the training data set. LFI highly correlated with fibrosis stages ($r=0.68$ with $p<0.001$). AUROC of LFI for F0-1 *vs.* F2-4 was 0.82. LFI seemed not to be correlated with inflammation in this study [21].

LFI was developed and validated initially in chronic hepatitis C patients. A large, multicenter study confirmed that LFI obtained through RTE is valuable for the diagnosis of hepatic fibrosis also in patients with chronic hepatitis B [22]. The AUROC of LFI for predicting significant fibrosis was 0.858, while for cirrhosis, it was 0.862.

Another study was conducted by Yada *et al.* on 245 patients with HCV and HBV chronic infection with liver biopsy used as the gold standard. The researchers used 9 parameters from the histogram and LFI. AUROC of LFI was 0.800 to discriminate between F0-1 *vs.* F2-4 and 0.846 between F0-3 *vs.* F4 [23].

Moreover, in 2014 the first ***meta-analysis*** regarding RTE was published, which included thirteen published studies [24]. The overall results suggested that LFI was excellent in diagnosing F≥3 and had moderate accuracy for F≥2 and F=4. However, LFI could not be applied to accurately differentiate F2 *versus* F0-1 and F=4 *versus* F0-3.

Another ***meta-analysis*** published by Kobayashi *et al.* in 2015 included 15 studies evaluating the diagnostic performance of RTE for staging liver fibrosis [25]. This meta-analysis demonstrated that RTE is not highly accurate for any cut-off stage of fibrosis; both summary sensitivity and specificity are roughly 0.80. Compared

with findings of meta-analyses on TE and ARFI, the overall accuracy of RTE seems to be nearly identical for the diagnosis of significant fibrosis (F≥2), but less accurate for the diagnosis of cirrhosis (F≥4).

Recently, two studies made on the phantoms showed that the quantitative techniques are superior to qualitative visual scoring in order to assess target stiffness by RTE [26, 27].

c. RTE for Liver Fibrosis Evaluation in Patients with Non-alcoholic Fatty Liver Disease (NAFLD)

Very little information is available to date. The group of Ochi evaluated the effectiveness of elastic ratio by RTE for liver fibrosis and portal hypertension assessment in a cohort of 187 patients with non-alcoholic liver disease [28]. Elastic ratio cut-off values by stage were: 2.47 for F1, 2.67 for F2, 3.02 for F3, and 3.36 for F4. Using these cut-off values, the diagnostic accuracies for fibrosis diagnosis were: 82.6% (F0 *versus* F1-F4), 92.3% (F0-F1 *versus* F2-F4), 94.7% (F0-F2 *versus* F3-F4) and 96.0% (F0-F3 *versus* F4), respectively. RTE could also be a useful tool for evaluating portal hypertension, but only 8 patients with NAFLD also had portal hypertension in this study.

d. RTE for Predicting Liver Cirrhosis Complications

Only one study was published regarding this topic to the best of our knowledge. Hirooka M *et al.* included 277 consecutive patients with chronic liver disease who underwent RTE of the spleen and correlated spleen elasticity and severity of portal hypertension with the hepatic venous pressure gradient (HVPG). The correlation between the two parameters was high (R = 0.855; 95% confidence interval: 0.767 - 0.911; P>0.0001) and the accuracy of diagnosing gastroesophageal varices was 90% when a cut-off value of 8.24 for splenic elasticity was used for predicting HVPG of more than 10 mm Hg [29].

Unlike other Ultrasound Societies, *The Japan Society of Ultrasonics in Medicine* (JSUM) state the importance of HI-RTE in the evaluation of liver fibrosis in the *JSUM ultrasound elastography practice guidelines*, published in 2013 [14]. In these guidelines, the authors believe that the liver fibrosis progression can be

easily observed visually or quantitatively by the use of the liver fibrosis index, elastic ratio, or strain ratio. In Japanese experts' opinion, HI-RTE accurately measures liver fibrosis with no regard to the adverse effects of ascites accumulation, inflammation, jaundice, and liver congestion.

In conclusion, RTE can find its applicability in the non-invasive assessment of liver fibrosis. RTE is readily available with the ultrasound machine, is easy to use, cost-effective and, moreover, painless. In the future, a large, prospective, international multi-center study is essential to achieve a further evaluation of the potential diagnostic value of HI-RTE.

Main advantages and weaknesses of liver fibrosis evaluation by means of HI-RTE:

Advantages	**Weaknesses**
-integrated into an standard ultrasound system - real-time elastographic method - feasible in patients with ascites, inflammation, jaundice, liver congestion - good results for non-invasive liver fibrosis evaluation in patients with chronic hepatitis B and C -promising results for non-invasive liver fibrosis evaluation in patients with fatty liver diseases	- inconsistency methods and data analysis among different research teams - a well trained examiner with sufficient experience is needed - not very accurate to differentiate patients without fibrosis and those with mild fibrosis and patients with moderate *vs.* mild fibrosis -no large multicenter studies are available

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare for this publication.

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CHAPTER 5

Combined Methods for Liver Fibrosis Evaluation

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Abstract: Biological tests, elastographic methods alone or in combination can be used for the non-invasive evaluation of chronic liver diseases, in order to increase their value.

Combinations of non-invasive tests were searched for in order to improve the diagnostic performance of significant fibrosis (F≥2) and severe fibrosis/cirrhosis (F3–F4) the most promising being *TE and serologic tests*. In chronic hepatitis C a clinical management algorithm was proposed, using the combination of TE (FibroScan®) and FibroTest as the first-line tests in the work-up strategy, thus avoiding liver biopsy in most patients (77%). In HBV inactive carriers, the combination of TE and FibroTest allowed the exclusion of significant fibrosis (F≥2) in nearly 80% of cases.

Another useful combination is of *two elastographic methods [TE and VTQ (ARFI)]*, which proved to be highly specific for predicting significant fibrosis (F≥2 Metavir). When both TE and VTQ (ARFI) values were higher than the proposed cut-offs, their combination had 93.3% Sp and 96.8% PPV for predicting F≥2, so that liver biopsy could be avoided in 60.5% of cases. For predicting cirrhosis (F4), the results were also very good, with 94.4% Sp, 94.4% NPV and 91.8% accuracy, so that the combination of TE and VTQ was able to confirm, and also to exclude the presence of liver cirrhosis.

Keywords: Combination methods, Elastography, Liver fibrosis, Serological tests.

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Since both serological tests and elastographic techniques are available for the non-invasive assessment of fibrosis severity in chronic liver diseases, many authors have tried to combine them to increase their diagnostic accuracy.

1. COMBINATION OF ELASTOGRAPHIC METHODS WITH SEROLOGICAL TESTS

FibroTest (a serological test that combines six biologic parameters) has been proved to be an accurate test to predict the presence of significant fibrosis (F≥2) as well as of severe fibrosis/cirrhosis (F3–F4) [1 - 3]. If TE and FibroTest results agreed (70 - 80% of cases), there was also a great similarity with liver biopsy results: 84% concordance in patients with significant fibrosis (F≥2); 95% concordance in patients with severe fibrosis (F≥3); and 94% concordance in cirrhotics (F=4).

Castera *et al.* evaluated the accuracy of two algorithms using non-invasive tests to predict liver fibrosis severity using liver biopsy (LB) as the gold standard: one including TE and FibroTest and the other including APRI and FibroTest (SAFE biopsy) [2]. The combination of TE and FibroTest saved 23% more liver biopsies than SAFE biopsy for predicting F≥2 Metavir (71.9% *vs.* 48.3%, $p<0.0001$), but its accuracy was significantly lower (87.7% *vs.* 97.0%, $p<0.0001$). The situation was reversed for predicting liver cirrhosis, where the accuracy of TE + FibroScan was significantly better than of SAFE biopsy (95.7% *vs.* 88.7% $p<0.0001$), while the number of saved biopsies was similar (78.8% *vs.* 74.8%; $p>0.05$).

Cross *et al.* performed a study that evaluated by TE and King score 187 patients with chronic hepatitis C, with LB considered as the reference method (Ishak score was used for staging liver fibrosis) [4]. The AUROCs for TE, King score and the combination of King score and TE for the diagnosis of significant fibrosis (F≥3 Ishak) were 0.83, 0.82 and 0.85, respectively, while for the diagnosis of cirrhosis (F≥5 Ishak) they were 0.96, 0.89 and 0.93, respectively. NPVs higher than 90% were obtained for the diagnosis of cirrhosis for the following cut-off values: 10 kPa for TE (NPV 98%); 24.3 for King score (NPV 91%); and 26.1 for the two combined (NPV 94%).

The combination of TE with FibroTest showed promising results in chronic

hepatitis C patients [5, 6] and also in HBV inactive carriers, in whom it allowed exclusion of at least significant fibrosis (F≥2) in approximately 80% of cases [7].

A number of 212 patients with chronic hepatitis C were evaluated in our department by means of LB, TE and serological tests (APRI score, Lok score, Forns score, FIB-4 score, Fibrosis Index score, King score, Bonacini score) [8]. The strongest correlation with liver fibrosis severity was observed for TE (r=0.62), King score (r=0.57) and APRI score (r=0.56). By multiple regression analysis, the following formula was obtained:

Prediction liver fibrosis score (PLF score) = 0.956 + 0.084 x TE – 0.004 x King score + 0.124 x Forns score + 0.202 x APRI score

The AUROCs of PLF score for predicting F≥1, F≥2, F≥3 and F=4 were 0.76, 0.78, 0.86, and 0.97 respectively. The PLF score had a better predictive value than TE for F≥2 Metavir (AUROCs 0.78 *vs.* 0.74, p=0.02); also for F≥3 Metavir (AUROCs 0.86 *vs.* 0.81, p=0.003), while for diagnosing cirrhosis the performance was similar (AUROCs 0.97 *vs.*0.97, p=0.28).

Liu *et al.* evaluated 111 subjects (95 with chronic hepatitis B and 16 healthy volunteers), by means of VTQ (ARFI), TE and APRI score [9]. Strong correlations were observed between fibrosis stage and ARFI (r=0.85, p <0.001), between fibrosis stage and TE (r=0.81, p <0.001) while only a moderate correlation was found between fibrosis stage and APRI (r=0.63, p <0.001). An optimal linear combination (LC) of the three methods was developed, and its diagnostic performance was evaluated by a 10-fold cross-validation:

LC: For F≥2: ARFI + 0.034 TE – 0.084 APRI

For F4: ARFI + 0.044 TE – 0.135 APRI

The calculated accuracies of LC for significant fibrosis (≥F2 Metavir) and cirrhosis (F4) were 83.86% and 91.88%, respectively, better than those of VTQ (ARFI) (83.50% and 88.76%, respectively); of TE (75.27% and 87.61%, respectively); and also than those of APRI score (73.29% and 81.67%, respectively) [9].

Takaki *et al.* evaluated 176 patients with chronic hepatitis by means of liver biopsy, VTQ (ARFI) and simple serological tests [10]. In the training set (120 subjects), LS expressed as Shear Waves Velocity (SWV) assessed by VTQ (ARFI), the INR and ALT independently and significantly correlated with liver fibrosis severity. Based on this data *VIA* index score was calculated.

***VIA* index** = - 1.282 + 0.965 × SWV + 1.785 INR + 0.00185 ALT

In the training set, an AUROC of 0.838 was calculated for *VIA* index as a predictor of significant fibrosis (F≥2); 0.904 for prediction of severe fibrosis (F≥3) and 0.958 for prediction of cirrhosis (F4). In the validation set (56 subjects), the AUROCs were 0.917 for F≥2, 0.906 for F≥3 and 1.000 for F4, respectively.

2. COMBINATION OF ELASTOGRAPHIC METHODS

In a study comprising 197 HCV patients, our group evaluated the ***combination of TE and VTQ (ARFI)*** [11]. This combination was highly specific for predicting significant fibrosis (F≥2 Metavir). If LS values obtained by both elastographic techniques were higher than the cut-off values of 6.7 kPa for TE and 1.2 m/s for VTQ (ARFI) (currently used for predicting F≥2), the combination had 93.3% Se and 96.8% PPV for F≥2, so that liver biopsy cold be avoided in those patients (60.5% of cases). Also, by combining the two elastographic methods for predicting cirrhosis (F4) (TE ≥12.2 kPa and VTQ (ARFI) ≥1.8 m/s), the results were very good, with 94.4% Sp, 94.4% NPV and 91.8% accuracy, so the combined methods are not only able to confirm, but also to exclude the presence of cirrhosis.

TE has been recommended in France - by the Hâute Autorité de Santé, as a first line method for liver fibrosis assessment in patients with chronic hepatitis C and no co-morbidities [12]. With regard to the other elastographic methods, such as VTQ (ARFI), studies have demonstrated its non-inferiority in comparison with TE [13, 14]. Probably, by combining different non-invasive methods for LS evaluation, the accuracy of those methods will improve.

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare for this

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CHAPTER 6

Comparison of Elastographic Techniques

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Abstract: Several elastographic techniques for liver fibrosis assessment are available (on different machines) and practitioners are interested in comparing these techniques with regard to feasibility but also with regard to accuracy in staging fibrosis. Comparative studies including at least three methods are presented in this chapter. Regarding feasibility, the most feasible technique seems to be ElastPQ (approximately 99%), followed by VTQ (approximately 93%) and TE and 2D-SWE (approximately 87%). VTQ, ElastPQ and 2D-SWE had similar accuracies for diagnosing at least significant fibrosis (F≥2) and cirrhosis (F4) considering TE as the reference method.

Keywords: ARFI elastography, Comparative studies, Liver elastography, Transient elastography, 2D-SWE elastography.

At this moment, when many elastographic techniques for liver fibrosis assessment are available (on different machines), practitioners are interested in published data comparing these techniques not only with regard to feasibility but also with regard to accuracy when compared to liver biopsy. Not so many comparative studies have been published to date. We will present in this chapter studies comparing at least three elastographic techniques.

In a study performed in France, 349 consecutive patients with chronic liver diseases underwent liver biopsy and liver stiffness assessment by 2D-SWE (Aixplorer® - Supersonic Imagine), ARFI technology (VTQ - Siemens) and TE

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(FibroScan® - Echosens®) (M probe for patients with BMI < 30kg/m^2 and XL probe for patients with BMI > 30kg/m^2) [1]. AUROCs were calculated and compared for each stage of fibrosis. 2D-SWE, TE, and VTQ correlated significantly with histological fibrosis score (r=0.79, p<.00001; r=0.70, p<.00001; r=0.64, p<.00001, respectively). In this study, AUROCs of 2D-SWE, TE and VTQ were 0.89, 0.86, and 0.84 for mild fibrosis; 0.88, 0.84, and 0.81 for significant fibrosis (F≥2); 0.93, 0.87, and 0.89, for severe fibrosis (F≥3) and 0.93, 0.90, and 0.90 for the diagnosis of cirrhosis, respectively. 2D-SWE had a higher accuracy than FibroScan® for the diagnosis of severe fibrosis (≥F3) (p=0.0016), and a higher accuracy than VTQ for the diagnosis of significant fibrosis (≥F2) (p=0.0003). Finally, no significant differences were observed for the diagnosis of mild fibrosis and cirrhosis using the three elastographic methods.

For daily practice, feasibility of ultrasound based elastography is crucial, so as to be able to evaluate a vast majority of patients that enter an elastography laboratory. In a comparative study performed by our team [2] we aimed to compare the feasibility of four elastographic methods used for liver fibrosis evaluation (Transient Elastography - TE; point Shear Waves Elastography (pSWE) using ARFI technique - VTQ and ElastPQ techniques, respectively; and 2D-SWE).We included in our study 151 consecutive subjects with or without chronic hepatopathies (excluding patients with ascites), in which liver stiffness (LS) was evaluated in the same session by means of 4 elastographic methods: TE (FibroScan®, Echosens®®), VTQ (Siemens Acuson S2000™), ElastPQ (Philips, Affinity) and 2D-SWE (Aixplorer®, SuperSonic Imagine S.A). Reliable LS measurements were defined as follows: for TE and VTQ – the median value of 10 LS measurements with a success rate ≥ 60% and an interquartile range < 30%, for 2D-SWE – the median value of 3 LS measurements acquired in an homogenous area and for ElastPQ - the median value of 10 LS measurements. For TE, M and XL probes were used. LS was expressed in kPa for TE, 2D-SWE, ElastPQ and in m/s for VTQ. All elastographic measurements were performed by experienced operators. In this study, reliable LS measurements were obtained in a significantly higher proportion of patients by means of ElastPQ as compared with TE, 2D-SWE and VTQ: 99.3% *vs.* 87.4% (p<0.0001), 99.3% *vs.* 87.4% (p<0.0001) and 99.3% *vs.* 92.7% (p=0.08). TE and 2D-SWE had similar rates of reliable LS

measurements 87.4% *vs.* 87.4% (p=0.86). Reliable LS measurements by all four shear waves ultrasound elastographic methods were obtained only in 72.2% (109/151) subjects. For TE and VTQ we used technical quality criteria (IQR and SR), but for the other two methods (ElastPQ and 2D-SWE) no quality criteria were used since none were published.

In another comparative study performed by our group [3] we compared the performances of point Shear Waves Elastography using ARFI technique (VTQ and ElastPQ, respectively) and 2D-SWE (SuperSonic Shear Imaging) considering Transient Elastography (TE) as the reference method. We included in this study 151 consecutive subjects (with or without chronic hepatopathies, none with ascites), who were evaluated in the same session by means of 4 elastographic methods: TE (FibroScan®, Echosens®®), VTQ (Siemens, Acuson S2000™), ElastPQ (Philips, Affinity) and 2D-SWE (Aixplorer®, SuperSonic Imagine S.A). For differentiating between stages of liver fibrosis we used the following cut-off values: for TE - significant fibrosis (F≥2) – 7.2 kPa and for liver cirrhosis (F4) - 14.5kPa [4]; for VTQ: F≥2 – 1.35m/s, F4=1.84m/s [5]; for 2D-SWE: F≥2 – 7.1 kPa, and F4=13.5 kPa (HCV,NAFLD) and 11.5 kPa in HBV [6]; and for ElastPQ F≥2-5.9 kPa, F4=12kPa [7]. In this study, considering TE as the reference method, the diagnostic accuracy of VTQ, 2D-SWE and ElastPQ for the diagnosis of absence or mild fibrosis (F<2) was similar: VTQ *vs.* 2D-SWE (86.2% *vs.* 82.5% p=0.57); VTQ *vs.* ElastPQ (86.2% *vs.* 84.4% p=0.85), 2D-SWE *vs.* ElastPQ (82.5% vs. 84.4% p=0.84). For significant fibrosis (F≥2) the values obtained were: VTQ vs. 2D-SWE (84% vs. 76.1% p=0.19); VTQ vs. ElastPQ (84% vs. 80.7% p=0.64), 2D-SWE vs. ElastPQ (76.1% vs. 80.7% p=0.50). For diagnosing cirrhosis we also obtained similar diagnostic accuracies: VTQ *vs.* 2D-SWE (96.3% *vs.* 93.6% p=0.54); VTQ *vs.* ElastPQ (96.3% *vs.* 94.5% p=0.75), 2D-SWE *vs.* ElastPQ (93.6% *vs.* 94.5% p=0.99). In this study, similar to previously published papers, the accuracy of elastographic methods increased with the severity of fibrosis, producing the best results in patients with liver cirrhosis. Finally, the conclusion of this study was VTQ, ElastPQ and 2D-SWE had similar accuracies for diagnosing at least significant fibrosis (F≥2) and cirrhosis (F4).

In another comparative study performed by our group [8] we aimed to compare

the performance of several ultrasound elastographic techniques and FibroTest in diagnosing compensated HCV liver cirrhosis (LC). At this moment, expensive treatments for HCV patients are prioritized considering the severity of fibrosis, and patients with compensated LC are the first to be treated. In this prospective study which included 40 consecutive patients diagnosed with LC by means of liver biopsy, TE (LS > 12.5 kPa [9]) or by clinical, biologic, ultrasonography and endoscopic criteria, all were evaluated with five elastographic techniques in the same session, while FibroTest was performed within a month of the elastographic methods. LS as a marker for fibrosis was assessed by: TE - FibroScan®, Echosens®®; by Point shear Waves elastography techniques: VTQ - Acuson S2000, Siemens and by ElastPQ technique - Affinity, Philips; and by 2D-SWE - Aixplorer®, Supersonic Imagine (SSI) and with the LogiqE9, General Electric (2D-SWE GE) system. In each patient we performed 10 valid measurements (VM) for TE, VTQ, ElastPQ and 2D-SWE.GE, and 3 valid measurements for SSI. For each elastographic technique the median value of VM was calculated. The following published cut-offs were used to diagnose cirrhosis: TE-12.5 kPa [9]; VTQ-1.81 m/s [5]; ElastPQ-12 kPa [7]; SSI-13.5 kPa [6]; 2D-SWE.GE-11.9 kPa [10]. In this study we compared the proportion of correctly classified patients by all non-invasive tests. In all patients, we obtained VM by all five elastographic methods. Subjects were correctly classified by: TE in 97% of cases, VTQ-97%, ElastPQ-82%, SSI-90%, 2D-SWE.GE-90% and FibroTest-85%. There were no significant differences between FibroTest - TE (85% *vs.* 97%, p=0.13), FibroTest - VTQ (85% *vs.* 97%, p=0.13), FibroTest - ElastPQ (85% *vs.* 82%, p=0.95), FibroTest - SSI (85% *vs.* 90%, p=0.73), FibroTest - 2D-SWE.GE (85% *vs.* 90%, p=0.73) respectively. The conclusion of this study was that all ultrasound based elastographic methods performed well for the diagnosis of compensated liver cirrhosis and thus can be used in daily practice.

All the studies presented in this chapter show the necessity of head to head comparison of different ultrasound based elastographic method for daily practice. For practitioners, a question has arisen: should one or more elastographic methods be used in daily practice [11]. And our answer is that many elastographic methods have proved their value for liver fibrosis assessment and very soon will be validated for daily practice.

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare for this publication.

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CHAPTER 7

Elastography in Focal Liver Lesions

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Abstract: The accurate characterization and the differential diagnosis between different types of focal liver lesions (FLL) are important aims that all imaging modalities available today should satisfy. Elastographic methods aim to exploit the elasticity differences between FLL and liver parenchyma in order to make the differential diagnosis between malignant and benign lesions. Currently, three elastographic methods have been evaluated and showed their applicability in this area: Acoustic Radiation Force Impulse (ARFI) Elastography, Real-time Elastography (RT-E) and Shear Waves Elastography (SWE). Many studies have shown that using one of the elastographic methods, for a chosen cut-off, the differentiation between malignant and benign nodules is possible. Other studies demonstrated that elastographic techniques are helpful to detect recurring hepatocellular carcinomas (HCCs), or to evaluate HCC or liver metastases after local or systemic treatment.

Keywords: Benign or malignant, Elastography, Focal liver lesions.

A focal liver lesion (FLL) refers to an area of damaged tissue identified into the hepatic tissue, with varying significance, depending on the patient's health condition and a variety of other factors. The differential diagnosis of a FLL can be narrowed down by several factors, including age, gender, use of birth control pills or hormone medications, travel history and the presence of cirrhosis, hepatitis or other chronic liver diseases. In many cases, FLLs are detected incidentally, during a routine abdominal ultrasound examination.

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FLLs are classified as ***benign*** or ***malignant***.

Benign (noncancerous) FLLs can be *solid* or *cystic* (meaning that the lesions are fluid filled). Within these types, the subtypes include hemangiomas (the most common), focal nodular hyperplasia (FNH), hepatic adenoma, focal fatty changes, and hydatid cysts and bile duct cysts.

Malignant liver tumors can be *primary* liver cancers or *secondary* liver lesions (metastases).

The most common *primary* malignant liver tumor is hepatocellular carcinoma (HCC) and the second most common type of liver malignancy is cholangio-carcinoma. Other rare liver cancers are: angiosarcomas and hepatoblastomas.

The liver is one of the most often affected organs in advanced cancers and most types of malignant tumors may spread into the liver in the late stages. The most common *secondary* liver tumor is colon cancer metastasis, but other cancers (such as pancreatic, gastric, thyroid, skin and kidney cancer) often spread into the liver.

The accurate characterization and the differential diagnosis between different types of FLLs are important aims, that all imaging modalities available today should satisfy [1].

Conventional ultrasonography (US) is often the first imaging modality performed to screen for, or to study hepatic lesions because of its low cost and wide availability. Color-Doppler, Tissue Harmonic Imaging and more recently, microbubble contrast agents (Contrast Enhanced Ultrasound-CEUS), have significantly improved the characterization of solid FLL. Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are second line imaging methods able to accurately characterize previously detected lesions, but they are more expensive and less available. Contrast enhanced imaging modalities, such as contrast-enhanced US, contrast enhanced-CT and contrast-MRI, assess lesion morphology and vascularization, with a high diagnostic accuracy owing to their specific features, well described in the literature. Nevertheless, invasive studies are sometimes required to make a definite diagnosis [1].

Neoplastic and inflammatory diseases can change the tissue's

composition/structure, and thus parenchyma stiffness of an organ. Elastography aims to assess these elasticity differences in order to be able to identify malignant transformation [2].

Many elastographic methods have tried to assess liver tumors' stiffness.

1. POINT SWE USING ACOUSTIC RADIATION FORCE IMPULSE (ARFI) TECHNOLOGY

Point SWE using Acoustic Radiation Force Impulse (ARFI) technology is an elastomeric technique incorporated into a conventional ultrasound (US) system, which permits real-time non-invasive quantification of tissue elasticity during US B-mode examination.

In order to evaluate such a lesion by VTQ (ARFI) technology, the FLL has to be visualized in abdominal US. After that the measurement box is placed in the lesion (Fig. **1**) and VTQ (ARFI) measurements are performed (median value of 10 acquisitions expressed in m/s). VTQ (ARFI) measurements should also be performed in the surrounding tissue.

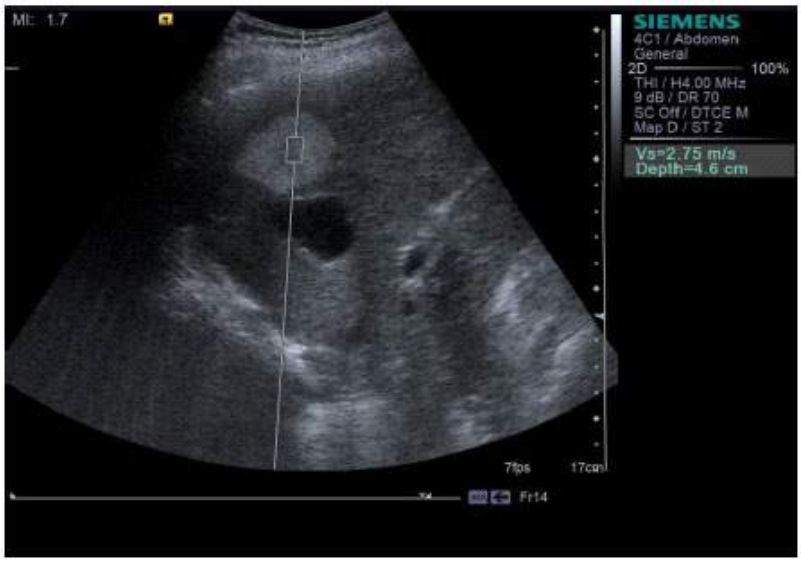

Fig. (1). VTQ (ARFI) measurement in hemangioma.

a. VTQ (ARFI) in Benign FLL

Hemangiomas are the most frequent benign FLLs. Imaging methods are very sensitive in the diagnosis of hemangiomas, starting with standard US, followed by CEUS, CE-CT or CE-MRI. In VTQ (ARFI) evaluation, a high variability of Virtual Touch Quantification (VTQ) values was observed for this type of lesion: **2.30 m/s**-Gallotti *et al.* [1], **1.30 m/s**-Zhang *et al.* [3], **1.83 m/s**-Park *et al.* [4]. Its stiffness depends on the amount of fibrotic septa which divide the dilated vascular spaces.

Focal Nodular Hyperplasias (FNH) was reported to be *the stiffest* lesions after metastases and cholangiocarcinomas, regardless of their dimensions and of the presence or absence of central scar (due to the presence of fibrotic content) (Fig. **2**).

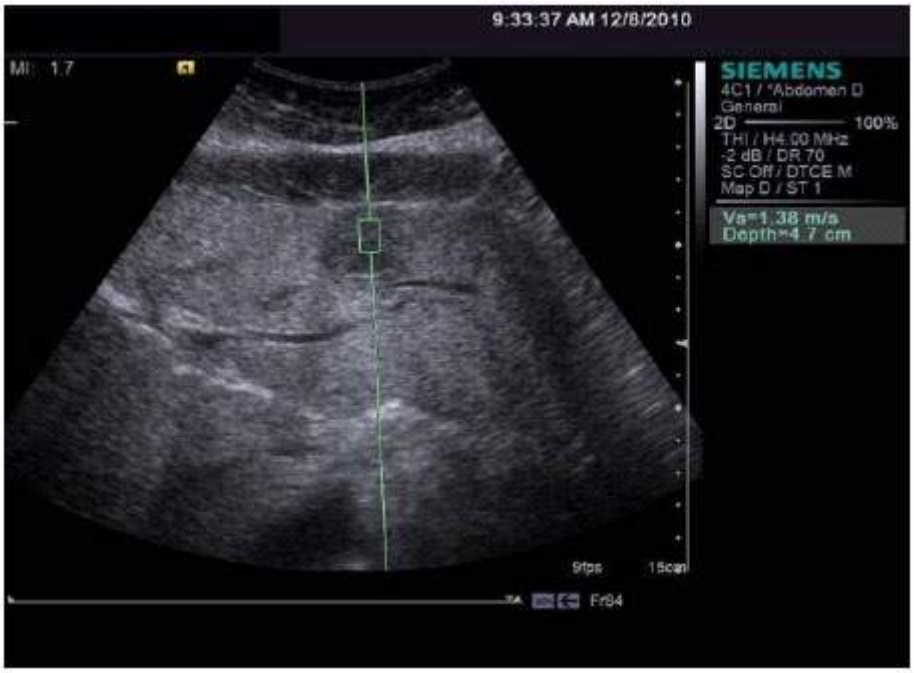

Fig. (2). VTQ (ARFI) measurement in Focal Nodular Hyperplasia.

Adenomas showed similar VTQ values to those observed in the surrounding liver (mean VTQ value of the lesion 1.25 m/s; mean VTQ value of the surrounding

parenchyma 1.40 m/s) [1].

In a multicenter prospective study performed by Gallotti *et al.*, 40 lesions were evaluated and a total of 400 measurements were obtained [1]. The lesions were: 6 HCCs (15%), 7 hemangiomas (17.5%), 5 adenomas (12.5%), 9 metastases (22.5%) and 13 focal nodular hyperplasias (32.5%). A significant difference ($p<0.05$) was found by comparing tissue stiffness in adenomas *vs.* other lesions. Their conclusion was that VTQ (ARFI) could provide significant information regarding tissue stiffness, useful for FLL differential diagnosis.

b. VTQ (ARFI) in Malignant FLL

Almost all ***HCCs*** evaluated *appeared as softer lesions* compared to the surrounding cirrhotic liver [1, 5] (mean VTQ value 2.17 m/s *vs.* 2.99 m/s respectively) (Fig. **3**).

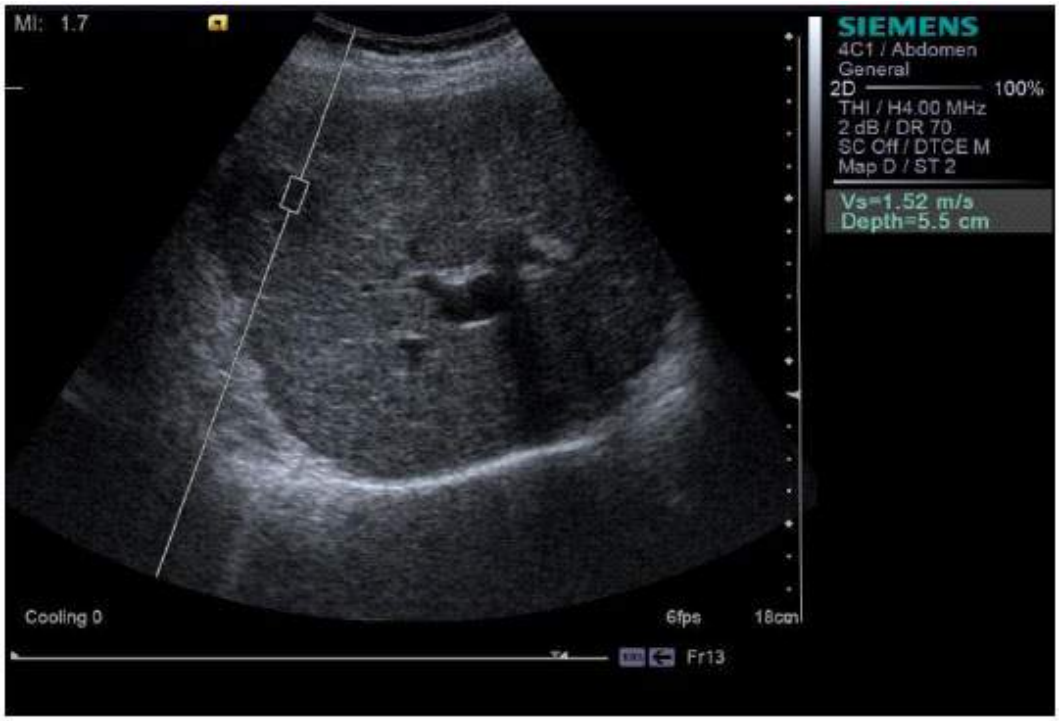

Fig. (3). VTQ (ARFI) measurement in HCC.

All ***metastatic lesions*** (Fig. **4**) ***and cholangiocarcinomas*** *were stiffer* than the surrounding liver: mean VTQ value of the lesion 2.87 m/s; mean VTQ value of the surrounding parenchyma 1.63 m/s) [1].

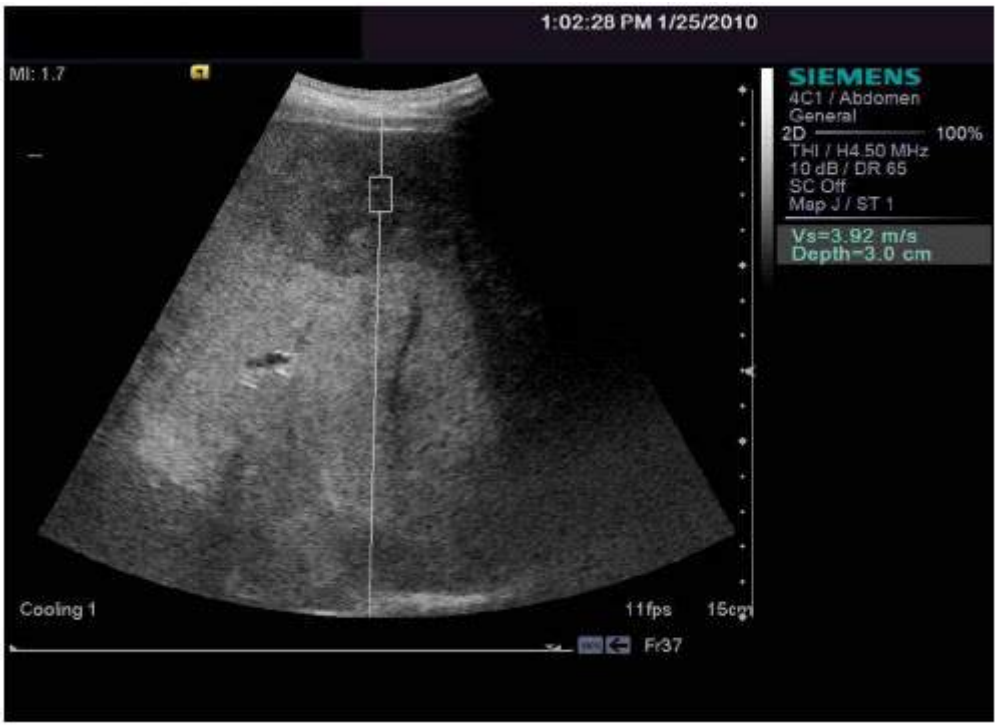

Fig. (4). VTQ (ARFI) measurement in liver metastasis.

Cho at all showed that in 72% of cases metastatic lesions and cholangiocarcinomas were stiffer than the surrounding liver [6]. In the same study which included 51 patients with 60 FLLs (17 hemangiomas, 25 HCCs, 15 metastases and three cholangiocarcinomas) a cut-off value of 2 m/s was obtained for malignancy, with an 89% positive predictive value and 81% specificity. Images obtained with VTQ (ARFI) elastography helped in tumor characterization regarding the stiffness and margins of liver tumors. By measuring shear Waves velocity it was possible to differentiate malignant hepatic tumors from hepatic hemangiomas.

Our group [7] evaluated 59 FLL: 40 HCCs, 10 liver metastases, 7 hemangiomas, 1 adenoma and 1 focal fatty lesion. Tumor stiffness (TS) was significantly lower in

HCCs than in the surrounding liver parenchyma (2.26±0.98m/s *vs.* 2.71±0.68m/s, p=0.01), but significantly higher in metastases (Fig. **2**) than in the liver parenchyma (2.82±1.11m/s *vs.* 1.69±0.64m/s, p=0.01). TS was significantly higher in metastases than in hemangiomas (2.82±1.11m/s *vs.* 1.47±0.67m/s, p=0.01) and in HCCs *vs.* hemangiomas (2.26±0.98m/s *vs.* 1.47±0.67m/s, p=0.04), but not significantly different in HCCs *vs.* metastases (2.26±0.98m/s *vs.* 2.82±1.11m/s, p=0.12). Using VTQ for ARFI measurements we found significant differences between the surrounding liver parenchyma and malignant FLLs, but this method cannot distinguish malignant from benign FLL.

Shuang-Ming *et al.* demonstrated in a study on 116 consecutive patients with 128 liver lesions (60 benign, 68 malignant) that VTQ (ARFI) can differentiate benign and malignant liver lesions [8]. For a cut-off value of 2.22 m/sec, 89.7% sensitivity, 95% specificity and 92.2% accuracy were observed for the diagnosis of malignancy.

Yu *et al.* evaluated by VTQ (ARFI) 89 patients with 105 FLL (28 HCCs, 13 metastasis, 35 hemangiomas, 15 focal nodular hyperplasias, 8 focal fatty sparing, 4 focal fatty deposits and 2 adenomas [9]. VTQ (ARFI) values showed significant differences between benign (1.73 ± 0.8 m/s) and malignant FLL (2.57 ±1.01 m/s) (P<0.001). For differentiation of malignant from benign nodules, TS measurements had 68% sensitivity, 69% specificity, 58% positive predictive value and 77% negative predictive value, if 1.9 m/s was chosen as a cutoff value. If the cut off chosen value was 2.72 m/s the sensitivity, specificity, positive predictive value and negative predictive value were 69%, 89%, 56%, and 93%, respectively.

Kapoor *et al.* performed a study designed to evaluate the role of VTQ (ARFI) in differentiating metastatic from non-metastatic liver nodules [10]. The study comprised 48 patients with liver nodules. Nodule stiffness was determined by real-time elastography (ES) using color maps and shear Waves velocity (SWV) (ARFI measurements). Nodules with marked stiffness or SWV higher than 2.5 m/s were diagnosed as metastatic. Fine needle aspiration cytology was used for the final diagnosis. There were no significant differences seen on elasto-maps in the stiffness of metastatic and non metastatic nodules (p=0.16), while SWV showed significant differences in the strain velocities of benign, metastatic and

hepatocellular carcinoma nodules p <0.0001 and p <0.008 respectively. If a cut off value of 2.5 m/s was chosen for SWV, the sensitivity, specificity and false positive to detect metastatic nodules by ES were 88%, 83% and 16%, respectively. When the SWV cut off value was set at 2.0 m/s, the sensitivity, specificity and false positive were 94%, 70% and 29%, respectively. The study showed that SWV was a useful tool in diagnosing both solid and necrotic metastatic liver nodules as compared with the color stiffness maps alone.

c. VTQ (ARFI) Ratio

VTQ (ARFI) Ratio the ratio between the median VTQ (ARFI) value in the liver and in the lesion seems to be more accurate in the evaluation of FLL. Lu *et al.* demonstrated that *diagnostic performance with stiffness values was significantly lower than that with stiffness ratio for discrimination of metastasis from primary liver cancers* [11].

VTQ (ARFI) seems to be a useful method in the following scenarios:

- for differential diagnosis between adenomas and FNHs;

- for the study of metastases;

- for the study of HCCs in cirrhotic liver [12].

Further studies are required in order to find the correct place of VTQ (ARFI) elastography in everyday clinical practice.

2. REAL-TIME ELASTOGRAPHY (RTE)

Real-time Elastography (RTE) has proven its utility in differentiating between benign and malignant pancreatic lesions and lymph nodes [13 - 15]. It was also proved to be useful in differentiating solid tumors located in the wall or nearby the gastrointestinal tract which can be also visualized and characterized by endoscopic ultrasound elastography [15]. There are several studies showing the usefulness of RTE in FLL characterization.

Gheorghe *et al.* performed a study in which it was demonstrated that US elastography is a promising method for the non-invasive diagnosis of early HCC

[16]. The study included 42 cirrhotic patients with 58 nodules (1-3 cm) which were evaluated by means of real-time elastography (Hitachi EUB-6500) - the mean colors intensity (red, blue, green) were measured using a semi-quantitative method. Histograms analysis for each color was performed in order to quantify the nodule elasticity as compared with the surrounding cirrhotic liver tissue. Mean intensity of blue color proved to be a good diagnostic tool for HCC (AUROC=0.94); for a cut-off value>128.9, 92.2% sensitivity, 78.9% specificity, 95.4% PPV and 68% NPV were observed.

Kato *et al.* studied the intra-operative application of RTE for the diagnosis of liver tumors [17]. Fifty-five liver tumors in 44 patients were examined with RT-E, concomitantly with routine intra-operative ultrasonography. Elasticity images were classified into four types: type A (even strain) to type D (no strain), according to the distribution and the strain level contrasted with that of the surrounding liver (***elasticity type of liver tumor*** (ETLT)). Twenty-one of 22 HCCs were classified as type B (with a sensitivity of 95.5%, a specificity of 90.9% and an accuracy of 92.7%), while all 24 metastatic adenocarcinomas were classified as either type C or type D (with a sensitivity of 100%, a specificity of 80.6% and an accuracy of 89.1%). Using a new criterion, ETLT, RTE was able to distinguish rather accurately between HCC and metastatic adenocarcinoma.

Fukuda *et al.* showed that common liver tumors had their own strain patterns which could help to make a differential diagnosis [18]. The study included 47 liver tumors (14 HCCs, 12 metastatic liver tumors and 21 hemangiomas). The strain images obtained by RTE were classified into five groups in contrast to the surrounding liver: category 1 (even strain pattern), category 2 (less strain area in the tumor <50%), category 3 (less strain area = about 50%), category 4 (50% <less strain area <90%) and category 5 (less strain area >90%). Of 14 HCCs, 12 were category 4. Of 12 metastatic liver tumors, 11 were category 4 or 5. Of 21 hemangiomas, 17 were category 1 or 2. RT-E revealed that HCC or metastatic liver tumors had less strain than the surrounding liver; in contrast, most hemangiomas did not.

Inoue Y studied the usefulness of RTE for the intra-operative characterization of small FLL [19]. In this study, 27 adenocarcinomas, 18 HCCs and 11 benign

lesions were included. Elasticity images were also classified into 4 types, from type A (more, or comparable strain relative to the background) to type D (no strain). Fourteen of the 18 HCCs were type B or C (with 83% sensitivity, 76% specificity and 61% accuracy), while 22 of the 26 adenocarcinomas were type D (with 85% sensitivity, 86% specificity, and 86% accuracy). For 15 lesions, clear images in B-mode intra-operative US (IOUS) were difficult to obtain, whereas RTE clearly visualized the elasticity differences. His conclusion was that the new RTE system serves as a supportive modality for B-mode IOUS.

Strain Elastography in the Evaluation of FLL: the strain index value (strain ratio of liver parenchyma *vs.* focal lesions) of each lesion was calculated. Mean strain index values of benign and malignant liver lesions were compared. The mean strain index value of malignant liver lesions ± SD (2.82 ± 1.82) was significantly higher than that of benign liver lesions (1.45 ± 1.28; P< .0001). Hemangiomas had a significantly lower mean strain index value than other benign lesions (P < .00 34). There was ***no significant difference between strain index values of different types of malignant lesions*** (P > .05) [23].

3. 2D-SHEAR WAVES ELASTOGRAPHY (2D-SWE)

2D-Shear Waves Elastography (2D-SWE) is another new elastographic technique used in FLL characterization.

In the study of Guibal *et al.* [20] whose purpose was to describe elastic map characteristics for FLLs assessed by 2D-Shear Waves Elastography (2D-SWE), 106 hepatic lesions in 85 patients were included (41 benign and 65 malignant): 17 hemangiomas (Fig. **5**), 14 focal nodular hyperplasias (FNHs), 10 adenomas, 16 HCCs including 2 HCCs in normal liver, 43 metastases (Fig. **6**), 6 cholangiocarcinomas. Tumor heterogeneity was assessed on elasticity 2D maps and tumor and parenchyma elasticity values were also quantified. Significant differences in stiffness were observed in:

- FNHs (33±14.7 kPa) *vs.* adenomas (9.4±4.3 kPa) (p=0.0002);

- HCCs (14.86±10 kPa) *vs.* CCCs (56.9±25.6 kPa) (p=0.0004).

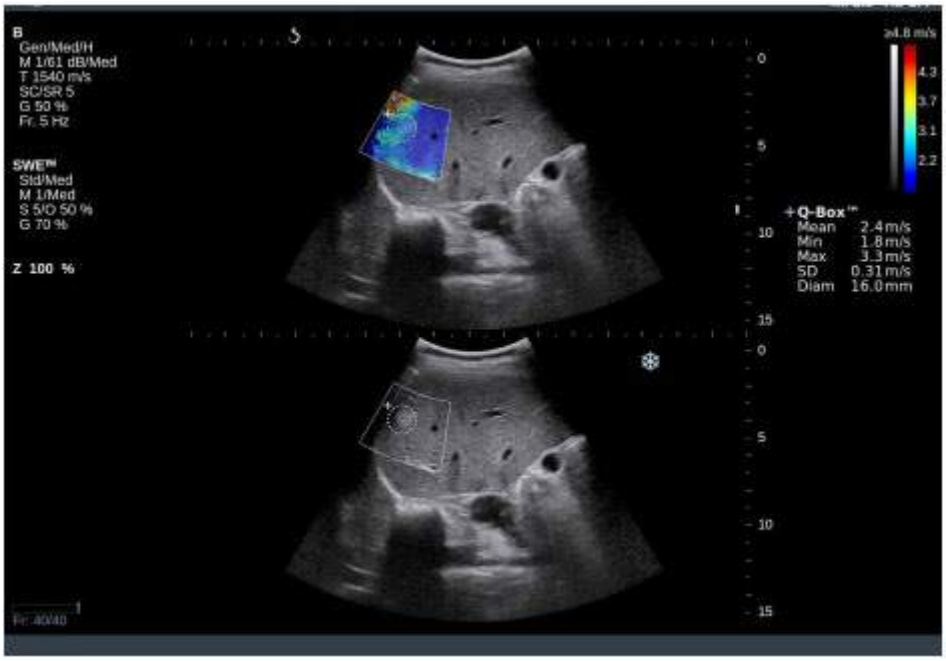

Fig. (5). 2D-SWE measurement in a hemangioma.

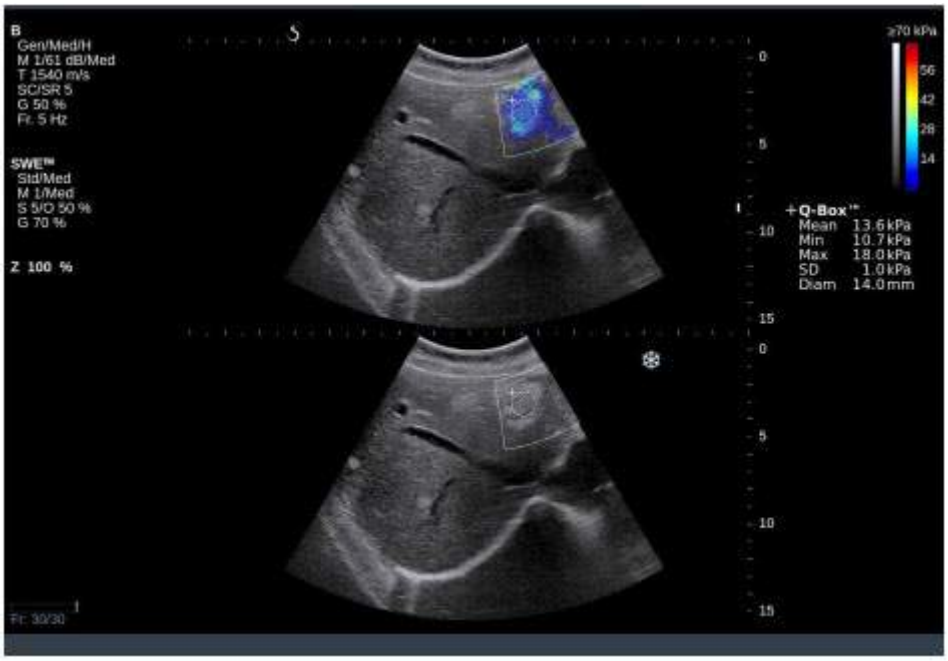

Fig. (6). 2D-SWE measurement in a liver metastasis.

Parenchyma elasticity values were 7.7 kPa in normal liver and 28.5 kPa in cirrhotic liver ($p<0.001$). On 2D elasticity maps, FNH had high central elasticity values.

Ronot *et al.* evaluated 73 patients with 105 FLLs and observed no significant differences in stiffness between benign *vs.* malignant FLLs ($p=0.64$); FNHs were significantly stiffer than adenomas ($p=0.014$) [21].

Also, the study of Brunel *et al.* [22] showed the usefulness of shear Waves elastography (2D-SWE) during ultrasound for differentiating between focal nodular hyperplasias (FNHs) and hepatic adenomas (HAs).

In conclusion SWE can provide additional information regarding FLLs.

4. ELASTOGRAPHIC METHODS USED FOR THE EVALUATION OF LIVER TUMORS TREATMENT EFFICIENCY

Many studies have demonstrated that for small HCCs, treatment efficacy of radio frequency ablation (RFA) is comparable to that of surgical resection [24 - 26]. MR and CT are considered 'gold standard' methods for post-therapy assessment of nodule viability and procedure's success [27, 28]. Contrast-enhanced US can be also an alternative for follow-up assessment of RFA procedures [29 - 31].

Fahey *et al.* suggested that Virtual Touch Imaging (VTI) by ARFI can be useful in RFA therapy [32]. US in combination with VTI (ARFI) imaging may be useful during several stages of RFA procedures since boundary definition is improved in VTI. Comparison of pre- and post-treatment VTI may provide information regarding the success of RFA. Large displacement contrast was observed in VTI of both pre-ablation malignancies (mean 7.5 dB, range 5.7 –11.9 dB) and post-ablation thermal lesions (mean 6.2 dB, range 5.1 – 7.5 dB) in the Fahey study.

Kwon *et al.* demonstrated that the VTI technique is helpful in detecting more easily recurred HCCs in patients with liver cirrhosis [33]. The study included 38 patients with HCC including recurred HCCs after RFA. They all had undergone VTI elastography. The tumor brightness was assessed and the shear Waves velocity was measured for stiffness quantification. According to the brightness, the tumors were classified as brighter, the same color or darker as compared with

the surrounding parenchyma. Using the same methods, 8 patients with recurred HCCs after RFA were evaluated regarding the brightness as compared with adjacent RFA ablation area. From the 38 patients with HCCs, in 20 (52.6%) cases the HCCs were brighter than surrounding cirrhotic parenchyma. Another 13 (34.2%) were darker. The others (5 cases, 13.2%) were seen as the same color as the adjacent liver parenchyma. Post-RFA lesions were darker than the previous tumor and the surrounding parenchyma in all 38 cases. However, recurring HCCs were brighter than the treated site in all 8 cases.

The study of Xiaohong Xu *et al.* explored VTI elastography in assessing residual tumors after radiofrequency ablation (RFA) in 83 HCC lesions [34]. All patients were examined with VTI, contrast enhanced ultrasound (CEUS), and CT or MRI. For all lesions virtual touch tissue imaging (VTI) and shear Waves velocity (SWV) were assessed before and at one month after RFA procedure. After RFA there were 14 lesions with residual tumors detected by CT or MRI, but VTI was not able to detect residual tumors in these cases. This study concluded that VTI technique cannot demonstrate residual tumor post RFA and also that VTQ (ARFI) elastography cannot replace imaging methods with contrast (CEUS, CT or MRI) in assessing response to ablation therapy.

In a study performed by Leen *et al.*, 22 patients with colorectal liver metastases undergoing radiofrequency ablation (RFA), were evaluated by 2D-SWE, using the Aixplorer® ultrasound system [35]. The lesions' elasticity (kPa) was quantified using the on-board quantification software, following placement of regions of interest over the whole lesion before, during and immediately after RFA. The size of the ablation zone was measured using the parametric image of the RFA zone. The reference method used was CEUS, performed before and after each RFA, to assess the size of lesions and of the ablation zones. There was a significant increase in the elasticity of the ablated zone as compared with the pre-RFA tumor measurements (21.5±5.8 *vs.* 4.6±2.7; $p<0.0001$). The elasticity was maximal towards the end of the ablation procedure.

The size (cm) of the ablated zone based on both CEUS and 2D-SWE (4.5±0.75 & 4.9±0.80) was significantly increased as compared with that of the pre-RFA (3.1±1.0) ($p<000.1$), without a significant difference in the size of the ablation

zones as determined with either 2D-SWE or CEUS. Using the parametric imaging of 2D-SWE, the margins of the RFA zones matched those obtained by using CEUS in all cases.

In another study performed by Leen *et al.*, whose aim was to assess the value of 2D-SWE in monitoring the effects of systemic treatment in liver tumors, 22 healthy volunteers, 30 colorectal cancer subjects with liver metastases (mCRC) (16 pre treatment and 14 post 6 cycles of systemic chemotherapy) were evaluated with 2D-SWE, using an Aixplorer® ultrasound scanner [36]. In all cases the elasticity (kPa) of the liver parenchyma was quantified using the on-board quantification software, following placement of fixed size regions of interest (ROI) over the right lobe of the liver. The metastases' elasticity was also quantified following placement of ROI over the whole lesion. Using B-mode scanning, the liver parenchyma was classified into normal, fatty change or cirrhotic, using a scoring system. Eleven of the 14 subjects who had been treated had B-mode evidence of liver steatosis. The elasticity of those with mCRC and fatty changes was significantly elevated as compared to that of the normal liver parenchyma for the volunteers (12.6±3.7 *vs.* 7.7±3.6; p=0.0025). The elasticity values of tumors which had been treated were also significantly higher as compared with those of untreated metastases (50.7±21.6 *vs.* 4.7±2.8; p=0.0001). This data suggest that 2D-SWE can be used to assess the effects of systemic chemotherapy on liver parenchyma and on treated metastases.

2D-SWE permits the real-time detection of coagulation necrosis produced by radiofrequency and could potentially be used to monitor US-guided thermal ablation [37].

Selective internal radiation therapy (SIRT) is a loco regional radio-embolism technique that is used for the treatment of unresectable liver metastases and as a treatment option for extensive liver metastases that are refractory to first and second line chemotherapies [38].

Recent papers evaluated the tissue stiffness changes measured by 2D-SWE in patients receiving SIRT for hepatic malignancy. In this study tumor stiffness increased throughout the study period and this increase is assumed to be due to

fibrosis as a part of tissue healing process [39].

2D-SWE can also be useful in the evaluation of anti-angiogenic therapy for hepatic malignancy [40].

Despite the fact that FLL elastography is at the beginning of its clinical use, these preliminary results seem to show some usefulness for the differential diagnosis of these kinds of lesions. Regarding which elastographic method is better, we shall find out in the future. Ultrasound based elastographic methods have the advantage of being incorporated into an ultrasound machine and thus, that they can be performed immediately after basic liver evaluation with ultrasound waves. Concerning the clinical use of ultrasound based elastography for FLL, this method seems not to be ready yet for daily medical activity, but only for research.

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare for this publication.

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CHAPTER 8

Guidelines on Liver Elastography

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Abstract: Scientific papers regarding ultrasound based elastographic techniques have been published in great numbers since new elastographic methods are constantly appearing in the market. Thus it is mandatory that professional societies and experts in the field should try to organize the available data in order to assess the clinical usefulness of elastography. In this regard, guidelines were issued by national and international ultrasound societies, as well as by other professional societies. These guidelines are presented in this chapter.

Keywords: EASL guidelines, EFSUMB guidelines, Liver elastography, WFUMB guidelines.

Liver elastography became more and more a clinical procedure. Transient Elastography (TE) was the first method recommended by national or international guidelines (EASL) as an alternative to LB, but the development of other elastographic methods (point or 2D SWE) made guidelines mandatory in order to clarify the value and limits of any elastographic method.

The European Federation of Societies of Ultrasound in Medicine and Biology (EFSUMB) prepared the first guidelines on ultrasound based elastography, as a proof of this technique's development in Europe. They were elaborated by a group of experts from European countries, based on the most relevant scientific papers

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and on their own experience in this field. These guidelines were divided into two parts, the first covering the basics of elastography (physics and technology) [1] and the second one describing the clinical applications of elastography in some organs [2].

In the first part, the authors classified the ultrasound based elastographic methods into strain elastography and shear waves elastography (SWE). From a practical point of view, strain elastography is used especially for nodules (breast or thyroid) assessment, while SWE for the evaluation of the liver. SWE was further divided into Transient Elastography (TE), point SWE using Acoustic Radiation Force Impulse (ARFI) technology and 2D SWE (or Real Time elastography). This very clear classification of ultrasound based elastographic techniques has attempted to ease the clinicians' approach to a very technical domain.

In the clinical part of EFSUMB guidelines, the authors presented the usefulness of elastography in fields where scientific proof is strong enough to recommend its use in the clinical workflow. According to these guidelines, elastography can be used for the evaluation of the liver, breast, thyroid, lymph nodes, pancreas (by endoscopic ultrasound - EUS), bowel, musculoskeletal. But it must be mentioned that the body of evidence has not the same strength for all the organs presented in the guidelines. Liver and breast are the fields where elastography plays a crucial role in the diagnostic workflow and where this technology is implemented in daily practice.

The EFSUMB guidelines present data available on elastography up to 2012, when they were published. Many papers have been made available regarding TE, but only a few regarding ARFI assessment of the liver, mainly in diffuse liver diseases. The body of evidence was not strong enough to recommend elastographic techniques for focal liver lesions (FLL) assessment. This observation is also valid for the guidelines that appeared later.

Because new data on liver elastography became available at a high rate, national societies made their own guidelines for practitioners, in a field where new technologies and new ultrasound machines constantly arrive in the market. The Japanese Society of Ultrasound issued the first national guidelines on liver

elastography [3]. In these guidelines the authors present data available regarding strain elastography and SWE, giving practical advice and tips for the clinical use of liver elastography. Strain elastography for diffuse liver diseases is presented first, since this is a field where Japanese authors were the pioneers who proved this method's value for liver fibrosis assessment, using the liver fibrosis index (LFI) [4, 5]. The Japanese guidelines also cover SWE, presenting results of Virtual Touch Quantification (VTQ), ElastPQ or 2D SWE, taking into consideration the type of ultrasound machine that was used. Many of the studies included in these guidelines were published by Asian or Japanese groups, so that these recommendations seem to be valid mostly in Asian patients.

The Romanian guidelines and recommendations were published in 2014 [6]. They were the first national European guidelines that tried to combine the large national experience with published papers on this topic from around the world. They also cover only the liver and were written by practitioners with large personal experience in different types of liver elastography. At the end of these guidelines, the authors make practical recommendations regarding the practical approach to liver elastography and its value in clinical practice.

In 2015, the World Federation on Ultrasound in Medicine and Biology (WFUMB) published its own guidelines on ultrasound based elastography. These guidelines were divided into three parts, covering elastography basics [7], as well as clinical application of elastography in the liver [8] and breast [9]. In the part covering the liver, the authors included significant papers published in this field and finally made recommendations regarding the clinical use of different elastographic techniques.

All the guidelines presented above were issued by societies of ultrasound. At the same time, another professional society - the European Society for the Study of the Liver (EASL) issued its own guidelines considering inside information regarding the value of liver elastography using ultrasound waves. In the guidelines concerning the non-invasive tests used for evaluation of liver disease severity, a panel of experts made practical recommendations on the use of biological tests and elastographic methods, summarizing their main advantages and disadvantages (Table **1**) [10].

Table 1. EASL Guidelines on non-invasive tests for evaluation of liver disease severity [10].

Serum biomarkers	Measurement of liver stiffness			
	Transient elastography	ARFI (pSWE)	2D-SWE	MR elastography
Advantages				
• Good reproducibility • High applicability (95%) • No cost and wide availability (non-patented) • Well validated • Can be performed in the outpatient clinic	• Most widely used and validated technique: standard to be beaten • User-friendly (performed at bedside; rapid, easy to learn) • High range of values (2-75 kPa) • Quality criteria well defined • Good reproducibility • High performance for cirrhosis (AUROC >0.9) • Prognostic value in cirrhosis	• Can be implemented on a regular US machine • ROI smaller than TE but location chosen by the operator • Higher applicability than TE (ascites and obesity) • Performance equivalent to that of TE for significant fibrosis and cirrhosis	• Can be implemented on a regular US machine • ROI can be adjusted in size and location and chosen by the operator • Measures liver stiffness in real-time • High range of values (2-150 kPa) • Good applicability • High performance for cirrhosis	• Can be implemented on a regular MRI machine • Examination of the whole liver • Higher applicability than TE (ascites and obesity) • High performance for cirrhosis
Disadvantages				
• Non-specific of the liver • Unable to discriminate between intermediate stages of fibrosis • Performance not as good as TE for cirrhosis • Cost and limited availability (proprietary) • Limitations (hemolysis, Gilbert syndrome, inflammation…)	• Requires a dedicated device • ROI cannot be chosen • Unable to discriminate between intermediate stages of fibrosis • Applicability (80%) lower than serum biomarker: (obesity, ascites, operator experience) • False positive in case of acute hepatitis, extra-hepatic cholestasis, liver congestion, food intake and excessive alcohol intake	• Unable to discriminate between intermediate stages of fibrosis • Units (m/sec) different from that of TE (kPa) • Narrow range of values • (0.5-4.4 m/sec) • Quality criteria not well defined • Prognostic value in cirrhosis?	• Further validation warranted • Unable to discriminate between intermediate stages of fibrosis • Quality criteria not well defined • Learning curve? • Influence of inflammation?	• Further validation warranted especially in comparison with TE • Not applicable in case of iron overload • Requires a MRI facility • Time-consuming • Costly

In 2015, EASL issued a new guideline regarding the treatment of chronic hepatitis C [11] in which liver elastography is a recognized method for fibrosis assessment. According to these guidelines liver fibrosis can be evaluated by non-invasive methods (elastographic or serologic), while liver biopsy should be used only in inconclusive cases (recomandationA1).

As shown above, guidelines in the field of liver elastography are published almost every year, which is mandatory since important papers are constantly being published in this field, regarding new 2D SWE or point SWE techniques available in new ultrasound machines with elastographic modules. We presented in this chapter the guidelines published until the end of 2015. Maybe new guidelines will be published in the near future, assisting medical practice in the field of liver elastography.

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare for this publication.

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