"VICTOR BABEȘ" UNIVERSITY OF MEDICINE AND PHARMACY FROM TIMISOARA

FACULTY OF MEDICINE

Department VII - Internal Medicine II

LAZĂR I. ALIN

PHD THESIS

DYNAMICS OF LIVER FIBROSIS IN CHRONIC LIVER DISEASES

– A B S T R A C T –

Scientific Coordinator:

PROF. DR. ȘIRLI ROXANA LUCIA DENISA

T i m i ș o a r a

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Chronic liver diseases (CLD) represent an important cause of death among mankind, even if new antiviral treatments are available, treatments that can induce a sustained viral response in a few weeks in the case of hepatitis C virus (HCV), as in the case of antiviral treatment for hepatitis B virus (HBV) which, indeed, does not cure the disease, but is able to maintain undetectable viremia, or, at least, a stable low viral load. Besides alcohol abuse, an emerging recognized cause of CLD is metabolic dysfunction-associated steatotic liver disease (MASLD), due to the increase in cases of type 2 diabetes mellitus, metabolic syndrome and obesity. Continuous liver injury causes a number of inflammatory reactions, which contributes to the progression to liver fibrosis and cirrhosis. Therefore, it was necessary to develop new tools for the diagnosis as well as for the monitoring of liver stiffness as a marker of fibrosis in patients with CLD.

Complications of liver fibrosis can be serious, hepatocellular carcinoma (HCC) develops on a fibrotic or cirrhotic background in over 80 percent of cases; thus, liver cirrhosis is the primary risk factor for HCC which represents the 5th most frequent solid tumor and the second leading cause of cancer-related mortality. Furthermore, the structural and functional changes in liver cirrhosis cause portal hypertension. Other clinically important consequences of portal hypertension include ascites, variceal bleeding, hepatic encephalopathy and renal failure. Portal hypertension is associated with a further increase of liver stiffness.

The widespread use of Vibration Controlled Transient Elastography (VCTE) for liver stiffness measurement (LSM) as a marker of fibrosis, represents a significant shift in physician approach to assessing liver disease, offering the convenience of repetitive stiffness measurements in weaned patients and also during antiviral therapy.

The aim of this research was assessing the usefulness of VCTE in the dynamic evaluation of liver stiffness as a marker of fibrosis in certain chronic liver diseases, as well as the usefulness of VCTE and Controlled Attenuation Parameter (CAP) as a reference method in somewhat newer systems for evaluating liver stiffness (LS) and staging liver steatosis.

GENERAL PART

Chapter 1. Epidemiology of Chronic Liver Diseases in Romania

Chronic liver disease (CLD) has a wide range of causes, including toxins, long-term alcohol consumption, infection, autoimmune and genetic or metabolic disorders. HBV, HCV, MASLD, and alcoholic liver disease (ALD) are the most often involved in the etiology, however the role of viral hepatitis, MASLD, and ALD in the total burden of CLD is rapidly changing.

In Romania, it was established a prevalence of rate of 4.4 and 27% for HBsAg and antibodies to the hepatitis B core antigens respectively, representing a high figure throughout the European Union. Also, a study carried out in 2020, shows a prevalence of 1.93% of HCV infection in Romania in adult population, with differences between the areas of development. In a Romanian study of 3005 hospitalized patients without known hepatic disease, the frequency of MASLD was found to be 20%, comparable to the European general population.

Chapter 2. Assessment of Liver Fibrosis

The evaluation of liver fibrosis is carried out by two methods: invasive methods (liver biopsy) and non-invasive (elastography and biological tests)

Liver biopsy

Liver biopsy (LB) still represents the gold standard for evaluating necro inflammatory activity, steatosis and fibrosis in chronic liver diseases. However, it is an invasive method and may have complications such as pain or bleeding (0.3%). Considering that under current conditions the biopsy is performed under ultrasound guidance, the risk of organ perforation is minimal. Death after the procedure was reported in 0.01% of cases. Other shortcomings: the intra- and interobserver variability, the sampling variability: a liver sample is appropriate for pathological investigation if it is longer than 25 mm and has more than eight or eleven portal tracts.

Non-invasive methods

Non-invasive liver fibrosis tests have become more popular in clinical practice because they avoid multiple disadvantages of liver biopsy. Non-invasive methos are classified into two types: blood-based testing (serum fibrosis markers) and elastography procedures, which evaluates the physical properties of liver tissue.

There are basically two types of elastography methods: ultrasound (US) based and magnetic resonance elastography (MRE).

Ultrasound (US) based Elastography

Elastography has proven to be a valuable tool in the diagnosis and monitoring of multiple medical conditions, especially in the field of chronic liver diseases, but also in thyroid, breast, prostatic pathology. Regarding CLD, US based elastography evaluates liver stiffness as a marker of fibrosis.

US based elastographic methods can be classified into two large categories:

1.   Shear wave elastography (SWE): Vibration Controlled Transient Elastography (VCTE), SWE techniques based on ARFI (Acoustic Radiation Force Impulse) – all of them extensively used for fibrosis assessment in CLD

2.   Strain techniques: Strain elastography – much less used for liver fibrosis assessment

Vibration Controlled Transient Elastography (VCTE)

VCTE represents the most established elastographic method used for fibrosis staging in chronic liver diseases, which was developed in the early 2000s. Liver stiffness is measured with the FibroScan™ device (EchoSens, Paris, France), the device includes a probe equipped with a vibrator and a single element ultrasonic transducer. The result of the examination is expressed in kPa ranging from 2.5 to 75 kPa and represents the median of 10 measurements. The following criteria are used to validate successful measurements: at least ten valid shots and an interquartile range (IQR) less than 30% of the median liver stiffness measurement (LSM) value. Unreliable LSM defined as less than 10 valid shots, with an IQR > 30%, and failed LSM define as zero valid shots.

As advantages, VCTE it is a simple method, easy to learn, which can be performed at bedside with good reproducibility, high performance for cirrhosis (AUROC >0.9) and good prognostic value in compensated cirrhosis. Like every method, VCTE also has disadvantages: it requires a dedicated device, it is operator dependent, the region of interest (ROI) cannot be selected, it cannot be performed in patients with perihepatic ascites, it overestimates fibrosis severity in patients with acute hepatitis, hepatic congestion, intrahepatic cholestasis, excessive alcohol consumption.

SWE techniques based on ARFI

In these techniques, the pulse that deforms the tissue is an ultrasound impulse centered at a certain depth, generating shear waves that move away from the application area. Depending on the technique of measuring the speed of these shear waves, ARFI-based SWE techniques are: point SWE (p-SWE); the acoustic pulse is centered at a point where the shear wave speed is measured in a ROI, yielding a value in m/s that can be converted to kPa; and 2D-SWE and 3D-SWE, in which the tissue stimulation is performed in several points and the shear wave speed is measured in a larger area. This type of elastography became possible with the technical advances, the result of the evaluation being a numerical value (either in m/s or in kPa), but also an elastogram (a color-coded map)

The biggest advantage that 2D-SWE offers is that it evaluates a larger area of the liver (up to 10 cm^2). Typically, harder tissue is represented in red and softer tissue in blue. Minimal training in abdominal ultrasonography (> 300 examinations) is required to be able to obtain good elastograms. IQR/M ratio < 30% and measurement depth < 5–6 cm is recommended as quality factors.

Strain elastography (SE): This method depends on the clarity of the B image, for the evaluation of liver fibrosis index (the current standard analytic method of SE) nine features are needed: mean and standard deviation of the relative strain value, complexity of the blue area in the ROI, skewness, kurtosis, entropy, inverse difference moment, angular second moment. Limitations: while heart activity is the most often used stimulus for liver tissue deformation, a weak pulse might have a negative impact on SE image quality. SE is useful for assessing patients with ascites and narrow intercostal space, although it can be challenging to get quality pictures in highly obese individuals. The experience of the operator can influence the results, it's necessary to learn to avoid artifacts.

Magnetic resonance elastography (MRE)

MRE represents a new and advanced non-invasive method of evaluating liver fibrosis, it may be integrated into a standard Magnetic resonance imaging (MRI) system with minor hardware and software modifications, having been proven reliable for detecting and staging hepatic fibrosis caused by various chronic liver diseases, with higher applicability than other methods (ascites, hepatodiaphragmatic interposition of the bowel loops and obesity). Limitations of the method: MRI can be too low in iron overload of the liver tissue, in diseases such as hemochromatosis or hemosiderosis, it is a time consuming and costly method.

Serum markers of fibrosis

Another non-invasive method of evaluating liver stiffness is represented by biological tests, serum markers, both patented (FibroTest®, FibroMeter, HepascoreTM and ELFTM test) and non-patented, such as FIB-4 and Forns' index, which have adequate accuracy in the diagnosis of advanced liver fibrosis. Advantages of serum markers: good reproducibility, 95% applicability, well validated, can be conducted in outpatient clinic, good prognostic values for severe fibrosis/cirrhosis in some etiologies of CLD. Disadvantages of serum markers: non-liver specific, expensive (patented), the performance is not as good as in the VCTE in the diagnosis of liver cirrhosis, false positive results.

Chapter 3. Assessment Of Liver Steatosis

The importance of assessing liver steatosis is not only because MASLD is a common CLD, but also that steatosis is frequently associated with other chronic liver disorders, including HCV, and metabolic steatosis, rather than viral steatosis, leads to a low response rate to antiviral treatments.

Quantification of liver steatosis using ultrasound-based techniques

Conventional B-Mode Ultrasound is a simple technique, used for a long time, available in many medical centers and with low costs. The typical ultrasound signs for liver steatosis are the "bright liver" with "posterior beam attenuation", also the "vessel blurring" sign. Under normal conditions, the liver and right kidney have the same echogenicity, therefore the kidney/liver ratio can provide a more precise evaluation.

Semi-Quantitative Ultrasound Methods are represented by: Hepatorenal index, Hamaguchi score and US-FLI.

Quantitative Ultrasound-Based Techniques

There are two main methods for assessing liver steatosis using the attenuation coefficient: the **controlled attenuation parameter** (CAP) utilizing A-mode ultrasound and **B-mode ultrasound-guided attenuation imaging**.

Controlled Attenuation Parameter (CAP)

CAP is included in the Fibroscan device since 2010, representing a physical parameter intended to evaluate liver steatosis using the effects of steatosis on ultrasound propagation. The CAP is measured according to the same criteria and on the same signals used for LSM, results are displayed simultaneously and are expressed in decibels per meter (dB/m), varying from 100 to 400 dB/m.

B-Mode Ultrasound-Guided Attenuation Imaging

Lately, more and more software tools are implemented in ultrasound machines for the evaluation of ultrasound attenuation using the B module. Of these, worth mentioning are: ultrasound-guided attenuation parameter (UGAP) - General Electric, attenuation imaging (ATI) - Canon, Attenuation (ATT) - Fujifilm, tissue attenuation imaging (TAI) - Samsung, attenuation coefficient (AC) - Siemens, liver fat quantification (LFQ) - Philips, ultrasound attenuation parameter (UAP) – FibroTouch.

Magnetic Resonance Imaging Proton Density Fat Fraction (MRI-PDFF)

Magnetic resonance imaging quantifies liver steatosis by measuring the proton density fat fraction (PDFF). PDFF is the ratio of protons attached to fat divided by total protons in the liver as shown on MRI. The results are in percentage, ranging from 0 to 100 percent. As a disadvantage, MRI can't be used often for screening and follow-up for liver steatosis because to its limited availability and low price-effectiveness. Given the rising frequency of MASLD, a more available and cost-effective non-invasive diagnostic tool is necessary for the assessment of liver steatosis.

SPECIAL PART

The key objectives of the component studies of this thesis were the assessment of liver stiffness dynamics in chronic liver diseases (CLD) and quantification of liver steatosis using new elastography techniques

Chapter 4. General Objectives

(1)   To evaluate the dynamic changes of liver stiffness as a marker of fibrosis measured by VCTE in patients with chronic hepatitis B undergoing nucleos(t)ide analogues therapy

(2)   To evaluate the reliability and significance of VCTE in real-world clinical settings.

(3)   To establish the usefulness of 2D-SWE measurements obtained using a new ultrasound technique, using VCTE with Controlled Attenuation Parameter (CAP) as the reference method

(4)   To determine the usefulness of a new liver steatosis quantification system - Ultrasound-Guided Attenuation Parameter (UGAP)

Chapter 5. Materials and Methods

Subjects

Before participating in the research, all individuals provided written consent for participating in LS measurements, as well as ultrasonographic and biochemical exams. The inclusion and exclusion criteria were customized to each study's aims. This research was conducted in accordance with the most recent edition of the World Medical Association Declaration of Helsinki and was approved by the local research ethics committee.

Abdominal Ultrasound

Before measuring the liver stiffness, the subjects were evaluated by fully abdominal ultrasound scan, evaluation of the liver being done initially by subcostal and afterwards by intercostal approach, observing the signs that could affect the LSM: the existence of liver lesions, biliary obstruction and/or ascites, as well as the presence of steatosis.

Grading of liver steatosis can be performed using a qualitative grading system: Grade 1: US imaging shows a minor increase in fine echoes with normal visualization of the diaphragm and intrahepatic vascular limits; Grade 2: US imaging shows moderate diffuse increase in fine echoes with slightly impaired visualization of the diaphragm and intrahepatic

vessels and Grade 3: US imaging shows marked increase in fine echoes with poor or no visualization of the diaphragm, intrahepatic vessel limits and posterior portion of the right lobe of the liver.

Vibration-Controlled Transient Elastography (VCTE) and Controlled Attenuation Parameter (CAP)

VCTE (EchoSens®, Paris, France) with or without CAP represents the elastographic reference method in this research. All patients were evaluated in fasting conditions for at least four hours, in supine position, with the right arm under the head, in maximum abduction, utilizing an intercostal approach to the right liver lobe. The operator utilizes, depending on the body mass index (BMI), the M or XL probe, ultrasonography M-mode and A-mode images to find the liver and initiates the measurement when pressing the dedicated button.

It is customary to determine 10 values, expressed in kPa in a range between 2.5-75 kPa (for VCTE) and dB/m (for CAP) in a range between 100 and 400 dB/m. Reliability criteria for the median value of the 10 measurements is an interquartile range to median ratio (IQR/M) of ≤30%. A failed VCTE measurement was defined if no valid value was obtained after at least 10 shots, and a measurement was considered unreliable if fewer than 10 valid shots could be obtained and/or IQR/M ≥ 30%.

2D-SWE with Attenuation Imaging (ATI)

The ultrasound examinations and liver stiffness measurements were performed using the Canon Aplio i800 system with the Multi-Frequency Slim Face Convex PVI-475BX (i8CX1) 4 MHz probe, the patient having not eaten for no less than 4 hours, positioned supine with the right arm behind the head, while holding his breath 5 measurements are taken (with IQR/M ≤ 30%), the results being the median values of the measurements, expressed in kPa. For the Attenuation Imaging, the acquisition protocol was the same as for the SW, reliable measurements were defined as the median value of 5 measurements with an IQR/M <30% and R^2>0.90, the ATI ROI just below the orange liver capsule artifact, numeric values expressed in dB/cm/MHz.

Ultrasound-Guided Attenuation Parameter (UGAP)

UGAP evaluates the attenuation coefficient using a reference phantom containing glass bead particles of known attenuating materials. It utilizes an automated measuring method to determine and analyses the optimal measurement range. The diaphragm is automatically eliminated, and the slope is measured within the optimal range to get a realistic attenuation coefficient. UGAP measurements were realized using a LOGIQ E10 ultrasound machine (GE Healthcare, Wauwatosa,WI, USA), with a C1-6-D convex array probe, in fasting conditions, patients are in supine position and in the right liver lobe, in a homogenous area, a colored-coded attenuation map is positioned.

Reliable UGAP measurements were defined as the median value of 10 measurements, with an IQR/M <0.30. UGAP values are presented in dB/cm/MHz or dB/m.

Study characteristics

In the **first study**, we included 87 subjects with chronic hepatitis B which had up to seven LSM, evaluated before starting therapy and followed-up every year during the follow-up period for a median interval of 64 months (range 12-108). At the initial VCTE evaluation, the following cut-off were used: 7.9 kPa for F≥2, 8.8 kPa for F≥3 and 11.7 kPa for F4.

Exclusion criteria were patients with less than 1-year follow-up, patients with chronic renal failure, patients younger than 18 old, heavy alcohol consumption (ethanol intake more than 210 grams/week in men and 140 grams/week in women), patients with perihepatic ascites, biliary obstruction, patients with elevated aminotransferase levels more than five times the upper normal limit, patients with focal liver lesions, patients with heart failure generating liver congestion and patients with pacemakers.

The second study is a retrospective study realized in a period 2007–2019 and included patients who presented to our center for liver stiffness measurements (LSMs). Exclusion criteria: age under 18 years, focal liver lesions, biliary obstruction, perihepatic ascites, pregnant women, patients with pacemakers and recent alcohol abuse. Furthermore, were included patients with repetitive LSM over the period.

When studying the LSM values over the years, 3 types of medical conditions were separately investigated: chronic viral hepatitis (VH); autoimmune diseases (AID) and fatty liver diseases (FLD).

We utilized the Tsochatzis meta-analysis cut-offs to differentiate liver fibrosis stages based on VCTE: F2 ≥ 7 kPa; F3 ≥9.5 kPa; and F4 ≥12 kPa.

The third study, included 112 subjects with reliable LSM, 44 healthy subjects and 68 subjects with chronic liver diseases.

For the healthy subjects the inclusion criteria were: age higher than 18 years, normal biological liver tests (aspartate aminotransferase, alanine aminotransferase, total bilirubin, γ-glutamyl transpeptidase, alkaline phosphatase) and LS values by VCTE <7 kPa. Exclusion criteria for patients with CLD: age under 18 years old, undergoing antiviral treatment, patients with ascites, focal liver lesions, biliary obstruction or/and liver congestion due to heart failure. The VCTE cut-off values used to differentiate between the liver fibrosis stages: F≥1: 7 kPa, F ≥ 2: 8.4 kPa and F=4: 13.2 kPa. For discriminating liver steatosis stages, the CAP cut-off values propose by Eddowes et al, were used: S1 (mild): 274 dB/m, S2 (moderate): 290 dB/m, S3 (severe): 302 dB/m.

The Multi-Frequency Slim Face Convex PVI-475BX (i8CX1) 4 MHz probe was used to evaluate LS via 2D-SWE, for Attenuation Imaging (ATI) acquisition the patient is in the same position as in SWE acquisition, with the probe perpendicular to the liver surface, the ATI ROI was located immediately below the orange liver capsule artefact.

The fourth study was composed of 179 subjects with CLD or healthy liver subjects, who undergone UGAP, VCTE and CAP in the same session. Inclusion criteria for all subject was age over 18, informed consent signed. Inclusion criteria for healthy liver subjects were:

no history of liver disease, negative tested for hepatitis B and/or C, a normal abdominal ultrasound examination and LS values <6.5 kPa.

The following VCTE cut-off values were used to differentiate between the liver fibrosis stages: F≥1: 7 kPa, F ≥ 3: 9 kPa and F=4: 11.8 kPa. For discriminating liver steatosis stages, the CAP cut-off values propose by the manufacturer, were used: S1 (mild): 230 dB/m, S2 (moderate): 275 dB/m, S3 (severe): 300 dB/m.

UGAP measurements were taken using a LOGIQ E10 ultrasound machine (GE Healthcare, Wauwatosa, WI, USA) with a C1-6-D convex array probe. Reliable UGAP measurements are defined as the median value of 10 measurements taken in a homogenous region of liver tissue, with an IQR/M <0.30. UGAP values are displayed in dB/cm/MHz or dB/m.

Chapter 6. Results

Study 1: Dynamic changes in Liver Stiffness measured by Transient Elastography in patients with chronic hepatitis B undergoing antiviral therapy with nucleos(t)ide analogues: a 10-year longitudinal study

The response to treatment according to HBV-DNA serum levels, from the 87 patients with detectable HBV-DNA at baseline, undetectable HBV-DNA was achieved in 64 patients (73.5%) at year one, 72 (82.7%) at year two, 79 (90.8%) at year three.

The patients were followed up for a median interval of 64 months (range 12–84), The mean liver stiffness values decreased significantly between the first and second measurements. Starting from the second year, LSM remained generally consistent.

When the cohort was divided into 2 sub-cohorts depending on the initial evaluation of liver stiffness: cirrhosis (24 patients) vs. non-cirrhosis (63 patients), the LSMs significantly decreased in patients with liver cirrhosis after only one year, 24.6 ± 4.3 kPa vs. 13.5 ± 4.2 kPa, p= 0.007. In patients without cirrhosis, the decrease was not significant after one year, 7.31 ± 3.62 vs. 6.80 ± 2.41, p = 0.27.

When analyzing the initial viral load, in patients with viremia under 100.000 UI (38 patients), LSMs decreased significantly after one year of treatment: 13.2 ± 5.2 kPa vs. 8.6 ± 4.4 kPa, p < 0.0001; compared with the LSMs in patients with higher viremia (49 patients): 9.7 ± 5.9 kPa vs. 8.4 ± 3.8 kPa, p = 0.25.

In patients with values of transaminases above the upper limit of normal (35 patients), LSMs decreased significantly after one year: 13.0 ± 5.8 kPa vs. 9.3 ± 4.3 kPa, p = 0.02; and in patients with normal levels (52 patients) LSMs also decreased, but with no statistical significance, 10.6 ± 9.5 kPa vs. 7.9 ± 3.8 kPa, p = 0.06. Also, a strong and direct correlation was found in the group with high transaminases values at the beginning between the decrease in liver stiffness and the decrease of transaminases, r = 0.81, p < 0.0001.

Study 2. The Prevalence of Liver Fibrosis Stages on More than 23,000 Liver Stiffness Measurements by Vibration-Controlled Transient Elastography: A Single Center Study

Determinants and Frequency of Unreliable and Failed Measurements

The database contained 23,420 measurements, from among these, valid LSMs were obtained in 90.91% (21,291/23,420) of the measurements, and 2129 (9.09%) of the measurements were deemed failed or unreliable. LSMs were obtained by the M probe in 16,635 (71%) cases and by the XL probe in 6785 (29%) cases. 68.6% (1460/2129) of the invalid measurements were obtained by the M probe, and 31.4% (669/2129) were obtained using the XL probe.

Patients with a BMI over 30 kg/m2 had 2.5 times the odds of unreliable LSMs in univariate analysis, though in multivariate analysis, this risk was reduced but remained significant.

The age above 60 years slightly increased the odds of unreliable LSMs, with minor differences between univariate and multivariate analysis. The male sex had a small increase in odds for unreliable LSMs in univariate analysis. Though, the odds ratio is slightly below 1 in multivariate analysis, which suggests that the male sex may not significantly increase the risk when considering other factors. Patients with metabolic disease had 1.5 times the odds of unreliable LSMs in univariate analysis, also this increased risk remains notable in multivariate analysis.

Based on the VCTE cut-off values propose by Tsochatzis et al, the stages of liver fibrosis in our group of measurements (n = 21,291) were: F < 2: 10,308 measurements (48.4%); F2: 3,342 measurements (15.7%); F3: 1,842 measurements (8.7%) and F4: 5,799 measurements (27.2%)

Dynamics of LSMs over the Years

In the year 2008, the mean LSM value increased to 15.20 kPa, more than double that of 2007. The substantial increase is accompanied by a significant standard deviation (SD) of 15.14, showing a broad dispersion of data points in 2008. Between 2009 and 2014, the mean LSM levels remained stable at about 13-14 kPa. Since 2015, there has been a progressive decrease trend, with 2019 showing the lowest mean value of 10.58 kPa throughout the whole time. A sub-analysis of HCV patients was conducted. Patients treated with direct-acting antiviral (DAA) LSMs dynamics decreased (16.55 ± 9.67 kPa vs. 11.12 ± 7.78 kPa, p = 0.02), while non-treated HCV patients showed a substantial increase in LSMs dynamics (13.37 ± 10.62 kPa vs. 20.78 ± 12.13 kPa, p < 0.001). In HBV patients receiving antiviral treatment, LS gradually decreased, especially in those with high baseline alanine aminotransferase levels and viral load.

LSM Values according to Etiologies

When analyzing the LSM values according to etiologies and gender, no matter how many measurements over time were performed in viral hepatitis patients, only the type of viral hepatitis and the masculine gender were associated with LSM values.

When analyzing the LSM values according to AID, we observed that no matter the autoimmune disease type, or the gender of time, none of the factors are associated with the LSM value.

For fatty liver disease, the factors associated with the LSM value were the type of FLD.

Study 3: Quantification of Steatosis and Fibrosis using a new system implemented in an ultrasound machine

This study aims to evaluate the usefulness of 2D-SWE (Aplio i800 Canon Medical Systems) for the noninvasive evaluations of LS and steatosis, using VCTE with the CAP as the reference method in a cohort of subjects composed of 112 adults with reliable LSM, 44 healthy subjects and 68 subjects with chronic liver diseases.

The mean LSM values obtain with 2D-SWE were similar with those obtain with VCTE (7.21±4.3 vs 7.47±8.13, p=0.66) and the mean difference between VCTE and 2D-SWE was 0.3±0.01.

There was a substantial positive connection between the liver stiffness values obtained using VCTE and 2D-SWE: r=0.88, p<0.0001 and between the coefficients evaluating steatosis (CAP vs. ATI), r=0.81, p<0.0001.

Regarding the hepatic steatosis, the best cut-off values by ATI were: for S≥1 - 0.79 dB/cm/mHz and for S3 -0.86 dB/cm/mHz.

Study 4. Ultrasound-Guided Attenuation Parameter (UGAP) for the quantification of liver steatosis using the Controlled Attenuation Parameter (CAP) as the reference method

The mean UGAP values were significantly lower than the mean CAP values: 231.5±40.9 dB/m vs. 268.6±61.7 dB/m, p<0.001. Using CAP as the reference method for quantifying hepatic steatosis, mean UGAP values increased with severity, with a good correlation between the 2 values (r=0.73, p<0.0001).

The UGAP cut-off values obtain for predicting the grades of liver steatosis, using CAP as the reference were: S1 - 192.5 dB/m; S2 – 231 dB/m; S3 – 248 dB/m.

Chapter 7. Discussions

7.1. Study nr. 1 – Dynamic Changes in Liver Stiffness in Patients with Chronic Hepatitis B Undergoing Antiviral Therapy

The study's most notable outcome was that liver stiffness decreased within the first two years after starting antiviral treatment. During the follow-up, hepatic stiffness must be assessed in order to monitor disease progression and treatment effectiveness.

The levels of transaminases and viremia load can be used to evaluate antiviral efficacy, therefore, in our study in patients with high levels of transaminases LSM decrease meaningful after one year of therapy, this decrease can also be explained by the decrease of liver inflammation during treatment, because in subjects with normal levels of transaminases,

where the inflammation was not as severe, the LS reduced after one year, although with no statistical significance. Regarding the viremia load, after a year of follow-up, patients with lower initial HBV DNA levels experienced a significant decrease in LSM, while those with higher initial levels did not. Patients with lower viremia levels also had higher initial liver stiffness values (13.2 ± 5.2 kPa) compared to those with high levels (9.7 ± 5.9 kPa).

Even if this study did not include histological evaluation, other studies in the literature have shown that antiviral treatment with NUCs can lead to the regression of liver fibrosis, helping the prognosis of patients with chronic hepatitis B.

Another limitation, we didn't use a non-invasive method to assess inflammation, which may have contributed to the ongoing decrease in liver stiffness. Another issue is the lack of data on liver function tests throughout follow-up.

7.2. Study nr 2 - The Prevalence of Liver Fibrosis Stages on More than 23,000 Liver Stiffness Measurements by Vibration-Controlled Transient Elastography: A Single Center Study

From the 23,420 measurements, the feasibility of LSMs by VCTE with M and XL probes was 91.2%. The data indicates that reliability improved with time, with the largest percentage of failed and unreliable measurements occurring around 2012. By 2019, this percentage had decreased to its lowest point. The highest percentage of unreliable LSMs was in 2012, 12.45% of LSMs were unreliable (265 measurements). After the year 2012, there was a decline in the percentage of unreliable LSMs, the most significant decrease happening between 2014 and 2015, this possible due better training, technological advancements. The M probe had a higher percentage of inaccurate and failed measurements compared to the XL probe, which is acknowledged for its difficulty in obtaining correct LSM in individuals with higher BMI. Another important factor is that the XL probe has only been available since 2012 in our clinic.

Similar to other studies, the majority of invalid/unreliable LSM were obtained with the M probe, in direct relation to the patients' BMI. The study shows a significant decrease in the failure rates of LSMs over the years. While the introduction of the XL probe could be one potential reason, another possible explanation is the increased experience of operators during time, as clinicians got used to with the technique, and therefore, the reliability of measurements was likely improved.

The majority of the unreliable or failed LSMs were in HCV patients (34.5%) representing also the majority of patients with repeated measurements across the 13-year periods of time, followed by LSMs in NAFLD patients (22.4%). One explanation in patients with NAFLD might be the higher BMI. The factors found involved in the unreliable results are age BMI, gender and metabolic disease.

Furthermore, we evaluated the kinetics of repeated LSMs in period of time in patients with chronic viral hepatitis; autoimmune diseases and fatty liver diseases. Out of 1374 patients with chronic viral liver diseases, 1048 had at least 3 LSMs performed over time, while the

remaining had 5 LSMs. We analyzed both groups (3 vs. 5 LSM over 13 years) because the longer the duration, the higher possibility of obtaining a good modelling of reality based on the acquired and processed data. LSMs values are strongly dependent on the etiology of the infection in viral liver diseases. In the majority of cases, HBV patients have high levels of transaminases compere to HCV patients the transaminases can be normal.

There is a definite link between time periods and LSM in individuals with autoimmune hepatopathies and fatty liver from various sources. Patients with ALD showed a decrease in variability of LSM values with time, which can be attributed to their alcohol abstinence.

The study has some limitations: due to its retrospective nature and long follow-up period, patients have been evaluated for liver fibrosis only using VCTE and not by using liver biopsy. However, it is difficult to conduct serial liver biopsies in a large cohort for follow-up in clinical practice. Another limitation of the study was the heterogeneity of patients, variability in etiologies and most importantly the variability of LSM at different time periods.

7.3. Study nr. 3 - Quantification of Steatosis and Fibrosis using a new system implemented in an ultrasound machine

The study demonstrates that evaluating liver fibrosis and steatosis using Canon's Aplio i800 ultrasound machine is a highly practical approach.

Results from the publications in the literature show a strong correlation between 2D-SWE elastography and histological evaluation or VCTE. A meta-analysis shows an excellent diagnostic accuracy for 2D-SWE (using liver biopsy as the reference procedure) for stages of liver fibrosis in HBV, HCV and NAFLD patients. Furthermore, 2D-SWE had a higher AUROC than VCTE for detecting severe fibrosis ($p=0.001$) and cirrhosis ($p=0.022$) in all cases, comparable to our data that showed AUROCs of 0.89 for severe fibrosis and 0.94 for cirrhosis.

Furthermore, based on VCTE cut-off values published by previous research, we were able to establish 2D-SWE cut-off values for different stages of fibrosis in our cohort.

Regarding the quantification of hepatic steatosis, a good correlation of ATI from Canon with CAP: $r=0.81$, $p<0.0001$, was observed, the ATI values are ascending with the severity of liver steatosis.

The limitations of this study were: liver biopsy was not used as a reference method for evaluating stages of liver fibrosis and steatosis. Also, a limited number of individuals with low incidence of liver cirrhosis and intermediate stages of fibrosis and steatosis may have affected the results.

7.4. Study nr. 4 - Ultrasound-Guided Attenuation Parameter (UGAP) for the quantification of liver steatosis using the Controlled Attenuation Parameter (CAP) as the reference method

In this study UGAP had a high feasibility rate (98.8%), comparable to CAP. The severity of steatosis was associated with higher UGAP values. The AUROC of UGAP for predicting grade 3 hepatic steatosis was over 0.90, indicating great diagnostic accuracy. Also,

in a study that included 182 patients with NAFLD-related CLD or HCV who had undergone UGAP, CAP, computed tomography and a liver biopsy the AUROCs of UGAP for diagnosing liver steatosis grade were ≥ 0.90.

The main limitation of our study is the lack of liver biopsy or magnetic resonance imaging proton density fat fraction (MRI-PDFF) as the reference technique for liver steatosis quantification. Another drawback is the minimal number of patients included with various etiologies.

Chapter 8. Conclusions

1. In patients with chronic hepatitis B undergoing antiviral treatment liver stiffness assessed by Vibration-Controlled Transient Elastography decreased significantly in the first two years of treatment after that remaining stable during a median interval of 64 months (range 12–84) follow-up

2. In real-world clinical settings VCTE has an important reliability and significance, with a feasibility over 90%

3. VCTE provides both a diagnostic answer and is a dependable tool for following disease development over time. Using VCTE in clinical practice helps identify fibrosis and cirrhosis early, leading to better patient outcomes and prompt therapies.

4. The two-dimensional shear wave elastography (2D-SWE) technique with Attenuation Imaging (ATI) is a highly feasible method, with excellent performance in diagnosing and staging liver fibrosis, strongly correlating with VCTE and CAP results

5. Ultrasound-Guided Attenuation Parameter (UGAP) seems to be a good method for liver steatosis quantification which correlates strongly with CAP values. Feasibility is very high and the examination can be performed immediately after a standard ultrasound examination.

6. The multiparametric ultrasound systems should enter the daily practice because they may offer important information about the liver in a short period of time. They can be used by physicians, allowing a complex evaluation of liver stiffness and steatosis in a few minutes.