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# **DOCTORAL THESIS**

## **ROLE OF ULTRASOUND-BASED METHODS FOR THE EVALUATION OF FOCAL LIVER LESIONS**

### **A B S T R A C T**

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# **ABSTRACT**

## **GENERAL PART**

Incidental focal liver lesions (FLL) are frequently encountered in the clinical setting, posing a challenge in the daily workflow of the Gastroenterology and Hepatology department. The widespread utilization of various imaging modalities, including ultrasonography (US), contrast-enhanced ultrasonography (CEUS), computed tomography (CT), and magnetic resonance imaging (MRI), has contributed to an elevated incidence of FLL detection.

Accurate characterization of FLL holds significant importance in establishing a definitive diagnosis and determining patient prognosis. To optimize the detection and characterization of FLL, imaging assessment should be conducted using appropriate equipment and adhering to protocols designed based on the specific clinical context. Contemporary US techniques have witnessed the integration of multiple novel procedures and techniques within ultrasound machines, such as CEUS, elastography methods, parametric imaging, and Doppler, which collectively have the potential to expedite the diagnostic algorithm for FLL. Notably, the introduction of ultrasound contrast and US elastography, encompassing both strain and shear-wave elastography, represents noteworthy advancements in the field of US over recent years.

CEUS has significantly contributed to enhancing the diagnostic capabilities of ultrasound-based methods for FLLs. Currently, CEUS serves as an excellent diagnostic tool for FLL identified through standard US in patients without liver cirrhosis or a history of malignancy. It is particularly effective for diagnosing hepatocellular carcinoma (HCC) in at-risk patients and liver metastases (LM) in individuals with cancer. Moreover, CEUS is cost-effective and positively impacts patient management, offering a safe, rapid, and highly accurate alternative to sectional imaging techniques for diagnosing FLL. Consequently, the reliance on invasive or irradiative procedures, as well as the associated stress and waiting times for obtaining a definitive diagnosis, are reduced.

The CEUS diagnosis of HCC can be challenging. To address this, algorithms based on CEUS examination have been developed with the objective of standardizing the interpretation and documentation of HCC in high-risk patients.

Furthermore, ultrasound-based elastography has emerged as a non-invasive and reproducible method with an established role in assessing liver fibrosis. However, the precise role of elastography in characterizing FLL remains unclear. Recent studies have focused on evaluating the performance of different elastographic techniques for characterizing FLL.

The integration of these techniques within a multiparametric ultrasound (MPUS) approach holds considerable value in achieving a non-invasive diagnosis of FLL. However, it is essential for practitioners to possess comprehensive and accurate knowledge regarding the current state-of-the-art ultrasound evaluation techniques, which poses a challenge. Nonetheless, embracing this challenge is crucial to ensure that patients have access to the most effective diagnostic and therapeutic options available.

## **SPECIAL PART**

This research encompasses three distinct studies conducted on separate cohorts, necessitating the organization of the corresponding section into individual subsections to provide a clear presentation of each study.

## GENERAL OBJECTIVES

The primary aims of this research are as follows:

1. To investigate the role of non-invasive ultrasound-based methods in the evaluation, characterization, and differentiation of focal liver lesions (FLL).
2. To establish a standardized protocol utilizing ultrasound-based methods, including standard ultrasonography (US), contrast-enhanced ultrasonography (CEUS), and shear-wave elastography (point SWE method), for the characterization and differentiation of FLL.
3. To assess the diagnostic performance of the 2017 ACR CEUS LI-RADS algorithm for non-invasive diagnosis of hepatocellular carcinoma (HCC) in high-risk patients.
4. To determine the intra- and interobserver reproducibility of the VTQ point shear-wave elastography technique (ARFI technique) in assessing FLL and identify factors that may influence reproducibility.
5. To characterize hepatocellular carcinoma using elastographic point shear-wave elastography (pSWE) with the VTQ method implemented in the US Acuson S2000TM system (Siemens AG, Erlangen, Germany), and investigate factors influencing intratumoral elasticity variability in patients with liver cirrhosis.
6. To evaluate the improved cost-effectiveness of FLL diagnosis utilizing the proposed ultrasound-based methods.
7. To explore the role and usefulness of the Multiparametric Ultrasound (MPUS) concept in the diagnosis of FLL.

By addressing these objectives, this research aims to contribute to the understanding and advancement of non-invasive ultrasound-based approaches for FLL evaluation and diagnosis.

**1. The utility of implementing a standardized contrast-enhanced ultrasonography (CEUS) reporting algorithm for stratifying focal liver lesions (FLL) in patients at high risk of hepatocellular carcinoma (HCC) progression. Study I- focuses on evaluating the LI-RADS v2017 CEUS algorithm as a non-invasive diagnostic tool for HCC in individuals with chronic liver disease.**

### 1.1 Background and Objectives

An issue often encountered with contrast-enhanced ultrasonography (CEUS) in hepatocellular carcinoma (HCC) diagnosis is the potential for false positive results in cases of cholangiocellular carcinoma (CCC). To address this concern, the American College of Radiology developed the CEUS LI-RADS (Liver Imaging Reporting and Data System) diagnostic algorithm for stratifying at-risk lesions evaluated by CEUS. The main objectives of this study were as follows: to assess the performance of the CEUS LI-RADS v2017 algorithm in a cohort of patients at high risk of developing HCC; to evaluate the accuracy of the LR-5 category for diagnosing HCC; to determine the distribution of HCC nodules across each LI-RADS category; to analyze the frequency of different types of HCC in the context of liver cirrhosis; and to investigate non-HCC malignant lesions (LR-M category).

### 1.2 Materials and Methods

#### Study Design and Patient Selection

This retrospective study was conducted at the Gastroenterology and Hepatology Department of the Pius Brînzeu Timisoara Emergency Clinical County Hospital (SCJUPBT). It involved a re-evaluation of CEUS examinations performed on patients at high risk of developing HCC, specifically those with liver cirrhosis of any etiology or chronic hepatitis B

or C with severe fibrosis, who were diagnosed with at least one focal liver lesion (FLL) through standard abdominal ultrasound.

#### **CEUS Examination of FLLs**

All patients with FLLs included in the study underwent standard US and CEUS examinations. The CEUS examination protocol adhered to the recommendations provided by the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) for characterizing FLLs using contrast-enhanced ultrasound. The CEUS examinations and subsequent report writing were performed by four experienced physicians with expertise in hepatobiliary ultrasound and CEUS (classified as Level III experts according to EFSUMB and SRUMB classification). During the study period, two different ultrasound systems were used for CEUS: the Acuson S2000 US system (Siemens AG, Erlangen, Germany) and the LOGIQ E9 US system (General Electric Healthcare, Chalfont St Giles, UK). SonoVue® (Bracco Spa, Milan, Italy) was used as the contrast agent, with the volume administered varying depending on the ultrasound system used (2.4 ml or 1.6 ml).

#### **CEUS LI-RADS Algorithm v2017**

The CEUS LI-RADS diagnostic algorithm, developed by the American College of Radiology (ACR), was employed for characterizing and stratifying FLLs in patients at risk of developing HCC. This algorithm categorizes HCC into eight distinct diagnostic categories: LR-1 (definitely benign), LR-2 (probably benign), LR-3 (intermediate probability of malignancy), LR-4 (high probability of HCC), LR-5 (definitely HCC), LR-NC (cannot be classified due to image degradation), LR-TIV (venous tumor invasion - malignant portal thrombosis), and LR-M (probable or definite features of malignancy but not specific for HCC). An independent physician, not involved in the initial CEUS review, re-evaluated all CEUS studies (including written reports, videos, and images) and classified the FLLs into the eight diagnostic categories according to the CEUS LI-RADS v2017 algorithm criteria.

#### **Reference method for FLL diagnosis**

The diagnosis obtained using the CEUS LI-RADS algorithm was compared with the final diagnosis based on the reference diagnostic method, which included contrast enhanced CT/MRI, or histology.

#### **Statistical Analysis**

Statistical analysis was performed using IBM SPSS® for Windows, Version 20.0 (Armonk, NY: IBM Corp), and Microsoft Office Excel 2007. The results were expressed as percentages, and quantitative variables were presented as mean  $\pm$  standard deviation, absolute numbers, and percentages.

### **1.3. Results**

Out of a total of 499 CEUS examinations that were re-evaluated based on the inclusion criteria, 464 focal liver lesions (FLLs) evaluated by CEUS in 382 patients at high risk of developing HCC were included in the final analysis. The mean age of the patients was  $63 \pm 9.6$  years, with a predominance of male sex in 68.3% of cases (261/382).

Liver cirrhosis was diagnosed in 85.3% of the patients, while the remaining cases (14.7%) were diagnosed with chronic liver disease in the form of severe fibrosis. Malignancy was observed in 85.4% (396/464) of the FLLs, with HCC nodules accounting for 90.6% (359/396) of the malignant cases.

Applying the diagnostic criteria of the CEUS LI-RADS algorithm, the 464 FLLs were classified into LI-RADS categories as follows: 22 FLLs in LR-1, 8 FLLs in LR-2, 26 FLLs in LR-3, 106 FLLs in LR-4, 264 FLLs in LR-5, 11 FLLs with associated malignant portal thrombosis in LR-TIV, and 38 FLLs in LR-M.

Typical CEUS behavior with defining criteria for the LR-5 category was observed in 264 (56.8%) of cases, and this corresponded to a final diagnosis of HCC in 258 (97.7%) of cases. The remaining 6 lesions in this category included 4 liver metastases and 2 regenerative nodules with high-grade dysplasia. Out of the total 464 FLLs, CEUS behavior corresponding to LR-3 and LR-4 categories was observed in 26 (5.6%) and 106 (22%) of cases, respectively. The risk of HCC progressively increased from the LR-3 to LR-5 category, as indicated by the number of HCC nodules included in these categories, with 42.3% and 80.2% of HCC nodules found in LR-3 and LR-4 categories, respectively.

The diagnostic accuracy of the LR-5 category for the diagnosis of HCC was 76.9%, with sensitivity, specificity, positive predictive value, and negative predictive value of 71.9%, 94.3%, 97.7%, and 49.5%, respectively. The positive likelihood ratio was 12.5, and the negative likelihood ratio was 0.3 for the diagnosis of HCC. By combining the LR-4 and LR-5 categories, a higher accuracy of 90.7% with improved sensitivity and negative predictive value can be achieved, but with a lower specificity. The combined LR-4 and LR-5 categories yielded sensitivity, specificity, positive predictive value, and negative predictive value of 95.5%, 74.3%, 92.7%, and 82.9%, respectively.

Features corresponding to the LR-5 category were described in 264 (56.9%) of all 464 nodules, with a corresponding diagnosis of HCC in 258 (97.7%) of cases. The remaining 6 cases in this category included 4 liver metastases and 2 nodules with high-grade dysplasia. No cases of HCC were classified as LR-5. Among the study cohort, 9 out of 464 (1.9%) were cholangiocellular carcinomas (CCCs), with 8 lesions included in the LR-M category and 1 lesion in the LR-4 category. The behavior of CCC cases in CEUS examination showed moderate peripheral enhancement (rim-like pattern) with heterogeneity during arterial phase, followed by washout during portal and late venous phases. No malignant lesions were included in the LR-1 and LR-2 categories.

The initial reports issued during the on-site CEUS examination demonstrated a conclusively correct diagnosis of HCC in 73.2% of cases (263/359), while the LR-5 category of the CEUS LI-RADS algorithm allowed for a correct diagnosis of HCC in 71.9% of cases (258/359), with no significant difference observed ( $p = 0.96$ ).

#### 1.4. Discussions

Recommendations from major hepatology societies endorse the use of US for HCC screening and surveillance in patients with liver cirrhosis or chronic liver disease with advanced fibrosis. CEUS examination is frequently employed for FLL characterization and has demonstrated excellent diagnostic performance supported by multiple studies and meta-analyses.

Several studies have established that the diagnostic performance of CEUS evaluation for HCC is comparable to that of computed tomography (CT) with single or dual-phase contrast or magnetic resonance imaging (MRI) with dynamic contrast. Given the hypervascular nature of HCC nodules, arterial phase hyperenhancement is a crucial element for its diagnosis. CEUS has shown greater sensitivity than CT or MRI in detecting arterial phase hyperenhancement. In our study, arterial phase hyperenhancement was observed in 327 out of 359 HCC cases. Washout in HCC is typically slow, mild in intensity, incomplete, and late, occurring > 60 seconds after contrast injection. However, the absence of washout has been reported in well-differentiated HCC. According to the CEUS LI-RADS definition, typical washout for HCC was present in 71.8% of HCC cases (258/359).

Our study demonstrated excellent Sp and good Se of the LR-5 category for HCC diagnosis, consistent with findings from other relevant studies. The intermediate and high risk for HCC, as defined by the CEUS LI-RADS LR-3 and LR-4 categories, was confirmed in our study by an increasing proportion of HCC nodules from the LR-3 to LR-5 category. The percentage of HCC nodules in the LR-3 category was 42.3%, in the LR-4 category it was 80.2%, and in the LR-5 category it was 97.7%. Thus, from an imaging perspective, the likelihood of an FLL being HCC progressively increases from the LR-3 to LR-5 category.

The diagnostic accuracy of the LR-5 category for HCC diagnosis was 76.9%, with Se, Sp, PPV, and NPV of 71.9%, 94.3%, 97.7%, and 49.5%, respectively. The positive likelihood ratio was 12.5, and the negative likelihood ratio was 0.3 for the diagnosis of HCC. Merging the LR-4 and LR-5 categories yielded Se, Sp, PPV, and NPV of 95.5%, 74.3%, 92.7%, and 82.9%, respectively. These results are comparable to reported data on the diagnostic performance of LI-RADS algorithms for HCC using CT and MRI.

Other published studies comparing the performance of CEUS and CT/MRI with single or dual-phase contrast for FLL characterization using the LI-RADS reporting system have reported similar findings. Tan et al. concluded that CEUS is a useful tool for reassessing LR-3 or LR-4 lesions cleared by CT/MRI, indicating that an FLL reclassified by CEUS examination tends to be HCC. Ding et al. reported good intermodality agreement for

the LI-RADS category between CEUS and contrast-enhanced CT/MRI (kappa value of 0.319;  $p < 0.001$ ).

Differential diagnosis between HCC and cholangiocellular carcinoma (CCC) in patients with liver cirrhosis is challenging. For this reason, CEUS examination has not been recommended as an imaging method for the diagnosis of HCC in previous guidelines from the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD). Several studies have focused on detailed analysis of the contrast behavior of these tumors to aid in the differential diagnosis of HCC and CCC. The literature indicates that, unlike HCC, CCC exhibits more rapid washout, often occurring within one minute after contrast injection, and greater hypoenhancement in the portal venous phase. In our study, there were no cases of CCC included in the LR-5 category. The prevalence of CCC cases was 1.9% (9/464), with most of these lesions being included in the LR-M category (8/9), and 1 lesion in the LR-4 category.

The overall malignancy rate in our study group was 85.4% (396/464). HCC was the most common etiology among all FLLs (359/464; 77.4%) and accounted for 90.6% (359/396) of malignant FLLs, consistent with findings from other published studies. Although HCC is the predominant form of FLL in patients with liver cirrhosis, other non-HCC lesions, both benign and malignant, occur with varying frequencies in cirrhotic livers.

The final diagnosis of non-HCC lesions classified in the diagnostic categories LR-3, LR-4, and LR-M aligns with the results reported in other published studies. However, differences were observed regarding the types of malignant FLLs included in the LR-M category. Our study indicated a higher proportion of liver metastases and a lower number of HCC nodules included in the LR-M category.

## **1.5. Conclusions**

Temporal characteristics of focal liver lesion FLL behavior during CEUS play a crucial role in the CEUS LI-RADS reporting system, serving both for FLL stratification and the differentiation of hepatocellular carcinoma (HCC) from other malignancies.

The LR-5 category of the CEUS LI-RADSv2017 algorithm exhibits good Se, Sp, and excellent PPV for the accurate diagnosis of HCC, thereby minimizing the risk of false positive diagnoses with CCC. The prevalence of CCC in the context of liver cirrhosis was found to be 1.9% (9/464), and none of these cases were included in the LR-5 category. As the LI-RADS category progresses from LR-3 to LR-5, the risk of the FLL being HCC gradually increases.

In our study group, the overall percentage of malignancy was 85.4%, with HCC being the most frequently observed etiology, followed by liver metastases (LM) at a rate of 5%. Benign FLLs were described in 14.6% of cases, with regenerating nodules, hepatocellular adenoma (HA), and other benign entities being the most commonly encountered non-malignant lesions, in descending order of frequency.

## **2. The role of ultrasound based elastography for FLL evaluation. Study II - Elastographic Features of Hepatocellular Carcinoma. Evaluation of Intra- and Interobserver Reproducibility of Point Shear-Wave Elastography Using the Virtual Touch Quantification (VTQ) Method (ARFI Technique) for Tumor Assessment and its Influencing Factors.**

### **2.1 Background and Objectives**

Sonoelastography has been validated for the assessment of liver fibrosis in hepatology. However, its usefulness in clinical practice for characterizing and differentiating liver tumors is still not well-established. Therefore, there is a need to closely evaluate the elastographic characteristics of focal liver lesions (FLL) in various clinical settings. This study aims to assess the stiffness of hepatocellular carcinoma (HCC) in the context of liver



cirrhosis using the point shear-wave elastography (pSWE) technique, specifically the Virtual Touch Quantification (VTQ) method implemented in the Acuson S2000TM ultrasound system (Siemens AG, Erlangen, Germany). Additionally, the study aims to determine the intra- and interobserver reproducibility of the VTQ method for assessing FLL stiffness and identify any influencing factors.

## **2.2 Materials and Methods**

### **Study Type and Patient Selection**

This prospective study was conducted at the tertiary Gastroenterology and Hepatology Center of SCJUPBT from June 2016 to November 2019. A total of 115 patients with liver cirrhosis were evaluated, and 121 FLLs were detected during ultrasound screening for HCC. However, only 88 lesions met the inclusion criteria and were included in the final study cohort.

### **Inter-Observer Reproducibility of VTQ**

To evaluate the interobserver reproducibility of the VTQ method in assessing tumor stiffness compared to the adjacent liver parenchyma, a separate group of 44 patients was included in the study. This assessment was conducted from January 2017 to December 2017 at the Gastroenterology and Hepatology Clinic of SCJUPBT. The patients underwent VTQ measurements at the level of 48 FLLs initially detected by standard ultrasound (US) and at the level of the adjacent liver parenchyma.

### **pSWE Elastography: VTQ - Virtual Touch Quantification (ARFI Technique)**

The same pSWE method, VTQ using Acoustic Radiation Force Impulse (ARFI) technology, was used for elastographic characterization of HCC and for assessing interobserver reproducibility. The VTQ measurements were performed using the Siemens Acuson S2000TM ultrasound system with a 4C1 convex probe. For elastographic assessment of HCC in the context of liver cirrhosis, a single expert operator with extensive experience in liver elastography (>1000 SWE determinations) performed all VTQ measurements in both the FLLs and adjacent liver parenchyma.

During the elastographic measurements, patients were positioned in the supine or left/right lateral decubitus position based on the FLL location. After identifying the lesion using conventional ultrasound, VTQ measurements were performed by placing a fixed-size region of interest (ROI) box (10x5 mm) in the solid part of the lesion, avoiding vascular or necrotic tissue. Patients were instructed to briefly hold their breath to minimize motion artifacts during the measurement.

Ten measurements were performed in both the FLL and adjacent liver parenchyma at approximately the same depth but at a distance. If multiple FLLs were present, the largest lesion or the one with the best visualization on standard ultrasound was chosen for elastographic evaluation. In cases of FLLs larger than 2 cm, the ROI box was positioned at different points to obtain multiple measurements. For the interobserver reproducibility analysis, two examiners (a novice and an expert) performed ten measurements each at the level of the FLL and adjacent liver parenchyma. The VTQ measurement results were expressed in meters per second (m/s) as the average value of the ten measurements.

### **CEUS Assessment**

The CEUS examinations were conducted by highly experienced physicians specializing in hepatobiliary ultrasound and CEUS (Level III experts according to EFSUMB and SRUMB classification). A standardized protocol following the EFSUMB guidelines for the characterization of circumscribed liver lesions was followed. SonoVue® (Bracco Spa, Milan, Italy) was used as the contrast agent for all CEUS examinations. The vascular behavior of the focal liver lesions (FLL) during CEUS was evaluated in three vascular phases. The assessment involved recording videos and images of the three vascular phases and generating a written report that described the vascular "pattern" of the FLL and provided the conclusion of the CEUS examination. The final CEUS diagnosis was determined based on the criteria outlined in the EFSUMB guidelines.

### **Statistical Analysis**

IBM SPSS® for Windows, V 20.0 (Armonk, NY, USA: IBM Corp) and Microsoft Office Excel 2010 were used for the statistical analysis of the obtained data. Descriptive statistics

were employed to present the baseline characteristics of the analyzed FLLs. Quantitative variables were expressed as mean  $\pm$  standard deviation, absolute numbers, and percentages. The intra- and interobserver reproducibility of the VTQ method was assessed using interclass correlation coefficients (ICC) with 95% confidence intervals (CI) and lower and upper limits of agreement (LOA). The interpretation of ICC values was as follows: poor (ICC 0-0.20), reasonable (0.21-0.40), good (0.41-0.75), and excellent (ICC 0.75). The Friedman test was used to compare the first five and last five measurements obtained during the elastographic evaluation conducted by the operator. A p-value less than 0.05 was considered statistically significant.

## **2.3. Results**

### **Elastographic characterization of HCC**

The final study group included 88 patients with a confirmed diagnosis of liver cirrhosis in whom 88 HCC lesions were detected and assessed using VTQ. The success rate of VTQ examination for HCC was 72.7% (88/121).

### **Tumor Stiffness - VTQ Values in HCC**

The mean value of VTQ measurements at the HCC nodule level was  $2.16 \pm 0.75$  m/s. The stiffness at the tumor level was significantly lower compared to the adjacent liver parenchyma:  $2.16 \pm 0.75$  m/s vs.  $2.78 \pm 0.92$  ( $p < 0.001$ ). The mean size of HCC nodules in the study group was  $4.9 \pm 2.2$  cm. A threshold value of 3 cm was chosen to analyze potential differences in VTQ measurements based on tumor size. It was observed that HCC nodules larger than 3 cm exhibited greater intratumoral variability of VTQ values compared to smaller nodules ( $2.05 \pm 0.67$  vs.  $2.21 \pm 0.78$ ,  $p < 0.001$ ).

### **CEUS Assessment of HCC**

A typical behavior to ultrasound contrast and a conclusive diagnosis of HCC were obtained in 76.1% of cases (67/88) compared to the results obtained through the reference methods (CT/MRI with contrast). Comparison between MEs performed in HCC with conclusive CEUS vs. inconclusive CEUS examination revealed no statistically significant differences ( $2.12 \pm 0.58$  vs.  $2.10 \pm 0.62$ ,  $p = 0.72$ ).

### **Intra-Observer Reproducibility**

The intra-observer reproducibility of the VTQ measurements for assessing tumor and liver parenchymal stiffness was excellent, with ICC values of 0.902 (0.87-0.950) and 0.964 (0.943-0.960), respectively. This indicates that the method is reliable for assessing both liver and tumor stiffness in the context of liver cirrhosis. No statistically significant differences were observed between the first and last five measurements, and the ICC value was excellent (0.926, 95% CI: 0.890-0.960,  $p = 0.75$ ). Based on these results, five VTQ measurements at the tumor level are considered sufficient for elastographic evaluation. The ICC values for the measurements indicate that VTQ is a reproducible method for assessing tumor stiffness.

### **Inter-Observer Reproducibility**

The mean stiffness values obtained through the VTQ method were similar in both sets of measurements for tumor and adjacent liver parenchyma assessment. The ICC values demonstrated very good inter-observer reproducibility for the VTQ method at both tumor and liver parenchyma levels, with ICC values of 0.950 (0.910-0.972) and 0.989 (0.980-0.994), respectively. The impact of several factors on inter-observer reproducibility was analyzed. Overall, very good ICC values were observed, with slightly reduced but not statistically significant values in the case of heterogeneous FLL, FLL size  $\geq 3$  cm, and deeply located FLLs at  $\geq 6$  cm. Among the tumor types, the studied group included 32 HCC, 7 HH, and 9 LM, with higher variability observed in HH cases.

## **2.4. Discussions**

The utility of elastography in distinguishing focal liver lesions (FLL) is based on the concept that tissue elasticity changes with alterations in tissue structure or composition, which occur through various pathological processes such as inflammation or malignant transformation. Among the available elastographic methods, point-SWE and 2D-SWE have

been extensively studied for characterizing FLL. Accurate characterization of FLLs detected in the context of liver cirrhosis is crucial for subsequent management, including follow-up and selection of appropriate therapeutic options. However, liver biopsy is limited in patients with liver cirrhosis due to potential complications, although it should be considered when a definitive diagnosis cannot be established using contrast imaging techniques.

In our study, we assessed the stiffness of hepatocellular carcinoma (HCC) in the context of liver cirrhosis using the point-SWE - Virtual Touch Quantification (pSWE-VTQ) method. The mean pSWE-VTQ values in HCC indicated significantly lower stiffness compared to adjacent liver parenchyma, with values of  $2.16 \pm 0.75$  m/s and  $2.78 \pm 0.92$  m/s, respectively ( $p < 0.001$ ). These findings are consistent with a study by Gallotti et al., which demonstrated that HCC tumors have lower stiffness than the surrounding liver parenchyma, with a mean pSWE of 2.17 m/s in HCC compared to 2.99 m/s in the liver parenchyma. Previous studies have also shown that malignant HCCs are generally stiffer than benign lesions, with a decreasing order of tumor stiffness as follows: liver metastasis > HCC > hepatocellular adenoma > focal nodular hyperplasia. In the context of liver cirrhosis, HCC nodules may exhibit lower stiffness compared to adjacent liver parenchyma and other malignant liver lesions (such as liver metastasis and HCC).

The interpretation of pSWE-VTQ measurements should consider the clinical context of the patient under examination. Our study specifically focused on the elastographic features of HCC in patients with liver cirrhosis who had a fibrotic liver background. The VTQ ratio, which represents the stiffness ratio between the HCC nodule and adjacent liver parenchyma, was found to be  $1.33 \pm 0.66$  m/s in our study, indicating lower stiffness of HCC nodules compared to adjacent liver parenchyma. This stiffness ratio in HCC cases aligns with other studies that have investigated the usefulness of VTQ ratios in differentiating tumors. The diagnostic performance of elastography is influenced by the clinical context, where factors such as age, associated pathologies, laboratory tests, and results of other imaging techniques should be considered for the differential diagnosis of HCC.

A strength of our study is the homogeneous distribution of the etiology of the included lesions, all of which were HCCs, despite the challenges encountered in clinical practice for diagnosing these lesions. Additionally, we were able to integrate elastography with ultrasound (US) and contrast-enhanced ultrasound (CEUS) to obtain a multiparametric US approach for analyzing FLLs, which can be easily applied in clinical practice for all analyzed lesions. Understanding the elastographic characteristics of HCC could be valuable in clinical practice, particularly in cases where CEUS examinations yield inconclusive results for FLL evaluation in patients with liver cirrhosis. The mean pSWE-VTQ values in HCC were not significantly different between cases with conclusive and inconclusive CEUS examinations ( $2.12 \pm 0.58$  vs.  $2.10 \pm 0.62$ ,  $p = 0.72$ ). Thus, suspicion of HCC may be reinforced when the mean VTQ values of examined nodules indicate less stiffness compared to the surrounding liver parenchyma. Elastography using pSWE can serve as a useful tool for guiding diagnosis and the need for prompt further evaluation. The tumor assessment using VTQ software can be easily integrated into imaging protocols that already involve standard US and CEUS for HCC evaluation, without incurring significant additional costs.

The feasibility of applying the pSWE-VTQ method in assessing tumors and liver parenchyma is supported by excellent intra- and inter-operator reproducibility, both in patients with liver cirrhosis and those without underlying liver pathology. Intra-observer reproducibility for mean stiffness measurements using VTQ in HCC was excellent, with an intraclass correlation coefficient (ICC) of 0.902 (95% CI: 0.87-0.950). Our study also demonstrated excellent inter-operator reproducibility of VTQ measurements, with an ICC of 0.950 (95% CI: 0.910-0.972). However, there was slightly higher variability in elastographic measurements at the tumor level, depending on factors such as size, heterogeneity, and depth of the lesion. Notably, clinical experience in pSWE elastography did not impact the inter-operator reproducibility of VTQ in tumor assessment, as evidenced by the minimal difference in mean VTQ values obtained by novice and expert elastography operators at both the tumor and adjacent liver parenchyma levels. Considering the time efficiency of the elastographic tumor assessment procedure, our study determined that obtaining five mean stiffness measurements using VTQ is sufficient to achieve reliable results.

Although our study included a relatively large number of HCC cases (n=88), it has certain limitations that should be acknowledged. First, there was no control group consisting of other types of benign/malignant hepatic lesions detected in the context of liver cirrhosis. Additionally, as no biopsies were available for the analyzed HCCs, we were unable to establish a correlation between VTQ values and the degree of tumor differentiation. However, the primary objective of our study was to characterize the elastographic properties of HCC in the context of liver cirrhosis and assess the feasibility of the VTQ method for evaluating tumor stiffness. Future research should focus on prospective multicenter studies that analyze the elastographic characteristics of different types of FLLs in various clinical settings. This will enable the accurate integration of elastography into the diagnostic algorithm for HCC.

## **2.5. Conclusions**

The VTQ elastographic method provides complementary information to standard ultrasound evaluation for assessing tumor and liver stiffness. In HCC nodules, the mean VTQ values were found to be  $2.16 \pm 0.75$  m/s. Understanding the stiffness of HCC can support an algorithm-based approach to evaluating FLLs in patients with liver cirrhosis. Excellent ICC values for mean stiffness measurements demonstrate that VTQ is a highly reproducible method for assessing both tumor stiffness and liver parenchyma. Five mean stiffness measurements using VTQ are sufficient for tumor assessment. Elastography experience does not influence the reproducibility of the VTQ method in assessing tumors or liver parenchyma.

## **3. The concept of multiparametric ultrasonography (MPUS) for the diagnosis of focal liver lesions. Study III – Role of an MPUS approach for the diagnosis of FLL with inconclusive CEUS results using a binary decision tree-based classifier.**

### **3.1. Background and objectives**

The concept of multiparametric ultrasonography (MPUS) has gained significant attention as a valuable diagnostic tool for focal liver lesions (FLL). In addition to providing information on the morphology and topography of these lesions, MPUS offers functional and hemodynamic data that, when correctly interpreted in the relevant clinical and biological context, can facilitate a rapid and cost-effective final diagnosis of the nature and type of the tumor. This diagnostic accuracy is crucial for patients in terms of proper diagnosis, selection of curative therapeutic options, and accurate prognosis.

The **objectives** of Study III were as follows: (1) to investigate the contribution of an MPUS approach to the diagnosis of FLL with inconclusive results from contrast-enhanced ultrasonography (CEUS) by utilizing a binary decision tree-based classifier to determine the malignancy of the formations; (2) to evaluate the accuracy of MPUS in the differential diagnosis of tumor nature (benign or malignant); and (3) to determine the rate of correct identification of tumor type using this algorithm.

**Study design and patient selection.** This retrospective study involved a re-evaluation of FLL with inconclusive CEUS results based on the diagnostic criteria outlined in the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) guidelines. The evaluation spanned a period of two years (2017-2018). All FLL included in the study underwent cross-sectional imaging evaluation with contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) with specific diagnostic criteria or underwent biopsy with histopathological examination, which served as reference methods for establishing the final diagnosis of FLL.

The ultrasound (US) exploration and CEUS examination of FLL were performed by experienced hepatobiliary ultrasound experts with over 10 years of proficiency in CEUS. The CEUS examination protocol adhered to the recommendations stipulated in the EFSUMB

guidelines and has been described in detail in previous studies. In cases where CEUS results were inconclusive, the atypical vascular pattern of FLL was reported in the CEUS interpretation. The US equipment employed for FLL assessment was the LOGIQ E9 system from General Electric Healthcare (Chalfont, St. Giles, UK). Notably, the operator was blinded to the final diagnosis during the post-processing analysis of FLL.

#### **The MPUS approach stages.**

**In Stage I**, the assessment of liver stiffness aimed to confirm or exclude the presence of liver cirrhosis (fibrosis F = 4). Liver stiffness (HR) was measured using SWE elastography methods, which were performed and interpreted according to the EFSUMB guidelines for liver elastography. Two elastography techniques were utilized: (a) 2D-SWE.GE technique integrated into the US LOGIQ E9 system, employing a C1-6-D probe, and (b) transient elastography - FibroScan®, utilizing M or XL probes depending on patient weight.

**Stage II** involved TIC analysis, where a quantitative analysis of tissue perfusion in the late phase of CEUS assessment was conducted. This analysis aimed to identify the presence of the washout phenomenon at the level of FLL. Time-intensity curve (TIC) analysis was performed on the late vascular phase (>120 s) of each FLL, with curve fitting parameters such as time to peak, area under the curve, peak gradient, and curve gradient assessed to identify the presence and degree of washout. The washout curve fit was calculated using the formula: Washout:  $F(t) = A \exp(-kt) + B$ . Two regions of interest were selected: one corresponding to the tumor area and another reference region adjacent to the parenchyma, perceived at the same depth.

**In Stage III**, parametric imaging analysis was conducted to evaluate the kinetics of FLL perfusion. This analysis was based on a color-coded map provided by the parametric imaging application implemented in the GE-LOGIQ E9 US machine. The arterial phase (onset 10-20s after contrast injection, lasting up to 30-45s) of each FLL with inconclusive CEUS results was reassessed and analyzed using the parametric imaging application. Classification of FLL was performed based on their typical vascular behavior in response to ultrasound contrast.

The classification process relied on a binary decision tree classifier (BDTC) after completing all the steps of the MPUS algorithm and clustering the data. This BDTC model facilitated computer-aided decision-making by utilizing the MPUS data to determine the presence of cirrhosis, malignancy of the formations, and ultimately, the type of formation.

#### **Statistical Analysis**

For statistical analysis, MedCalc v19.3 software from MedCalc Software Ltd., Ostend, Belgium, was employed. Categorical variables were compared using the chi-square test or Fisher's exact test, while continuous variables were assessed using the t-Student test or Mann-Whitney test. A p-value less than 0.05 was considered statistically significant. Linear regression analysis was employed to analyze ICT and factors associated with malignancy. To evaluate the performance of the MPUS score, receiver operating characteristic (ROC) analysis was conducted. Optimal cut-off values were determined from the corresponding ROC curves, and various performance metrics, including Se, Sp, PPV, NPV, and Ac of the algorithm, were calculated.

### **3.3. Results**

The study cohort comprised 91 patients who had been diagnosed with focal liver lesions (FLL) using standard ultrasound (US) and required contrast-enhanced ultrasound (CEUS) evaluation for further characterization due to inconclusive results. The average age of the patients was  $62.3 \pm 6.4$  years, and there was a relatively equal distribution of genders. Liver cirrhosis was diagnosed in 34 out of 91 cases (37.4%).

Among the study group, 56% (51/91) of the FLL were found to be malignant, with hepatocellular carcinoma (HCC) being the most common type (34/51), followed by liver metastases (13/51). The most prevalent benign FLLs were hepatic hemangiomas (7/40) and regenerating nodules (7/40). The final diagnosis of the 91 FLLs with inconclusive CEUS results, determined using reference methods such as biopsy and imaging criteria, included 34 HCCs, 13 metastases, 7 hemangiomas, 7 regenerating nodules, 5 areas of patchy

steatosis, 3 fatty-free areas, 4 cholangiocarcinomas, 2 abscesses, 5 adenomas, and 11 other benign formations (e.g., complex biliary cysts, parenchymal infarct areas, and vascular abnormalities).

Statistical analysis of the TIC-CEUS parameters revealed a significant difference between malignant and benign lesions only in the AREA parameter ( $25.08 \pm 37.98$  and  $7.08 \pm 42.6$ ,  $p = 0.04$ ). Other parameters such as A, B, k, and TtoPk did not show statistically significant differences between the two groups.

Regression analysis demonstrated a significant relationship between the area of the FLL and the area of the liver parenchyma ( $p = 0.02$ ). The regression equation ( $y = -12.21 + 0.16 x$ ) indicated that for each additional unit in the parenchymal area, the area of the lesion increased by an average of 0.16 dB.

The optimal cut-off value for the FLL area in predicting malignancy and the presence of washout in inconclusive lesions was determined to be  $>19.3$  dB, with an area under the receiver operating characteristic curve (AUROC) of 0.58, a sensitivity of 74.0%, and a specificity of 45.7%. Comparing different ultrasound-based methods, standard US had a sensitivity of 15.6% and an AUROC of 0.52 in detecting malignant lesions, while elastography showed an AUROC of 0.64 and a sensitivity of 74.5%. By combining elastography results with the cut-off value of the FLL area from CEUS-TIC analysis, improved diagnostic performance was observed, with an AUROC of 0.72 and a sensitivity of 90.2% for detecting malignant lesions with washout. The multiparametric ultrasonography (MPUS) approach correctly classified 66 out of 91 FLLs, resulting in an overall accuracy of 72.3%. The BDTC algorithm considered CEUS-TIC analysis, elastography results, and the presence of washout in determining the nature (malignant or benign) of the tumor. With this algorithm, 71 out of 91 lesions were correctly classified as malignant or benign, with an accuracy of 78.0%. There was no statistically significant difference in the diagnostic performance between malignant and benign lesions.

Regarding the specific types of FLLs correctly diagnosed using the BDTC algorithm, 20 cases (out of the total 91 FLLs with inconclusive CEUS) were accurately identified, resulting in a detection rate of 28%. Among these correctly classified cases, 9 were HCC, 6 were liver metastases, 2 were hepatic focal nodular hyperplasia (HFN), and 2 were fatty-free areas. In terms of distinguishing between malignant and benign FLLs, the algorithm predominantly detected HCC (23 cases classified as malignant), liver metastases (9 cases classified as malignant), and regenerating nodules (5 cases classified as benign).

The performance of the BDTC algorithm in differentiating the nature of the tumors was as follows: Ac 78%, Se 62%, Sp 45%, precision 80%, and F1 score 69.8%.

### 3.4. Discussions

Recent advances in US technology have enabled the development of new applications that provide a comprehensive understanding of structural, functional, and hemodynamic changes in FLLs. In this study, we employed a classifier based on a MPUS approach to re-evaluate FLL that initially yielded inconclusive results during CEUS examination. The MPUS analysis encompassed CEUS evaluation, liver elastography, and additional applications such as time-intensity curve (TIC) analysis for quantifying contrast agent and tissue perfusion, as well as perfusion imaging (PI) to document arterial phase contrast arrival time and uptake pattern using a color-coded map. To facilitate the classification process, we developed a binary decision tree-based classifier (BDTC algorithm), which enabled us to determine malignancy in the first stage and FLL type in the second stage.

Although CEUS demonstrates excellent performance in characterizing FLL, there are cases where a definitive diagnosis cannot be established. Several factors contribute to inconclusive CEUS results, including liver cirrhosis, FLL size, histological differentiation, vascular abnormalities, or inadequate ultrasound imaging windows. The terminology and potential clinical applications of MPUS have been discussed in several editorials, emphasizing the novel techniques and their potential utility in clinical practice. To date, there has been only one report on multiparametric ultrasound in FLL, focusing on "de novo" lesions and categorizing tumors into solid and cystic lesions. In our study, we deemed this

categorization unnecessary due to the low incidence of malignant cystic lesions in daily practice and the ease of diagnosing simple cysts using standard US. Instead, we primarily dichotomized patients into those with or without cirrhosis, as liver stiffness measurements obtained through elastography provided quantifiable information.

In addition to the practical algorithm proposed, we investigated several parameters to assess late-phase CEUS using TIC for malignancy highlighting and PI for fLL type, objectively quantifying washout and wash-in signals. Our study focused on evaluating inconclusive FLL analyzed via CEUS, which presented greater challenges compared to standard cases, even for experienced CEUS examiners. The most common lesion among the inconclusive FLL analyzed in this study was HCC, accounting for 37% of all lesions analyzed. Considering regenerative nodules as potential HCC precursors, the percentage increased to 45%. Diagnosing HCC remained challenging in our study, consistent with other relevant studies. We employed the BDTC algorithm to assess FLL, which effectively detected benign and malignant lesions; however, its performance in determining tumor type was less satisfactory. Nevertheless, this classifier serves as a precursor to employing machine learning methods and ultimately neural networks for FLL assessment. Several limitations exist within this study, including its retrospective nature and evaluation of a relatively small sample size. Additionally, the MPUS features utilized in this study were based on applications implemented in a single US system (GE's LOGIQ E9), representing an important study limitation.

This study represents the first publication to investigate the MPUS strategy for evaluating inconclusive FLL in CEUS, providing a valuable contribution to time and resource savings. The MPUS approach can be regarded as a recommended option for liver assessment, combining basic and advanced US features in an AI and MPUS-based system, which could overcome limitations associated with traditional US.

### **3.5. Conclusions**

The reassessment of FLL with inconclusive CEUS results using the MPUS algorithm, incorporating a BDTC classifier, yielded successful diagnoses of malignancy in 78% of cases and tumor type in 28% of cases.

## **FINAL CONCLUSIONS**

1. The implementation of standardized reporting for CEUS interpretations demonstrates efficacy in stratifying FLLs in patients at risk for HCC development.
2. The LR-5 category of the CEUS LI-RADS algorithm exhibits a high predictive value for the diagnosis of HCC.
3. The prevalence of CCC among patients with liver cirrhosis was found to be 1.9% (9/464), with no cases included into the LR-5 category.
4. The risk of HCC progressively increases from LR-3 to LR-5.
5. Given the inability to exclude malignancy in focal liver lesions (FLL) categorized as LR-3, LR-4, and LR-M, liver biopsy should be considered for definitive diagnosis.
6. The pSWE VTQ method demonstrates excellent intra- and interobserver reproducibility, making it a valuable tool for assessing both FLL stiffness and liver parenchyma.

7. The assessment of five elastographic measurements using VTQ is sufficient for evaluating tumors.
8. The reproducibility of the VTQ method in assessing tumors and liver parenchyma remains consistent regardless of elastography experience.
9. Evaluation through the pSWE VTQ method enables effective elastographic characterization of HCC.
10. The average VTQ measurements at the level of HCC nodules were determined to be  $2.16 \pm 0.75$  m/s. Understanding the stiffness of HCC can support an algorithm-based approach to focal liver lesions in patients with liver cirrhosis.
11. The MPUS approach successfully classified 66 out of 91 FLLs with inconclusive CEUS results, achieving an accuracy of 72.3%.
12. By utilizing the binary decision tree-based classifier (BDTC) algorithm developed based on MPUS evaluation of FLLs, we were able to establish a diagnosis of malignancy in 78% of cases and determine the formation type in 28% of cases.